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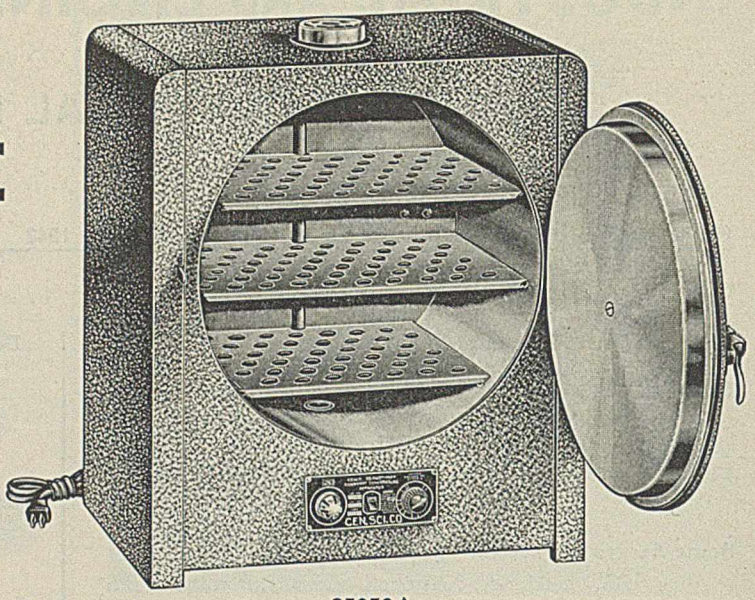

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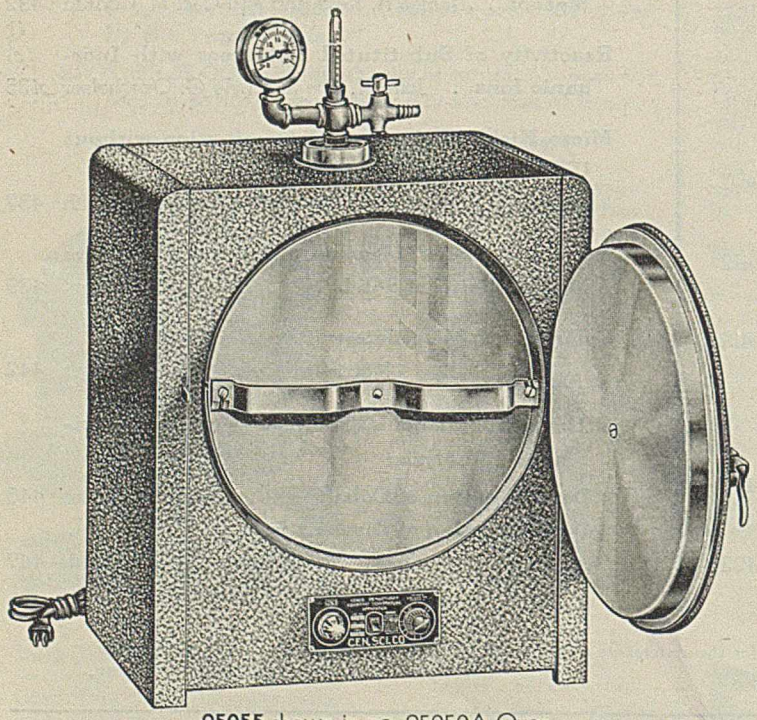

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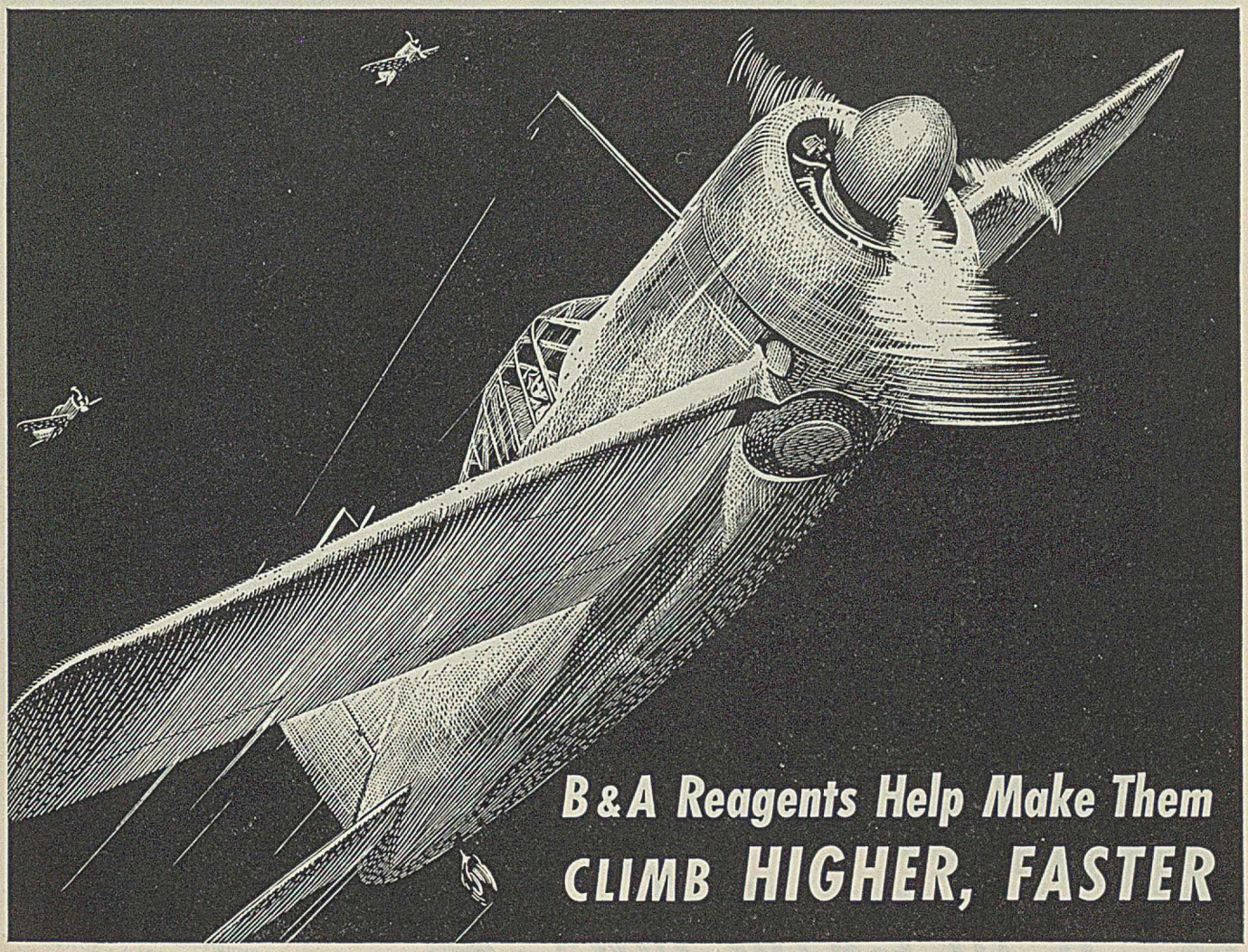

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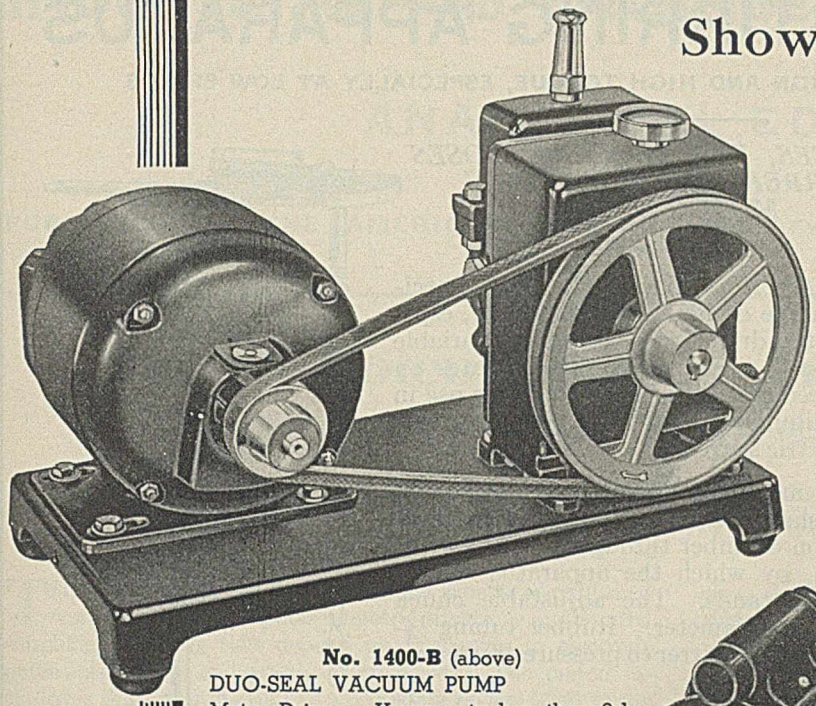

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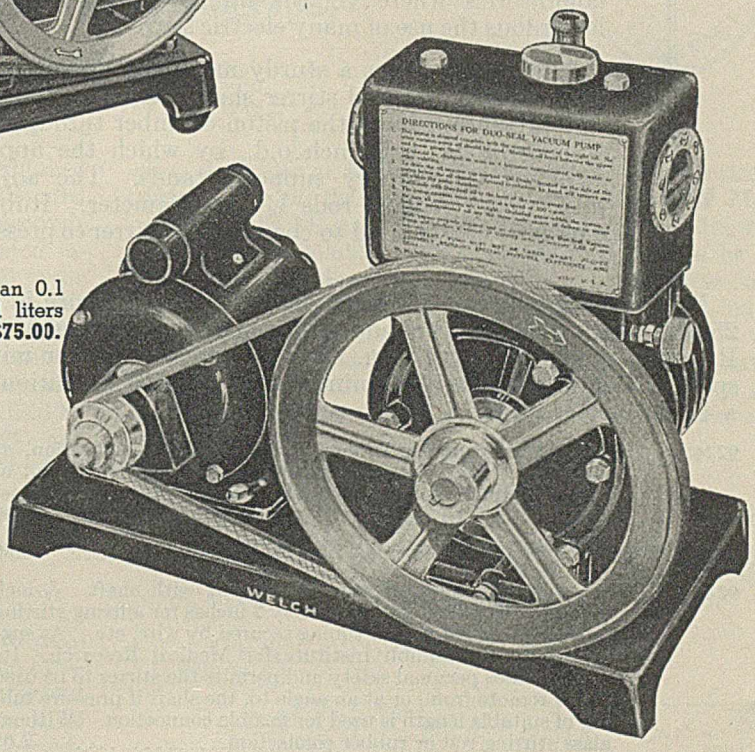

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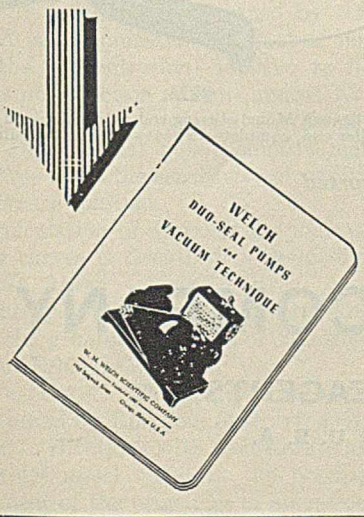

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Potentiometric Titrations

A Review with Bibliography

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THIS paper attempts to cite the chief aspects of published work in the field of potentiometric titrations and related simplified methods that depend upon changes in electrode potentials at the end points of titrations. The period included is that from the time of a preceding review (*162*) to the end of 1941. A partial review by the author based on this and other fields of electrotitration has appeared (*166*). More or less recent reviews of the field have been published by Lassieur (*329*), Fell (*135*), and Lederer (*333*). Mention should also be made of monographs or books in this field by Böttger (*47*), Hiltner (*229*), Kolthoff and Furman (*299*), and Müller (*387*). Useful data on oxidation-reduction potentials are to be found in Latimer's treatise (*330*).

The field will be reviewed under the general headings of Theory, Apparatus and Technique, and Applications.

Theory

THE ACID-ALKALI FIELD. A further detailed mathematical consideration of the appearance of inflections, the titration error, and the relative ultimate accuracy of indicator and e. m. f. methods has been given by Roller (*463*) and by Kilpi (*293, 294*). The latter has considered in detail the mathematical theory of the equivalence point, buffer action, the stepwise titration of dibasic acids, the theory of the titration error, and basic dissociation constants in glacial acetic acid (*293*). The conclusions of these recent studies are not widely different from those of Bjerrum (1911). In general, the appearance of an inflection is governed by the magnitude of Kc (where K is the ionization constant of the weak acid or base and c the concentration) relative to K_{solvent}. In aqueous solutions no inflection appears unless Kc is $> 27K_w$. For practical analytical purposes Kc should be greater than 10^{-10}. The classical potentiometric method is capable of about three times the sensitivity of titration to the color tint of a reference solution.

The calculation of approximate ionization constants from titration curves is described in detail by Hahn and Klockmann (*216, 219*).

The theory of the differential titration of a strong or a weak acid by the general procedure of MacInnes has been treated by Giraut-Erler (*185, 186*).

PRECIPITATION. The theory of second-class electrodes has been reviewed by Ringbom (*452;* cf. *450, 451*), with especial reference to titrations with lead nitrate as reagent.

The chief novel trend in the precipitation field is the development of the principles of operation of so-called third-class electrodes as indicators for titrations. The electrode systems are of the type: metal$_1$/solid M_1A/solid M_2A, where M_1 and M_2 stand for different metals whose salts with A are slightly soluble. The electrode is desired to be an indicator for the ions of M_2, for which no convenient solid electrode is available. The electrodes that have been investigated have been chiefly those which might indicate the concentration of calcium ion—e. g., silver–silver oxalate–calcium oxalate. The theory has been developed by LeBlanc and Harnapp (*332*). The two solubility products are:

$$S_1 = (B_1^+)\,(A^-) \text{ and } S_2 = (B_2^+)\,(A^-)$$

where B_1^+ and B_2^+ are the cations and A^- is the common anion. The effect of a small increase in the concentration of B_2^+ from $(B_2^+)_0$ to $(B_2^+)_0 + c$ is considered in terms of c, S_1, S_2, and $(B_2^+)_0$. The expression that results for c is:

$$c = \frac{2S_1 + S_2}{2(S_1 + S_2)}\,(B_2^+)_0 + \sqrt{\frac{(2S_1 + S_2)^2}{4(S_1 + S_2)^2}\,(B_2^+)_0^2 - \frac{S_1}{S_1 + S_2}\,(B_2^+)_0 + \frac{S_2^2}{S_1 + S_2}}$$

The condition $c = 0$ is the most favorable one for a good indicator electrode because this means that no change in the cation content of the solution occurs through the formation or solution of the precipitate. The ideal condition is most closely approximated when $\frac{2S_1 + S_2}{2(S_1 + S_2)} = 0.5$. A summary of the electrodes of this class that have been studied is given under Applications.

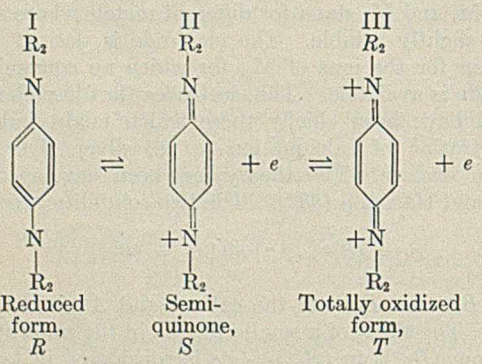
Polarometric Titrations. Although logically beyond the scope of this review, certain titrations based upon the dropping mercury electrode are closely analogous to titrations with polarized electrode systems. In the polarometric (amperometric) method it is often necessary to plot current *vs.* volume of reagent added with correction for the change in volume. The dropping mercury electrode may serve as an indicator for ions such as lead, calcium, barium, sodium, etc., for which in general no satisfactory direct indicator electrode can be prepared (*2, 298, 300–308, 328, 358, 411, 413*). This new technique is not limited to precipitations, but has been shown to be applicable also to oxidation-reduction titrations (*541, 553*). In certain cases the end point is marked by either an increase or decrease in current that is so abrupt that plotting of data is unnecessary (*306*).

OXIDATION-REDUCTION. Mohr (*379*) has pointed out again that the equivalence potential is not at the average between the two standard potentials of the higher and lower oxidation-reduction systems (cf. *299*, pages 45–6). As is well known, this point is at a weighted mean of the two standard potentials. In general, for the reaction $a\mathrm{Ox}_1 + b\mathrm{Red}_2 \rightleftharpoons a\mathrm{Red}_1 + b\mathrm{Ox}_2$, the equivalence potential is $E_E = \frac{bE_0' + aE_0''}{a+b}$, where E_0 is the standard potential for the higher and E_0'' for the lower of the oxidation-reduction systems.

Travers (*589*) has considered the question of making predictions from oxidation-reduction theory. Irreversible effects at electrodes and slow rate-determining steps often make predictions difficult.

The detailed mathematical theory of the course of e. m. f. during oxidation-reduction processes has been treated by Michaelis and associates (*369–371*) and by Geake (*180*). The polarographic technique has also proved to be significant in this field (*398*).

In the case of semiquinone formation, which was the chief point of interest in developing the theory, Michaelis formulates the transformations for substituted paraphenylene diamines, which are the types of compounds that were studied most exhaustively, as follows:



The respective concentrations of the three forms are symbolized by r, s, and t. The equilibrium constant is

$$K = \frac{s^2}{rt} \qquad (1)$$

The relation $r + t = 2s$ must also hold.

If the solutions are properly buffered during the oxidation studies, the two possible stages of the process are represented by

$$E = E_1 + \frac{RT}{F} \ln \frac{s}{r}$$

$$E = E_2 + \frac{RT}{F} \ln \frac{t}{s}$$

and for the complete process

$$E_{\text{total}} = E_m + \frac{RT}{2F} \ln \frac{t}{r}$$

where

$$Em = \frac{E_1 + E_2}{2}$$

Let the total concentration of substance be

$$a = r + s + t \qquad (2)$$

and let x be the molar concentration of oxidant added, starting with the pure reduced form of concentration r.

$$x = s + 2t \qquad (3)$$

Upon combining expressions 1, 2, and 3 with the electrode expression and letting x/a be represented by X, the following general equation is obtained:

$$E = E_0'' + \frac{RT}{2F} \ln \frac{X}{2 - X} + \frac{RT}{2F} \ln \frac{X - 1 \pm \sqrt{(X-1)^2 + 4X(2-X)/K}}{1 - X \pm \sqrt{(X-1)^2 + 4X(2-X)/K}} \qquad (4)$$

If the equilibrium constant of a particular semiquinone formation process is very small, Equation 4 reduces to the familiar equation for a process involving two electrons:

$$E = E_0' + \frac{RT}{2F} \ln \frac{\frac{x}{r+s+t}}{2 - \left(\frac{x}{r+s+t}\right)}$$

whereas at large values of K the curve is composed of two branches, each of form corresponding to

$$E = E_0'' + \frac{RT}{F} \ln \frac{(\mathrm{Ox})}{(\mathrm{Red})}$$

In Figure 1 is given Michaelis' plot of values of E *vs.* values of X for various values of the constant.

Geake (*180*) has given a detailed mathematical analysis of the possibilities, including formation of slightly soluble intermediate products.

Müller (*399–402*) has shown that an appropriate modification of Equation 4 may be applied to a study of semiquinone formation by the polarographic technique. This is not so accurate as the potentiometric method, but permits the study of labile radicals, and may also be applied in the range of over-voltage where the platinum electrode is not suitable.

Computation of Inflections. When the plots of e. m. f. *vs.* milliliters of reagent are not sharp, or the corresponding observations do not give a clear indication of the end point, it may still be possible to estimate the end point with fair precision, provided the reagent has been added in small equal increments. Hahn (*217*), Hahn and Klockmann (*218*), Fenwick (*136*), and later Shibata (*496*) have dealt with this question.

An illustration will make clear the simple application of the change in the second difference quotients of e. m. f. with milliliters of reagent. Let V_1, V_2, V_3, and V_4 be volumes read at equal

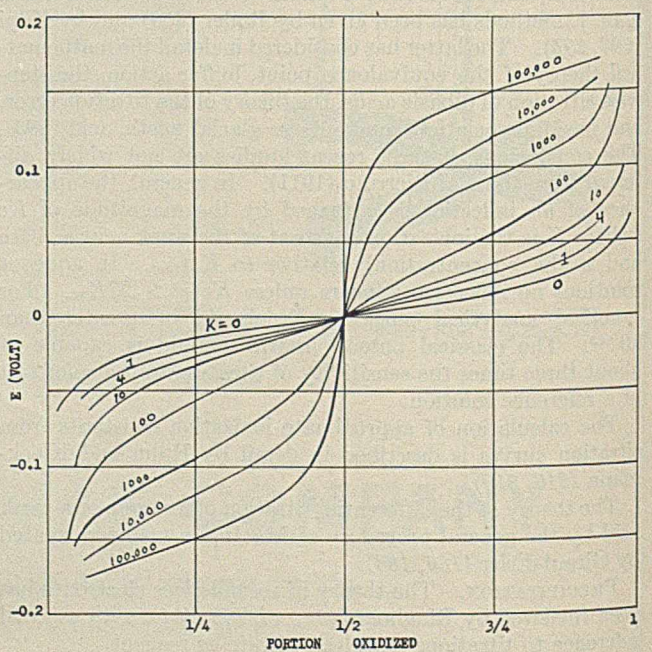


FIGURE 1. *E vs. X*, FRACTION OXIDIZED, FOR VARIOUS VALUES OF CONSTANT *K* OF SEMIQUINONE FORMATION

increments of reagent and the corresponding e. m. f. values be E_1, E_2, E_3, and E_4.

	E_1	E_2	E_3	E_4	E_5
E. m. f.	0.422	0.437	0.458	0.475	0.489
	V_1	V_2	V_3	V_4	V_5
Volume of reagent	30.20	30.40	30.60	30.80	31.00
$\Delta E/\Delta$ml.		7.5	10.5	8.5	7
Δ^2E/Δml.^{2}		3.0 (D_1)	−2.0 (D_2)	−1.5	

The end point lies between 30.4 and 30.6, since the values of Δ^2E/Δml.^{2} change sign, and the end point is calculated to be at $30.4 + 0.2 \times \frac{3}{3+2} = 30.5_2$. In general, the volume to be added to the last buret reading before the change in sign of the $\Delta^2E/$ml.^{2} values is $\frac{D_1}{D_1 + D_2} \times$ volume increment, using the numerical values of D_1 and D_2 without regard to their signs.

The deviation between the theoretical and actual volumes in titrations with unsymmetrical curves has been discussed by Murgulescu and Drăgulescu (*404*), and by Hahn, Frommer, and Schulze (*214*). Hahn (*206*, *213*) has treated the effect of errors and interferences upon accuracy, and the subject of titration errors has been handled mathematically by Shakhkeldian (*493*). Hahn (*209*) and Hahn and Klockmann (*215*) describe the methods of deriving equilibrium constants from titration data.

Apparatus and Technique

Electrode Devices. An excellent type of salt bridge with a ground-glass stopper built in each end of the bridge (Figure 2) has been described by Irving and Smith (*252*). A bridge with sintered-glass ends is described by Laitinen (*318*). Gotō (*190*) has described the use of micro filter tubes, or tubes provided with either ground-glass joint, sintered glass, or a stopcock to construct reference electrodes free from diffusion effects. Some of the commercially available forms of electrodes that have been developed for pH measurements may be used almost indefinitely in titrations without serious diffusion errors. Lykken and Rolfson (*347*) have advocated the use of such electrodes.

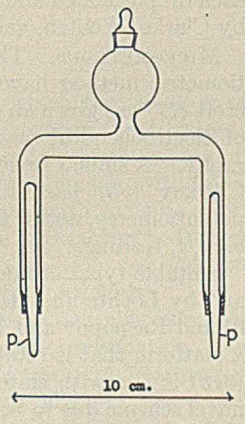


Figure 2. Salt Bridge

Tapered glass rods fit snugly through ground-glass connections.

Vance (*597*) has advocated the more general use of the silver–silver chloride electrode for reference in acid-alkali titrations. Capillary electrodes of platinum and silver have been proposed as indicator electrodes by Poupé (*434*).

Hiltner (*229*) attaches the upper ends of reference and indicator electrodes, with insulation to prevent short circuits, to a stout piece of wire bent to fit the rim of a beaker (Figure 3). Herzog (*228*) has proposed the use of flanged tubing, or tubes cut from the tops of test tubes, to hold electrodes which are mounted in rubber stoppers. The author has for many years mounted electrodes in holes drilled in Bakelite, the electrode mount being held in place by a ring of rubber tubing (*165*). Sholes (*499*) advocates protecting platinum electrode wires with glass to a point below the surface of the liquid that is titrated. The question of strain in glass and the failure of electrodes sealed through glass has been treated by Garrett *et al.* (*177*).

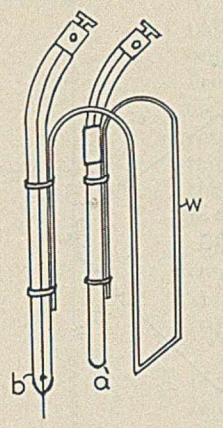


Figure 3. Simple Electrode Holder

Wire *w* serves as a support for electrodes *a* and *b* and will fit several sizes and types of beakers.

Atanasiu and Velculescu (*13*, *24*) have proposed the platinum-nickel electrode pair as a universal indicating system for neutralizations, oxidation-reduction titrations, and argentometry. In the last field the system appears to be unreliable, according to a few experiments by the author's students. Better combinations are available for the other fields.

Erbacher (*128*) has proposed the use of Ostwald decade boxes in a Wheatstone bridge arrangement for potentiometric titrations.

Simplified Devices for Determining End Points. The differential method has been further applied to the use of hydrogen electrodes in precision measurements by MacInnes and Cowperthwaite (*351*). Tungsten electrodes have been used by Abichandani and Jatkar (*1*). The method can be recommended in general for any type of problem where the use of a reference electrode presents difficulty. Orloff (*423*) uses the method, with platinum–lead peroxide electrodes, for controlling on an industrial scale the conversion of chromate to bichromate.

Guzmán and associates have used a thermocouple as a source of e. m. f. for polarizing electrodes (*203*) and have used the silver-platinum electrode system short-circuited through a sensitive galvanometer ($i = 7 \times 10^{-6}$ ampere per division) in the titration of the halides (*204*). The phenomena are analogous to the dead-stop method of Foulk and Bawden. The latter method has been extended by Clippinger and Foulk (*93*) to acid-alkali titrations with the addition of iodate and iodide or of hydrogen peroxide to serve as an electrometric indicator. In titrations with silver, cyanide acts as a depolarizer. In certain types of titration sodium nitrite is added to keep the anode depolarized.

Gay (*179*) has proposed the use of a small dry cell to polarize a tungsten-platinum electrode pair, in series with a 1500-ohm resistance, and a 0–1 milliammeter as indicating instrument. Under certain circumstances rather large currents can flow to or be drawn from the electrodes. The apparatus was found satisfactory for titrations with 0.1 *N* oxidants or reductants that show large breaks in potential, but was rather unsatisfactory for the titration of vanadyl solution with potassium permanganate (error 0.06 ml. in 25.00 ml.).

Multiple-electrode pairs have been proposed as a means of expanding the change in e. m. f. near the end point and thus making it possible to use cheaper instruments. Szebellédy and Jónás (*558*) placed the three duplicate electrode systems, connected in series, in the titration vessel and in two side tubes sealed to it. Upon shaking liquid into the side tubes three cells are created if electrical leakage across the wet glass surfaces is prevented by paraffin oil. Wolf (*621*) used four bimetallic electrode pairs connected in series and built as a stirrer. Upon stopping and lifting the stirrer or lowering the vessel, four cells are formed by cups, one below each electrode pair.

Stansby and Fitzgerald (*545*) have devised a semiautomatic arrangement with multiple electrodes for speeding the routine application of the method to large numbers of similar samples per day. A mounting for multiple electrodes in comparative

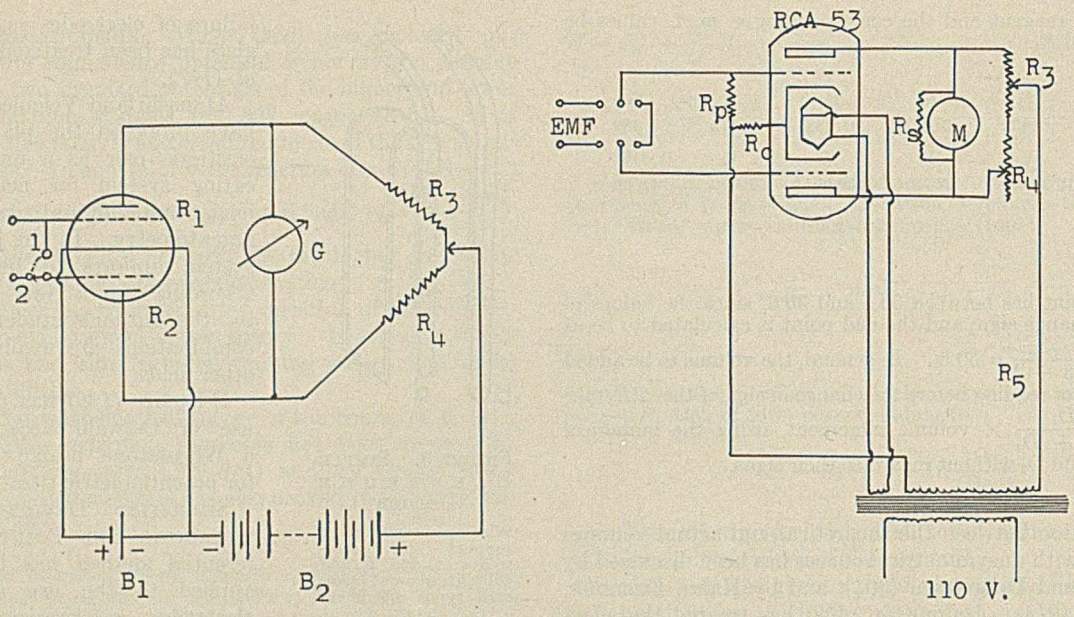


FIGURE 4. CIRCUITS FOR CONTINUOUS INDICATION IN POTENTIOMETRIC TITRATIONS

Left, Hiltner type (*230*)

R_p. From 50,000 to 200,000 ohms
R_c. 1000 ohms
R_s. 20 ohms
R_3. 2000 ohms

Right, Willard and Hager type (*615*)

R_4. 50 ohms
R_5. 480 (E_T − 220). E_T is transformer voltage
M. 0 to 150 microamperes

studies has been described in detail by Frediani and Warren (*150*). Tronstad (*594*) devised an automatic apparatus for estimating copper.

VACUUM-TUBE VOLTMETERS. As new types of radio tubes are developed, new or revised circuits are applied either in the field of pH measurement or for titration purposes. Circuits for measurement of pH with glass electrodes have reached a high degree of excellence. Detailed papers that have special merit are those of Cherry (*84*), Müller and Dürichen (*397*), Gilbert and Cobb (*184*), Stadie (*544*), Rosebury (*465*), and Morton (*384*); many other papers are important in this field. A review of this field is beyond the scope of this paper, and the reader is referred, for a critical discussion and review of the various types of circuits for use with the glass electrode, to the recent monograph by Dole (*112*).

The circuits that are finding most favor for continuous observation during titrations involve a Wheatstone bridge network in the output circuit. Some of the simpler circuits are shown in Figures 4 and 5. Circuits worthy of special mention are those of Baldinger (*31*), Compton and Haring (*94*), Ehrhardt (*126*), Garman and Droz (*175*), Goodwin (*189*), Hahn (*207*), Hiltner (*230*), Müller (*394*, *395*), Müller and Dürichen (*396*, *397*), Nottingham (*419*), Pollatschek (*433*), West and Robinson (*609*, *610*), and Willard and Hager (*615*).

The use of the visual tuning tube for indication of a sudden change in potential at the end of a titration was first described by Smith and Sullivan (*515*). Subsequently, Serfass (*486*) described still simpler circuits that give the same general performance as that of Smith and Sullivan. Instruments based on this principle are commercially available.

The idea of using the same vacuum tube as both polarizing and measuring device has been developed by Kassner *et al.* (*275*) and by Masaki and Hirabashi (*365*). The use of radio tubes to construct automatically operated titration assemblies has been described by Kordatzki and Wulff (*310*, *625*). Hahn (*211*) has criticized the work of Kordatzki and Wulff. A tube-operated circuit to close the stopcock of the buret was described by Shenk and Fenwick (*495*).

An ingenious circuit which gives a pulsation proportional to the change produced by each drop of reagent (pulse amplifier) has been developed by Baker and Müller (*30*). The maximum pulse per drop of reagent marks the end point. Another manner in which a vacuum tube voltmeter may be used to get differential readings is to reset the meter after each drop is added and a steady reading is found, as proposed by Clarke, Wooten, and Compton (*92*).

MICROMETHODS. The microchemical aspects of the potentiometric method have by no means been neglected. Ashcroft (*11*) has given an excellent review on the general subject of electrochemical methods in microchemistry, with bibliography. A similar review has been made by Ehrhardt (*125*). Chirkov (*85*) has studied microchemical applications of potentiometry, noting impulses in e. m. f. rather than plotting e. m. f. readings.

Suitable types of microburets are described by Mika (*375*) and by Lochte and Hoover (*340*). Krumholz (*313*) (Figure 6) and Raspopov and Finkelshtein (*447*) place the beaker on a platform that is rotated to effect stirring. The salt bridge (*313*) is one with sintered glass or small capillary ends. A novel scheme due to Schwarz (*482*) consists in supporting the small quantity of liquid to be titrated (1 drop) on a metal ring which is one of the electrodes. The other electrode tip and the buret make contact with the drop, which is agitated very gently by a magnetically operated vibrator to which the ring electrode is fastened. Micro hydrogen electrodes have been described by Frediani (*149*) and by Löbering (*342*) and micro quinhydrone electrodes by Mikawa (*376*), LaMer and Armbruster (*319*) for studies of heavy water solutions, Fuhrmann (*161*), and Itano (*256*).

A list of typical detailed applications of microtechnique in potentiometric titrations follows:

Acidimetry, using glass electrode (Partridge and Bowles, *427*)
Amino nitrogen (Roche and Roche, *462*)
Bromide, 0.25 mg. in the presence of 25 mg. of chloride (Vladimirov and Epstein, *603*)
Chloride, 0.25 ml. of 0.0001 *N* solution in alcohol or acetone titrated within a few per cent of the correct value (Schwarz and

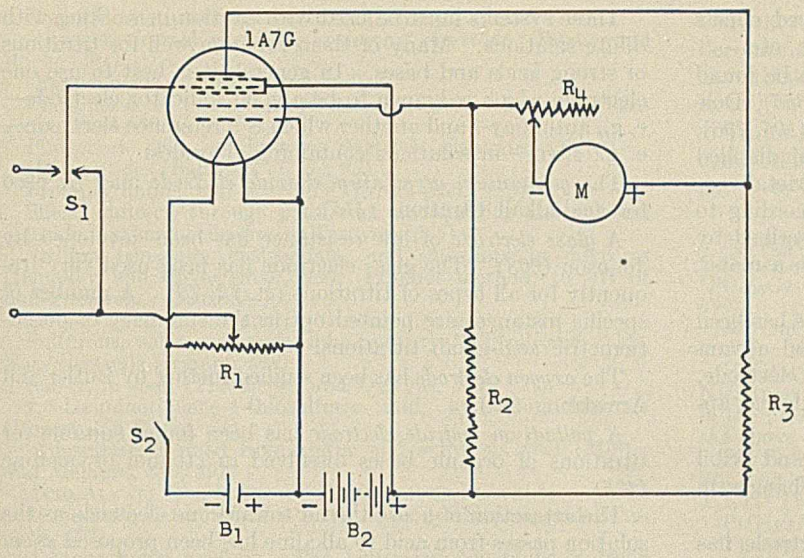


FIGURE 5. CIRCUIT OF CONTINUOUS-READING POTENTIOMETRIC INDICATOR (*176*)

B_1. 1.5 volts
B_2. 45 volts
R_1. 1000 ohms
R_2. 2000 ohms
R_3. 2500 ohms
R_4. 50,000 ohms
M. 0 to 50 microamperes

This circuit is easy to construct and has given good service in the hands of numerous workers.

with known mixtures. Sergeev and Yavorskiĭ (*488*) determined pH by trying various buffers until a galvanometer showed no deflection when connected between antimony electrodes in the buffer and the unknown, respectively. Oxygen in air may be determined by its effect upon the polarization of the carbon electrode of a dry cell, according to Moiseev (*380*).

ELECTRODES FOR pH MEASUREMENT. A complete review of this field is beyond the scope of this paper. A selection of important papers and reviews has been made.

Antimony Electrode. Good bibliographies of earlier work on this electrode are to be found in papers by Vellinger (*600*) and Guéron (*198*) and in Böttger's monograph (*47*). Papers that deal with the theory of the electrode and with precision measurements are those of Ball (*32*), Hovorka and Chapman (*249*), Parks and Beard (*425, 426*), Perley (*428*), and Wulff, Kordatzki, and Ehrenberg (*626*). Relative to the standard hydrogen electrode at 25° C., $E_{Sb} = 0.2552 + 0.05893$ pH from pH 2 to 8, according to Hovorka and Chapman (*249*). This work is in good agreement with work of Perley (*428*). King (*295*) has measured the salt error of the antimony electrode.

Gas Electrode. Krueger and Kahlenberg (*311*) studied the effects of various gases—hydrogen, oxygen, argon, and helium—upon metal electrodes. They reported small definite effects due to the inert gases as well as to oxygen and hydro-

Schlösser, *484*). For concentrations of chloride greater than 2.5 mg. per liter, maximum error 1.2 per cent (Bruevich and Vartolomeeva, *76*)

Lead, titrated with ferrocyanide (Schwarz, *482*)

Mercury, titrated with potassium iodide in range 1 microgram to 1 mg. (Schwarz and Kantor, *483*)

Silver in the range of a few micrograms (Zürcher and Hoepe, *630;* Spychalski, *543*)

Sodium, via one of the triple uranyl acetates and ceric sulfate (indirect titration of U^{IV} to U^{VI}), (Linder and Kirk, *336;* Kryulov and Kolarova, *314*)

Thallium (Berg and Fahrenkamp, *42*)

Titanium (Klinger *et al.*, *297*)

The paper by Schwarz (*482*), in which the titration of a single drop of liquid is described, covered the titration of halides with silver nitrate, sulfuric acid with sodium hydroxide, lead with ferrocyanide, ferricyanide with iodide, iodate, and thiosulfate, and bromate with arsenite. Prior to the period now under review some of these methods as well as others had been studied on a micro scale (cf. *299*, page 149; Hahn, *210;* and Zintl and Betz, for details).

CONCENTRATION CELLS. The use of suitable concentration cells makes possible a micro- or macroestimation of substances. Both titration procedures and direct measurements of the e. m. f. values of concentration cells may afford useful analytical information.

Johnson and Low (*267*) developed the work of Johnson (*266*) to make possible the estimation of the end point of the adjustment process in atomic weight studies. Furman and Low (*169*) developed a concentration-cell method for estimating traces of chloride in various salts. They noted effects of iodide ion upon the silver–silver chloride electrode. Hahn (*208*) noted that bromide ion affected the potential of the calomel half-cell and suggested that this type of effect might be used for the determination of bromides in chlorides. Low and Pryde (*344*) developed a concentration-cell method based upon the ferric-ferrous electrode for the indirect estimation of fluorides in various solutions. Drewski (*122*) used the concentration-cell technique for the estimation of iodine numbers. Valmari (*596*) used a somewhat similar idea for the estimation of nitrate and nitrite and the indirect estimation of potassium in the cobaltinitrite; a calibration curve was established

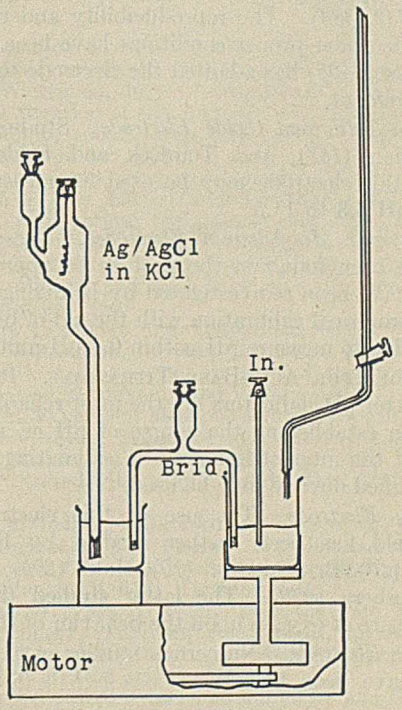


FIGURE 6. TYPICAL ASSEMBLY FOR POTENTIOMETRIC TITRATIONS ON MICRO SCALE

The beaker, holding 5 ml. of solution, is turned to cause stirring. The electrodes are of conventional design but of small dimensions.

gen. It is by no means certain that the observed effects were not due to trace impurities or electrode strain, etc.

Glass Electrode. Fundamental information is to be found in the papers by MacInnes and associates (*350–355*), Dole and associates (*112–120, 174*), Gross and Halpern (*195, 196*), and Laug (*331*). The measurement of the pH of distilled water has been studied by Burton *et al.* (*78*). Metal-connected glass films function as glass electrodes according to Thompson (*576*). Shielding by a solution is described by Adler (*5*). Detailed information is to be found in a monograph by Dole (*112*).

Hydrogen Electrode. A review of the applications has been given by Guéron (*198*). Because of the practical advantages that are associated with the use of the glass electrode, relatively little new work on the hydrogen electrode has appeared in the last decade.

Mercury–Mercuric Cyanide Electrode. Tomíček and Pribil (*586*) have shown that this electrode measures changes in hydrogen-ion activity in alkaline solutions.

Oxygen Electrode. The mechanism of this electrode has been studied by Hoar (*242*), who considers the well-known irreversibility as due to a film of oxide that is rather impervious except for cracks and pores. Tartar and associates (*567, 568*) have concluded that adsorption effects play a large role in the behavior of the electrode.

Quinhydrone Electrode. Important studies of this electrode have been made by Harned and Wright (*224*), Hovorka and Dearing (*250*), and Morgan, Lammert, and Campbell (*321, 322, 381, 382, 383*). The reproducibility and reliability of the electrode under proper conditions have been amply confirmed. Coons (*95*) has adapted the electrode to continuous pH measurement.

Tellurium–Tellurium Oxide Electrode. Studies by Bravo (*56*), Getman (*183*), and Tomíček and Feldmann (*580*) show that this electrode may be used for measurements in the range pH 5.3 to 11.5.

Tungsten and Molybdenum Electrodes. These electrodes which have been found in the past to be approximate pH indicators have been reinvestigated by Brintzinger and Rost (*62*). By empirical calibration with the aid of buffers it was found possible to measure pH within 0.1 pH unit.

ELECTRODES FOR ACID-BASE TITRATIONS. It is obvious that the better pH indicators are the most reliable electrodes to use when establishing the course of pH *vs.* milliliters of reagent. If the interest is merely in estimating end points, many simplified devices may be used.

Antimony Electrode. The use of this electrode in the titration field has been further studied by Böttger and Szebellédy (*49*), Britton *et al.* (*70*), Hahn (*205*), and Kauko and Knappsberg (*276*). The latter studied the effect of partial pressure of oxygen upon the behavior of the electrode.

Bimetallic Electrode. Numerous studies of possible combinations have been tested:

Aluminum *vs.* Rose metal (French and Hamilton, *151*)
Copper *vs.* tungsten (Teis and Vagner, *569*)
Ferroalloys and various alloys and metals—e. g., iron-nickel *vs.* molybdenum; iron-nickel *vs.* tungsten; also other ferroalloys (Banchetti, *36*). Brass, copper amalgam, iron oxide, pyrites; titanium, silver, iron, nickel, lead, copper, and tin in various pairs have been tested by Boltunov and Vorsina (*55*).
Gold *vs.* rhodium in fused salt baths (Lux, *345*)
Platinum *vs.* Carborundum (Khlopin, *289*)
Platinum *vs.* chromium (Wolf, *621*)
Platinum *vs.* graphite (Tikhonov, *577*)
Platinum-nickel (Atanasiu and Velculescu, *22*)
Platinum-antimony, platinum-tantalum (Boltunov and Krogius, *54*)
Platinum-nichrome (Mazzuchelli, *368*)
Platinum *vs.* V2A steel, ferroalloys, etc. (Banchetti, *36*)
Silicon *vs.* silicon carbide (Boltunov and Isakova, *53*)
Tungsten *vs.* nickel (Furman and Low, *168*)

These systems must be used with caution in working with dilute solutions. Many of them function well for titrations of strong acids and bases. In general, it is best to use one electrode which is known to be a good indicator electrode—e. g., antimony—and another which is a reference electrode—e. g., silver—in solutions containing chlorides.

The *germanium–germanium dioxide electrode* may be used for acid-alkali titrations (*415*).

A *glass electrode* of low resistance has been developed by Johnson (*268*). The glass electrode has been used very frequently for all types of titrations (cf. *72, 73*). A number of specific instances are pointed out in the summary of potentiometric acid-alkali titrations.

The *oxygen electrode* has been studied further by Butler and Armstrong (*79*).

A *palladium hydride electrode* has been found suitable for titrations of organic bases dissolved in ethanol or acetone (*475*).

The attraction of a *silk* thread toward one electrode as the solution passes from acid to alkaline has been proposed as an electrometric indication (*338*).

The *tellurium–tellurium oxide electrode* may be used for titrations (Brouwer, *75, 580*).

The *tungsten electrode* has been studied by Britton and Dodd (*64*).

Applications

Summary of Potentiometric Applications of Acid-Alkali Titrations

PRECIPITATIONS. Aluminum, using antimony indicator electrode (Kanning and Krath, *272*)
Aluminum, in presence of nitrates (Madzhagaladze, *356;* Stefanovskiĭ, *546*)
Aluminum, titration, using galvanometric method (Martin, *362*)
Aluminum, effect of various anions (Whitehead *et al.*, *611*)
Copper (Beebe, *39*)
Gold (Britton and Dodd, *65*)
Iron (Kanning and Krath, *272;* Shisakov, *497;* Zaides, *627*)
Lead as $Ph(OH)_2Pb(CNS)_2$ in presence of other ions (Hayek, 225)
Magnesium, antimony electrode, (Malvea and Withrow, *359;* Berraz and Christen, *43;* Itano and Tsuji, *258*)
Mercuric ion (Britton and Wilson, *73*)
Mercurous ion (Bennett, *41*)
Oxalates of lead, barium, etc., adsorption of hydrogen or hydroxyl ion (Tannaev, *561*)
Platinum (Britton and Dodd, *66*)
Uranium (Britton and Young, *74*)
Zinc (Prytz, *438*)
REACTIONS IN AQUEOUS SOLVENTS. Acids, amino and polypeptides (Balson *et al.*, *34, 35*)
Acids, dibasic (Ingold and Mohrhenn, *251*)
Dicarboxylic, unsaturated (Ashton and Partington, *12*)
Fatty (Lottermoser and Ghose, *343*)
Imino (Litzinger and Pickett, *339*)
In alkaloids (Drewski, *123*)
In colored liquids (de Lingo and Thaler, *337;* Thaler and de Lingo, *572;* Minnaev, *378*)
Alkali, in phenates, unsaturated oxygen electrode (Kargin and Usanovich, *273*)
Alkalinity, in sea water (West and Robinson, *610*)
Amino acids, polypeptides (Balson *et al.*, *33*)
Ampholytes (Konikov, *309*)
Antipyrine, antimony electrode (Gurevich, *199*)
Boric acid, quinhydrone electrode (Wilcox, *613, 614*)
Carbonates (Aumeras and Marcon, *27*)
Carbon dioxide, glass electrode (Wilson *et al.*, *618*)
Carbonic acid and sodium bicarbonate (Liander, *335*); glass electrode (Dorello and Rowinski, *121*)
Chromic acid, glass electrode used (Nuess and Rieman, *414*)
Dichromate, platinum electrode (Ruiss and Babalova, *467*); platinum–lead oxide electrode (Sabinina and Moralev, *472*)
Fumaric, maleic, and succinic acids (Cattelain and Couchet, *81*)
Gallic and gallotannic acids, differential method with hydrogen or tungsten electrodes (Sunthankar and Jatkar, *555*)

Hypochlorite, platinum electrode (Abribat, *3*)
Nitrogen, formol titration, antimony electrode (Roche and Roche, *461*)
Organic acids, volatile (Craig, *96*)
Phosphoric acid and phosphates, antimony electrode (Vengerova *et al.*, *601*)
Phosphoric acid, quinhydrone electrode (Sanfourche, *476*)
Polypeptides (Balson *et al.*, *34*, *35*)
Proteins, animal and vegetable (Errera *et al.*, *129*)
Silica, indirect, through potassium silicofluoride and calcium chloride, antimony electrode (Tarayan, *565*)
Sodium benzoate, bicarbonate, and salicylate in mixtures (Izmailov and Shvartsman, *260;* Pinkhof method, to pH of buffer reference electrodes)
Strong acids, galvanometric titration (Sergeev, *487*)
Sulfurous, selenious, and alpha-hydroxyalkyl sulfonic acids, glass electrode (Rumpf, *468*)
Sulfur, in steel (Thanheiser and Dickens, *575*)
2,5-Diaminotoluene-4-thiosulfuric acid, hydrogen electrode (Bogert and Sevag, *51*)
Thiosulfate, formed from sulfur and sulfurous acid (Müller and Melhorn, *390*, *391*)

Acid-Alkali Reactions in Nonaqueous Solvents. Acetic acid medium, determination of amino acids (Nadeau and Branchen, *407*)

Acetone-water medium. The ionization constants of weak acids—e. g., acetic and dichloroacetic—are made smaller while hydrochloric acid remains highly ionized (Izmailov and Bel'gova, *259*). Sulfuric acid in presence of lactic (Shkodin, *498*)

Alcohols, *N*-butanol, change in ionization constants (Hantzsch, *222;* Evans and Davenport, *131;* Wooten and Ruehle, *624*). Butyl Carbitol (Halford, *220*)

Alcohols, as medium for amino acid titration (Ogston and Brown, *421;* Neuberger, *412;* Balson *et al.*, *33*)

Alcohols, acetone, benzene, hexalin, and mixtures as media, using antimony or tellurium electrodes (Tomíček and Feldmann, *580*)

Alcohol-acetone (Shafershtein *et al.*, *489*)
Alcohol-anisole (Demarest and Rieman, *101*)
Benzene as medium (LaMer and Downes, *320*)
Cellosolves (glycolmonalkyl ethers) as media (Ruehle, *466*)
Propionic acid as medium for the titration of organic bases (acetanilide, acetamide, urea, acetoxime), (Terjesen and Sandved, *571*)
Oils, acidity in (Evans and Davenport, *130;* Clarke, Wooten, and Compton, *92;* Ralston *et al.*, *445*)

The foregoing section gives several illustrations of a new trend in research on the determination of acidity—namely, the deliberate use of a nonaqueous or a mixed solvent to spread the ionization characteristics of two or more solutes, so that a differential titration is possible in the medium and either impossible or less accurate in aqueous medium. The use of anhydrous acetic or propionic acid to enhance the relative sharpness of the titrations of bases which are very feebly ionized in water is another noteworthy advance in technique.

Titrations Involving Precipitation

Special Electrodes. The silver electrode for halide precipitations and the like and the platinum oxidation-reduction indicator electrode for ferrocyanide precipitations still are the chief indicating electrodes in this field. Mention has been made of acid-alkali processes above. Changes in pH due to adsorption during the precipitation of oxalates and sulfates of lead, calcium, strontium, and barium have been observed by Tannaev and Mirianashvili (*564*). Atanasiu (*14*) has proposed the platinum-nickel and the platinum–silicon carbide couples as suitable for ferrocyanide precipitations, and the platinum-nickel, platinum–silicon carbide, and platinum-graphite couples for halide and silver titrations. Second-class electrodes, as, for example, silver–silver chloride for halides or silver–silver sulfide for zinc, cyanide, or nickel titrations, are discussed by Hiltner (*231*, *232*). Chromium, molybdenum, and tungsten electrodes in contact with their insoluble salts are useful in the estimation of chromium, molybdenum, tungsten, and various heavy metals, according to Brintzinger and Jahn (*59*). It has been observed by Böttger and Schall (*48*), Kolthoff and Wang (*308*), and Obrucheva (*420*) that a platinum wire, after being used, becomes a good indicator for silver ions, perhaps because of an oxidation-reduction process involving a higher oxide of silver.

Third-Class Electrodes have been studied especially in connection with determination of calcium. The following list gives reference to separate publication in this field.

$Ag/Ag_2C_2O_4/CaC_2O_4,Ca^{++}$; $Zn/ZnC_2O_4,CaC_2O_4,Ca^{++}$; $Pb/PbC_2O_4,CaC_2O_4,Ca^{++}$; $Ag/Ag_3(PO_4)_2,Ca_3(PO_4)_2,Ca^{++}$; $Hg/Hg_3(PO_4)_2,Ca_3(PO_4)_2,Ca^{++}$; $Pb/Pb(IO_3)_2,Ca(IO_3)_2,Ca^{++}$ (Velíšek, *599*)

$M_1/CuC_2O_4,CaC_2O_4,Ca^{++}$ (M_1 is Pt, Au, or Ag); (Nierstrasz and Tendeloo, *416*)

$Hg(Pb)/PbC_2O_4,CaC_2O_4,Ca^{++}$ (Denina and Caris, *102;* Nierstrasz and Tendeloo, *416*). This system is said to function in the presence of chlorides which interfere with the majority of the above electrodes.

$Pb/PbSO_4,CaSO_4,Ca^{++}$; $Ag/Ag_2C_2O_4,CaC_2O_4,Ca^{++}$; $Hg(Zn)-ZnC_2O_4,CaC_2O_4,Ca^{++}$; $Hg/Hg(IO_3)_2,Zn(IO_3)_2,Zn^{++}$; $Hg/Hg_2I_2,-Tl|,Tl^+$ (Velíšek, *598*)

$Pt(Hg)/HgS,PbS,Pb^{++}$ (Ripan-Tilici, *457*)

Theory of third-class electrodes (LeBlanc and Harnapp, *332*)

Membrane electrodes formed of silver iodide and silver bromide, or of silver iodide and silver sulfide, and sealed to glass tubes with picein cement have been tested as indicating electrodes in silver and halide titrations by Skobets and Kleibs (*509*)

Tendeloo (*570*) has used a membrane of calcium fluoride in connection with the estimation of concentrations of calcium that are equal to or greater than 1 milliequivalent per liter. The electrode was applied in the study of gelatin and milk.

Applications of Potentiometric Method to Precipitations. Chromicyanide and cobalticyanide as reagents (Czaporowski and Wiercinski, *100*). Cobalticyanide was found to be useful for the titration of silver, cupric, and mercurous ions, but not for cadmium, cobalt, zinc, and nickel. The reactions of chromicyanide were too slow.

Cerium, lanthanum, and thorium determined as oxalates (Atanasiu, *16*, *17*, *19;* Jantsch and Gawolowski, *262*)

Chromate, with barium (Brintzinger and Jahn, *58*, *59*)

Ferrocyanide with mercuric, nickel, or cobalt (Masaki, *364*)

Ferrocyanide as reagent. Carbonate, indirect by adding excess of lead solution, and back-titration with potassium ferrocyanide (Ringbom, *450*)

Cadmium (Tannaev and Diaparidze, *562*)

Cerium, lanthanum, thorium (Atanasiu, *15;* Atanasiu and Velculescu, *21*). Acetone-water or alcohol and water (30 per cent alcohol) used (Shemyakin and Volkova, *494*)

Cerium in 30 per cent alcoholic solution (Spacu, *540*)

Cobalt and nickel (Atanasiu and Velculescu, *23*)

Copper (Fisher and Mavrin, *138*)

Copper, titrated with sodium sulfide (Jha, *263;* Prased and Jha, *435*)

Potassium, indirect (Nikolskiĭ and Lavrov, *417*)

Zinc (Brennecke, *57;* Fisher, *137;* Joassart and Leclerc, *265;* Kamienski, *270;* Kamienski and Karczewski, *271;* Saitō, *473*, *474;* Stefanovskiĭ, *547;* Tannaev, *561*)

The general types of precipitates that are formed with silver, lead, copper, cadmium, zinc, cobalt, nickel, and manganese salts when precipitated with hydroferrocyanic acid have been studied by Britton and Dodd (*67*).

Fluoride, precipitated by calcium ion (Ryss and Bakina, *471*); precipitated by cerous ion (Allen and Furman, *9*)

Lead, precipitated with sulfide (Maheshwari and Jha, *357*); precipitated with phosphate (Shakhkeldian, *491*)

Lead nitrate as reagent (Ringbom, *450*); a general study including details as to the determination of sulfate, carbonate, sulfite, chromate, tungstate, and molybdate (*500*)

Mercuric nitrate as reagent (Tomíček and Procke, *587*)

Mercurous salts (Michalski, *373*); iodide as reagent (Spacu and Spacu, *529*). Determination of arsenic with mercurous solution (Spacu, *537*)

Molybdenum, with lead nitrate (Ringbom, *450;* Senyuta, *485*)

Phosphate, with uranyl acetate (Atanasiu and Velculescu, *26*). The precipitate is HUO_2PO_4, temperature 60–70° C., pH 5.5 to 6. The same precipitate is formed with mono-, di-, or tribasic alkali phosphate.

Potassium, indirect, by its effect upon the titration of lithium ferrocyanide with heavy metals (Tannaev and Diaparidze, *563*)

Silver (Moser *et al.*, *385;* Raub, *448;* Weiner and Schmidt, *605*); determination of silver for coinage purposes (Robinson and Hugg, *460*). Gold *vs.* carbon treated with nitric acid as electrode system. Spychalski (*543*) titrated Bredig solution for silver with bromide or iodide with an accuracy of 0.5 to 1 per cent of the silver present. Hiltner and Gittel (*234*) developed a systematic potentiometric scheme for estimation of silver, bismuth, lead, copper, and cadmium in the same solution.

Silver nitrate as reagent. Arsenate, in acetate buffer plus alcohol (Hanson *et al.*, *221;* Spacu, *537*)

Azide (Moskovich, *386;* lead azide in 2 per cent barium nitrate solution, 0.04 *N* in nitric acid, and buffered with 0.2 *N* sodium acetate

Bromide, in presence of chloride (Flood and Sletten, *142*). The inflection for the bromide should be 15 mv. from the prolonged chloride curve. An empirical correction can be made accurately by a graphical method.

Chloride, effect of electrolytes on curve (Orlov, *424*)

Effects of acids (Guzeli, *202*)

Electrode system, silver *vs.* graphite (Khlopin, *280–282*)

Silver–silver chloride *vs.* quinhydrone, pH 3 (Itano, *257*)

In atomic weight studies (Johnson, *266;* Johnson and Low, *267*)

In brines (Hoff-Jørgensen, *245;* Tremblay, *592*)

In bromide mixtures (Schütza, *481*)

In sap (Neller, *410*)

In sea water (West and Robinson, *610*)

In soils (Snyder, *516*)

Cyanamide (Sinozaki, *508*)

Cyanide (see complex formation)

Cyanines, through alkyl iodides (Calzavara, *80*)

Cyanates (Ripan-Tilici, *454*); in presence of cyanide (Ripan-Tilici, *455*)

Ferrocyanide (Tomíček and Hubrova, *583*)

Hydrochloric acid, in alkaloids (Shafershtein *et al.*, *489*)

Hypophosphorous acid (Jung and Uspenskaya, *269;* Wolf *et al.*, *622;* Grundmann and Hellmick, *197*). The solution is neutralized to phenolphthalein and then disodium hydrogen phosphate is added. The halides, cyanide, and thiocyanate are precipitated before the hypophosphite reacts.

Iodide, accuracy of reaction at extreme dilutions (Kolthoff and Lingane, *302*). Precision studies accurate to 0.003 per cent (Lange and Berger, *327*)

Mercaptans. Mercaptans are precipitated prior to chloride (Tamele and Ryland, *559;* Tamele *et al.*, *560*). The latter paper deals with the use of aqueous alkaline solutions.

Mercaptobenzothiazole (Spacu, *532*)

Molybdate (Spacu, *533*)

Oxalate (Atanasiu, *18*)

Phosphate, as silver thallium phosphate (Spacu and Drăgulescu, *524*). As silver phosphate (Michalski, *372*)

Selenocyanate (Spacu, *536*). Determination of selenocyanate in presence of thiocyanate (Ripan-Tilici, *453*); in presence of cyanide (Ripan-Tilici, *456*); in presence of halides (Spacu, *538*); in presence of iodide (Spacu and Spacu, *531*, p. 179); in presence of chloride and bromide (Spacu and Spacu, *531*, p. 248); in presence of thiocyanate (Spacu, *536*)

Selenite (Ripan-Tilici, *458*)

Sulfide, with cyanide, thiocyanate, and chloride (Raines and Putning, *444*). Four breaks in e. m. f. are found, the first after silver sulfide is precipitated, the second after complex cyanide formation. The third follows the combined precipitation of cyanide and thiocyanate, and the fourth after the chloride has precipitated. Bursuk and Zan'ko (*77*) determined sulfide, thiocyanate, and chloride in a single titration with succession of end points.

Sulfide as reagent for heavy metals (Hiltner and Grundmann, *239*)

Sulfite (Spacu and Drăgulescu, *525*, *526*)

Tetrathionate, trithionate (Ishikawa and Murooka, *253*)

Thiocyanate. Indirect determination of cadmium via thiocyanate, $Cdpy_2(CNS)_2$ (Spacu and Spacu, *530*, p. 263)

Cobalt (Spacu, *535*)

Copper (Spacu and Spacu, *530*, p. 99)

Nickel (Spacu and Spacu, *529*, p. 270)

Copper direct with thiocyanate (Hiltner and Grundmann, *237*)

Thiosulfate and chloride (Petit, *429*)

Vanadates (Britton and Robinson, *71*). Metavanadate (Spacu, *539*)

Sulfates, precipitation with barium ion (Christensen *et al.*, *91;* Visyagin, *602;* Orestov, *422*); with lead, indirect (Shakhkeldian, *492*)

Tungstate, as barium tungstate (Brintzinger and Jahn, *58*); as lead tungstate (Noda, *418*).

Complex-Formation

Alkaloids. Maricq (*360*) has further extended the applications of the reagent potassium iodomercurate (K_2HgI_4) in the field of alkaloid chemistry to include stovaine, novocaine, pyramidone, antipyrine, and cinchophen. The alkaloid is precipitated with excess of the reagent and the excess is titrated, after filtration, with standard mercuric chloride.

Aluminum may be titrated with a standard solution of 0.5 *N* sodium fluoride to form sodium fluoaluminate in 50 per cent alcoholic solution saturated with sodium chloride according to Tarayan (*566*). The ferric-ferrous system is used for indirect indication with a platinum-calomel electrode system.

The potentiometric determination of *copper* in steels by the cyanide titration method is said to be more rapid than the colorimetric or the electrolytic method (Weihrich, *604;* Piccinini, *431*).

Glucose in simple solutions or in blood may be determined indirectly by the iodomercurate method, according to Maricq (*361*).

Fluoride may be titrated with uranous solution (U^{IV}) and at the completion of the reaction: $5F^- + U^{++++} + K^+ = KUF_5$ there is a jump of the potential of the uranyl-uranous electrode. Calcium, aluminum, iron, and phosphates interfere. The pH of the solution is controlled by a buffer of 0.5 *N* sulfanilate saturated with sulfanilic acid (Flatt, *140*). According to Ryss and Bakina (*471*), fluoride may be titrated with a standard calcium solution after saturating the solution with sodium fluosilicate, using the quinhydrone electrode as indicator.

Spacu and Drăgulescu (*527*) have studied the reaction between *mercuric chloride* and ammonium sulfite. The formation of two compounds is indicated, NH_4HgSO_3Cl and $(NH_4)_2Hg(SO_3)_2$. Mercury may be determined by titration with potassium iodide (Hiltner and Gittel, *235*). Spacu and Murgulescu (*528*) titrate iodomercurate ion with $Cu(en)_2$ sulfate or nitrate as reagent. The reaction is: $HgI_4^{--} + Cu(en)_2 = Cu(en)_2HgI_4$. A mercury electrode and a calomel half-cell are used. The reaction is sluggish near the end point.

The potentiometric determination of *nickel* by the cyanide method has been repeatedly studied. Bohnholtzer (*52*) used the conventional silver-calomel combination with a salt bridge. Chatterjee and Jha (*83*) found that the quinhydrone electrode could be used to indicate the formation of nickel cyanide when nickel was titrated with standard cyanide solution. The pH at the end point is about 7.5. Hiltner and Grundmann (*236*, *238*) recommended the use of the silver iodide and silver sulfide electrodes in connection with the cyanide method for nickel. Hiltner and Seidel (*241*) applied the cyanide method to the estimation of nickel in nonferrous alloys after the electrolytic removal of copper and lead. Khlopin (*283*) applied the galvanometric method of indication for the estimation of nickel in steels with standard cyanide solution. He also developed a back-titration method, using nickel as the final reagent after adding excess of standard cyanide to the ammoniacal solution (*285*, *290*). Weihrich (*604*) recommends the use of silver and silver iodide as the indicator electrode and gives tables of factors for converting various weights of copper to their equivalent amounts of nickel in this process.

Many organic acids enter into complex formation with ferric chloride, and Treadwell and Wettstein (*590*) have shown that certain of these processes may be followed potentiometrically—for example, the ratio of ferric ion to acid is 2 to 3 for citric acid, 1 to 3 for benzoic acid, 1 to 2 for malic acid, and 1 to 1 for oxalic acid. The platinum-calomel system

is used and air is excluded. The addition of alcohol or sugar is useful.

Wick (*612*) made a thorough study of the application of the potentiometric method to silver cyanide plating baths. Weiner and Schmidt (*605*) have also studied this question.

The reaction of sulfite ion with mercuric chloride solution has been studied potentiometrically by Spacu and Drăgulescu (*525*, *526*).

Oxidation-Reduction Reactions

The applications in this field are arranged approximately in the order of relative oxidizing power of the reagents with a numbered section for each reagent or group of similar reagents.

1. Permanganate Processes. The estimation of manganese after oxidation to the permanganate stage has been used repeatedly in steel analysis (cf. Section 13). The titration of the permanganate with ferrous solution in the presence of fluoride has been applied to the estimation of manganese in iron castings by Zan'ko and Stefanovskiĭ (*629*). The decomposition of permanganates may be studied in concentrated acids with a platinum–mercurous sulfate–mercury electrode system according to Chloupek (*88–90*). The effect of the presence of fluorides upon the reaction between arsenious and antimonous solutions or iodide or hydrogen peroxide and potassium permanganate has been studied by Pugh (*439*, *441*).

Iron. The determination of iron in feldspar has been considered by Lyle (*348*). Ryabchikov and Silnichenko (*470*) propose the use of cuprous chloride for the reduction of the iron, in the presence of sodium chloride and hydrochloric acid. Upon potentiometric titration two breaks are obtained, one after oxidation of the excess of cuprous salt and the other after re-oxidation of the iron.

Hydrogen Peroxide and Other Peroxides. Müller and Brenneis (*403*) found the permanganate method to be more suitable than many other methods (cf. Reichert *et al.*, *449*). Pugh studied the titration of permanganate with hydrogen peroxide in the presence of fluosilicate (*440*) as a buffer. There is one fairly sharp break at the end of the reduction to manganese dioxide and a sharper break in potential when the latter is reduced to manganous salt.

The potentiometric titration of *iodide* to iodine monochloride with standard permanganate is suitable in the presence of chloride or bromide, according to Flatt and Boname (*141*) and Hahn (*212*).

Nitrites may be titrated with permanganate, although the reaction is slow, according to Jimeno and Ibarz (*264*).

Nitroprusside complexes may be studied by potentiometric titration with permanganate (Masalskiĭ and Chernuiĭ, *366*).

Platinum. Bivalent platinum may be estimated with potassium permanganate or potassium bromate (Stelling, *549*). Grinberg and Ptitzuin (*191–193*) have used the potentiometric method for the study of various platinum and iridium complexes.

Uranium. This process has been reinvestigated by Luyckx (*346*).

Zinc. Tannaev (*561*) has proposed the titration of excess ferrocyanide with permanganate for this indirect estimation.

Titrations in Strongly Alkaline Solutions. Tomíček (*579*) studied the estimation of arsenite, antimonite, selenite, and tellurite. The direct titration of selenite was not successful.

2. Auric Chloride. That this reagent is useful in the titration of tocopherols was shown by Karrer and Jaeger (*274*). Certain carotenoids interfere. Some of the members of the group consume eight equivalents per mole, others two, and still others are not oxidized.

3. Cerate Systems. The sulfato ceric system has proved to be reliable and useful. General reviews of the applications have been given by Janssens (*261*), Willard and Young (*617*), and Furman (*164*).

Ceric (IV) *vs. Arsenious* (III). Lang and Zwerina (*325*) determine cerium with 0.1 *N* arsenite, using either iodine monochloride or manganous salt as catalyst.

Arsenic and antimony may be determined successively by titration with ceric sulfate, with addition of iodine monochloride after the antimony has been oxidized. The method is selective for antimony, provided the concentration of the arsenious acid is not too high (Furman, *163*).

Arsenite and ferrocyanide may be determined in the same solution (Lang, *323*).

The *ceric vs. ferrous* reaction has been recommended again for the estimation of cerium (Weiss and Sieger, *606*).

Various *combinations of elements* that are of interest in steel analysis may be determined in whole or in part with standard ceric solution: manganese, chromium, and vanadium (Dickens and Thanheiser, *110*). Vanadium, uranium, and iron (vanadium in ores) (Levenson and Kochmarev, *334*). Molybdenum, after reduction with stannous solution (Stehlik, *548*), with mercury (Furman and Murray, *172*), or with silver (Birnbaum and Walden, *44*).

Uranium (IV) after reduction of uranyl solutions may be titrated with ceric sulfate, as was shown by Ewing and Wilson (*134*) and by Furman and Schoonover (*173*). This reaction has been applied to the indirect potentiometric estimation of sodium by Furman, Caley, and Schoonover (*167*), and Kryulov and Kolarova (*314*).

Zinc may be estimated by the indirect ferrocyanide process (Sturges, *554*).

Nitrato and Perchlorato Cerates. Smith and Getz (*511*, *512*, *513*) have shown in a series of systematic studies that nitrato cerate ion, $Ce(NO_3)_6^{--}$, and the perchlorato cerate ion, $Ce(ClO_4)_6^{--}$, function at still higher potentials than the sulfate complex. With these reagents there is a better differentiation in the successive titration of ferrous iron and vanadyl ion in mixtures than with the sulfato reagent. The oxidation of organic compounds proceeds more smoothly and regularly, which makes possible improved methods for the titration of oxalate and other substances. Smith and Duke (*510*) were able to work out a rapid method for glycerol by oxidation with perchlorato cerate at 50° to 60° C.

4. Bichromate Processes. In the main the reaction bichromate *vs.* ferrous ion has been further studied or applied to direct and indirect estimations, as follows:

Study of asymmetry of the titration curve (Winter and Moyer, *619*). The reaction has also been studied by Shakhkeldian (*490*)

Barium, indirect estimation (Fisher, *137*)

Chromium (Khlopin, *288*, *290*). Platinum-tungsten system more exact than visual methods. In ferrochrome (Khlopin, *286*; Spindek, *542*); in plating baths (Khlopin, *278*)

Ferrous sulfate solutions (Shisakov, *497*)

Glycerol (*437*)

Iron, in ores (Sosnovskiĭ, *519*)

Oximes, indirect estimation of metals (Ishimaru, *254*)

Sulfite, sulfoxylate (Löbering, *341*; Mutschin, *406*)

5. Halogens and Their Oxy-Compounds. Periodate, estimation of glucose (*446*)

Potassium bromate. 8-Hydroxyquinoline (Smith and May, *514*)

8-Hydroxyquinoline, anthranilic acid (Kitajima, *296*)

Beta-naphtholsulfonic acids (Forrester *et al.*, *146–148*, *223*)

Platinous complex oxalates (Grinberg and Ryabchikov, *194*)

Thio acids, RSH (Hellström, *227*)

Thiocyanate, arsenite, and antimonite (Nakazono and Inoko, *409*)

Thionalide, indirect estimation of thallium (Berg and Fahrenkamp, *42*)

Tin (Raikhinstein, *443*)

Potassium iodate. Antimony (Furman and Miller, *170;* Singh and Ilahi, *503*)

Arsenic (Schoonover and Furman, *480;* Singh and Ilahi, *503*)

Cuprous thiocyanate (Hope and Ross, *248*)

Hydrazine (Stelling, *550*)

Iodide (to iodine chloride) (Flatt, *139*)

Iodide complexes, cadmium indirect (Kiba, *291*)

Iron (Singh, *501*)

Phenyl hydrazine and semicarbazide (Furman and Miller, *171*)

Stannous ion (Spacu and Drăgulescu, *521;* Singh and Ilahi, *503*)

Thiocyanate, tetrathionate, etc. (Singh and Ilahi, *504*)

Potassium chlorate. Estimation of various reductants: arsenic (III), antimony (III), ferrous, iodide, and thallous ions (Singh and Singh, *507*)

Estimation of phenol, *p*-nitroaniline, diphenylamine, and hydroquinone (Singh and Singh, *507*, p. 346)

Stannous ion (Kulvarskaya, *316*)

Estimation of chlorates and hypochlorites, using osmium tetroxide catalyst (Gaukhman and Stefanovskiĭ, *178*)

Chloramine T as oxidant. Estimation of antimony, arsenic, stannous, ferrous, ferrocyanide, and iodide ions (Tomíček and Sucharda, *588*)

Indirect determination of oxidants, titration of hydroquinone, hydrazine, potassium iodide, sodium bisulfite, sodium nitrite (added from buret) (Singh and Rehman, *506*)

Thallium (Aguado, *7;* Fresno and Aguado, *152*)

Hypochlorite as oxidant. Bromide (Afanase'v *et al.*, *6;* Chirkov and Spikel'man, *87*)

Various reductants: antimony, arsenic, ferrous, iodide, and thallous ions (Singh and Singh, *507*)

Iodine to iodine chloride, bromine to bromine chloride (Chirkov and Schnee, *86*)

Hydrazine, urea (Tomíček and Filipovic, *581*, p. 415)

Thiocyanate, thiosulfate, thallium, cyanide, selenite, and tellurite (Tomíček and Filipovic, *581*, p. 340)

Determinatlon of hypochlorite (Albribat, *3*)

Hypobromite as oxidant. Tomíček and Jasek (*584;* see also *581*) have studied the applications of this rather unstable reagent. In the estimation of thiosulfate, sulfite, and thiocyanate the results are 1 to 4 per cent high.

Bromine as oxidant. Hypophosphite (Blaser and Halpern, *45*)

Phosphite and thiosulfate (Nakazono, *408*)

Thiosulfate, reaction with bromine or potassium bromate (Cernatescu and Ralea, *82*)

Iodine as oxidant. Antimony (Furman and Miller, *170*) and tin (Pevtsov, *430*)

Mercurous salt (Michalski, *374*)

Tetraphenyl arsonium chloride, indirect estimation of metallic ions (Willard and Smith, *616*)

Thionalide, indirect estimation of metals (Kiba, *291*)

Thiosulfate (Fresno and Valdes, *160*). Indirect applications: antimony (Spacu and Drăgulescu, *522*); arsenic (Spacu and Drăgulescu, *523*); iodate (Spacu, *534*, *540*); oxygen (Perley, *428*); and thallium (Hollens and Spencer, *246;* Cuta, *99*)

Water, Karl Fischer reaction: $I_2 + SO_2 + 3C_5H_5N + H_2O = 2(C_5H_5NHI) + C_5H_5NOSO_2$ (Almy *et al.*, *10*)

Estimation of iodine, application to medicinals (Gjaldbaek, *187*)

Miscellaneous. Halogen solutions and sodium thiosulfate (Fresno and Valdes, *160*)

Chlorine electrode (Rius and Arnal, *459*). Electrode is not suitable for determination of chlorine in hypochlorous acid solution

Potassium iodide as reductant for palladium (Müller and Stein, *392*)

6. Vanadic Solutions as Oxidants. It has been shown that most of the more powerful reductants may be estimated with this oxidant: Ferrous, molybdenum (III, V), tungsten, uranium, vanadium (II, III), stannous, cuprous, and titanous solutions; hydrazine, hydroxylamine, hydrogen sulfide, phosphites, and sulfites (Syrokomskiĭ and Antropov, *556;* Syrokomskiĭ and Klimenko, *557*).

7. Hydrogen Peroxide, Permonosulfuric Acid, and Persulfuric Acid. Estimation of hydrogen peroxide by various methods (Reichert *et al.*, *449*).

Estimation of persulfuric and permonosulfuric acids, and hydrogen peroxide.

Permonosulfuric acid is reduced by excess of standard arsenite and potassium bromide in a solution 1.4 to 1.5 *N* in sulfuric acid. The excess of the arsenite is estimated with standard bromate.

Hydrogen peroxide is estimated by adding an excess of standard arsenite and sodium hydroxide. After 2 minutes the solution is acidified and the excess of arsenite is titrated.

Persulfuric acid. After the hydrogen peroxide reaction an excess of arsenite is added and after heating 40 minutes at 100° C. with the solution 1.4 to 1.5 *N* in sulfuric acid the excess of arsenite is titrated with bromate after cooling (Isida and Yukawa, *255*).

Müller and Holder's Procedure (*389*). Permonosulfuric acid may be titrated selectively in the presence of hydrogen peroxide with arsenite if the permonosulfuric acid is titrated directly with arsenite after neutralizing sulfuric acid with bicarbonate. After the determination of Caro's acid (H_2SO_5) sodium acetate is added to buffer and nitrate with bromine in potassium bromide solution to estimate hydrogen peroxide.

Bodin (*46*) estimates the hydrogen peroxide with potassium permanganate. The permonosulfuric acid is titrated by the potassium iodide–thiosulfate procedure after adding a few drops of iodine. To estimate the persulfuric acid a catalyst of cuprous iodide in potassium chloride is added and the iodine liberated from potassium iodide is titrated with thiosulfate.

Denisov (*103*) used the platinum-tungsten electrode system in connection with Gleu's method (*188*). This is the estimation of Caro's acid by the bromide-arsenite-bromate procedure; the estimation of hydrogen peroxide by potassium permanganate after adding manganese sulfate; indirect estimation of persulfuric acid by reduction with excess of standard arsenite in acidified solution at 100° C.

8. Nitrite as Reagent. Reaction between nitrite and hydrogen peroxide (Jimeno and Ibarz, *264*)

Diazotation. Aromatic amines (Singh and Ahmad, *502*)

Benzidine (Atanasiu and Velculescu, *20*); indirect determination of sulfate (Atanasiu and Velculescu, *25*)

Nitrite-ferrocyanide reaction for estimation of nitrite (Romon, *464*)

9. Potassium Ferricyanide as Reagent. Chromium by oxidation with ferricyanide in alkaline solution (Kutovskii and Kholmyanskaya, *317*)

Cobalt in steels and alloys (Dickens and Maassen, *104*, *105;* Scherbakov and Kholcheva, *479;* Tomíček and Freiberger, *582*)

Glucose in sodium carbonate medium (Britton and Phillips, *69*)

Manganese (Tomíček and Kalny, *585;* cf. *105*, *106*)

Sulfides. The sulfide is oxidized quantitatively to free sulfur (Scagliarini, *477*)

Uric acid. The reaction occurs best at pH 10.5 (Beccari, *38*).

10. Oxidation by Cupric Salts. Glucose, titrated with Fehling's solution (Britton, *63*). Hydroxylamine and hydrazine (Britton and Königsten, *68*). A nitrogen atmosphere is used. Hydroxylamine is oxidized to N_2O, and hydrazine to nitrogen. Indigo, sodium hydrosulfite. Air is excluded and Fehling's solution is used (Strafford and Stubbins, *552*).

Reduction by Cuprous Solutions. Chlorate and hypochlorite (Troberg, *593*); gold and platinum (Müller and Tänzler, *393*)

11. Miscellaneous Studies with Less Powerful Reductants. A number of the applications that belong in this section have been given under bichromate (ferrous reaction), peroxides (arsenite as reductant), and iodine (potassium iodide as reductants).

Arsenite for estimation of lead dioxide (Lang and Zwerina, *326*)

Ferrocyanide (See Section 9)

Hydroquinone, amino phenols. Estimation of gold (Ryabchikov and Knyazheva, *469*); estimation of iridium (Bogdanov and Krasikov, *50*)

Substituted hydroquinones (*436*)

Uranous sulfate, for ferric ion or bichromate (Ducloux, *124*)

Hydrazine, estimation of bichromate and vanadate (Holst, *247*); estimation of octavalent osmium (Crowell, *97*)

Sulfite, applied to estimation of bichromate, iodine, ferricyanide, cupric ion, and hydrogen peroxide (Singh and Malik, *505*)

12. More Powerful Reductants. *Vanadyl Sulfate in Alkaline Solution.* Chromate (Fresno and Mairlot, *154*, *157*, *158*)

Chromate and ferricyanide (Fresno and Mairlot, *155*, *159*)

Ferricyanide (Tomíček, *578*)

Gold (Fresno and Mairlot, *156*)

Gold, copper, silver, permanganate, chromate, and ferricyanide (Fresno and Mairlot, *158*)
Mercury (Fresno and Lafuente, *153*)
Selenite and tellurite (Tomíček, *578*)

Stannous Solution as Reagent. Chromium, vanadium, molybdenum (Trebiatowski, *591;* Sosnovskiĭ, *518*). Chromium and iron (Müller and Haase, *388*)
Nitroso-β-naphthol (Belen'kiĭ and Sokolov, *40*)
Molybdenum (Fogel'son and Kalmuikova, *144;* Krüll, *312;* Stehlik, *548;* Sosnovskiĭ, *520*)
Rhenium (Höleman, *244*). The action of various reductants and ReO_4^- was also studied by Höleman (*243*).
Titanium, iron, and molybdenum (Martynchenko and Shimko, *363*)

Sodium Hydrosulfite ($Na_2S_2O_4$). Copper, mercury, and silver (Murooka, *405*)

Potassium Tungsten Ennachloride. Permanganate, ceric ion, bromate, bichromate, ferric, and cupric ions (Uzel and Pribil, *595*)

Titanous Chloride or Sulfate. Dipicrylamine (potassium, rubidium, cesium indirect) (Kiba, *292*)
Food colors (Evenson and Nagel, *132, 133*)
Iridium (Woo and Yost, *623*)
Molybdenum (Stehlik, *548;* Wirtz, *620*)
Murexide (Kuhn and Lyman, *315*)
Palladium (Müller and Stein, *392*)
Uranium (Matula, *367*)

Chromous Solution as Reagent. Chromium, vanadium, and molybdenum (Zan'ko and Shylakman, *628*)
Copper (Miloslavskiĭ and Dolgova, *377*)
Iron and molybdenum (Brintzinger and Rost, *62*)
Iron, molybdenum, and titanium (Martynchenko and Shimko, *363*)
Iron and vanadium (Brintzinger and Rost, *60, 61*)
Osmium (Crowell and Brumbach, *98*)

Vanadous Solution. Estimation of ceric ion (Banerjee, *37*)
Estimation of cupric, ferric, silver, or bichromate ions (Maass, *349*)

13. Miscellaneous Applications of Oxidation-Reduction Methods, Principally in Steel Analysis. Application of systematic potentiometric analysis: aluminum, chromium, titanium, uranium, iron, manganese, cobalt, and nickel (Steuer, *551*). Silver, bismuth, lead, copper, cadmium, and iron-zinc groups (Hiltner and Gittel, *233, 234*). Lead, copper, nickel, zinc, and manganese (Hiltner and Seidel, *241*)

A general review of steel methods is given by Dickens and Thanheiser (*109*)

Steels and Ferroalloys. Cerium, manganese, chromium, and vanadium (Lang and Faude, *324*)
Chromium (Khitarov, *277;* Dickens and Thanheiser, *108*)
Chromium and vanadium (Fogel'son and Kalmuikova, *145;* Heczko, *226;* Werz, *607*)
Chromium, vanadium, and manganese (Adamovich, *4;* Hiltner and Marwan, *240;* Khlopin, *287*) polemic
Chromium, vanadium, molybdenum, and titanium (Gerke and Kardakova, *182);* Zanko and Shylakman, *628*)
Iron and alloys (Dickens and Thannheiser, *107*)
Manganese (Akhumov and Vasile'v, *8;* Avrunina and Zan'ko, *28, 29;* Genis *et al.*, *181;* Khitarov, *277;* Khlopin, *279, 284;* Soloveva, *517*)
Molybdenum (Dickens and Brennecke, *104;* Fogel'son and Kalmuikova, *143;* Werz, *608*)
Molybdenum and copper in steel (Schaefer, *478*)
Molybdenum and titanium in steel and alloys (Klinger *et al.*, *297*); in ores (Krüll, *312*)
Molybdenum in ferromolybdenum (Rabinovich, *442*)
Sulfur (Thanheiser and Dickens, *573*)
Vanadium (Eisermann, *127;* Thanheiser and Dickens, *574;* Dickens and Thanheiser, *107;* Fogel'son and Kalmuikova, *145;* Gutman and Mikeeva, *200;* Gutman and Piradyan, *201;* Fogel'son and Kalmuikova, *144;* Pinskaya, *432*)

Review of Methods for steel, etc. (Dickens and Thanheiser, *111*)

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Measuring the Specific Surface of Particulate Substances

A Water Vapor Adsorption Method

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SINCE the publication in 1921 of a photomicrographic method (*3*) of determining particle size, there has been considerable demand for a more rapid means for making such measurements. Though the authors agree that other procedures for making particle size determinations are desirable, they do not believe that simply a reduction in the time of measurement is the most important point involved. The real necessity for developing a new method of particle size measurement is that many particulate substances are not amenable to microscopical analysis. Gardner (*2*) lists approximately 2000 pigments, probably not more than 40 per cent of which could be measured with the microscope.

Photomicrographic Limitations

If a material such as a pigment is to be measured microscopically it must have, first, a suitable particle size. The individual particles must not be so small that they cannot be resolved and photographed clearly. Second, the pigment must possess a relatively high degree of uniformity; otherwise a representative sample, at the necessary magnification, cannot be obtained in a single photograph. Third, for white light and ultraviolet microscopy the material should preferably have a refractive index that permits the particles to be seen distinctly in suitable mounting media.

A water vapor adsorption method has been developed for determining the specific surface of particulate substances. No high-vacuum equipment is required, as the measurements are made at atmospheric pressure. This method makes possible determination of the surface shape factor and investigation of the particle size of materials too fine-grained to be measured with the microscope.

A table of the specific surfaces of a large number of particulate substances which were measured is included and checks with the microscope are given wherever possible. Methods of comparing microscopical measurements with adsorption measurements are discussed.

Though many pigments comply with the second and third requirements, they are sometimes so small in size that the individual particle cannot be seen with the usual microscopical equipment. Such pigments include the gas blacks, lithol reds, peacock blues, etc. The individual particles of these pigments can be detected with a microscope using a dark-field condenser or with an ultramicroscope, but these methods are not suitable for linear measurement of fine-grained materials, though they are successfully used for counting the number of particles in a given volume.

Electron microscopy is very well suited for the measurement of particles in the range between 0.01 and 0.1 μ, but this technique is too specialized and the equipment too costly for the average laboratory interested in particle size and specific surface measurement.

Notwithstanding the limited field of the photomicrographic method, it probably gives as complete an analysis of the subject as any method generally used at present. Its great advantage lies in the fact that it produces a frequency distribution curve from which the uniformity coefficient and all the various average diameters can be calculated. The method also involves certain quantities known as "shape factors", which are simply the proportionality constants to be used in conjunction with the horizontally measured diameters (*3*), so that these diameters can be employed for determining specific surface and the number of particles per gram of material.

It has been stated elsewhere (*4*) that the important unsolved problem in the subject of particle size determination has been to measure the surface shape factor, and that the solution would probably be found in an adsorption method. This statement was made from the microscopist's point of view. It could just as readily have been stated that the important problem is to measure specific surface—i. e., the number of square meters of particle surface per gram of material, or the square meters of surface per solid cubic centimeter of material.

Adsorption Method

The authors report here some work done on the development of a comparatively simple procedure for obtaining specific surface by an adsorption method. The measurement of specific surfaces of finely divided materials has been studied in great detail by Emmett and his co-workers in their classic work on the low-temperature adsorption of nitrogen and other gases. Adsorption from solution has also been employed for the surface measurements of finely divided materials (*5*). The work described here concerns the adsorption of water vapor at room temperature in a system which has a full atmosphere of pressure.

Some work was being done on the determination of the moisture content of various particulate substances. The method being studied was essentially one in which the material was mixed with calcium carbide which reacted with any water present. The amount of acetylene produced by this reaction was supposed to be proportional to the amount of water in the particulate substance. While attempting to check the accuracy of this method with a sample of iron blue which had a definite known moisture content, it was found that no pressure was produced. This condition was noted by James Massarene, one of the authors' co-workers. Further investigation showed that the gas which was generated produced no pressure because it was adsorbed by the sample. As a consequence, acetylene was first used in the adsorption apparatus but later it was found more convenient simply to use water vapor. The idea embodies measuring the number of molecules of water adsorbed on a known quantity of material and then multiplying this number by the cross-sectional area of the water molecule. Wherever possible, checks were made with the photomicrographic method of measurement.

The apparatus and method used in the determination of specific surface given in this paper depend on the fact that adsorption takes place on solid surfaces (*6*).

Apparatus

Essentially the apparatus consists of two adsorption flasks, a sensitive differential manometer, and a system for introducing the gas or vapor to be adsorbed.

The adsorption flasks, *A* and *B* in Figure 1, are connected through the differential manometer, *C*, with stopcocks, *a*, between each flask and the manometer.

Into the stoppers, which have ground-glass joints, are sealed stopcocks, *b*, and the three-way cocks, *c*. The stems of cocks *b* go well down into the flasks while those of cocks *c* are near the top, so that good circulation is obtained when any gas or vapor is passed through the system.

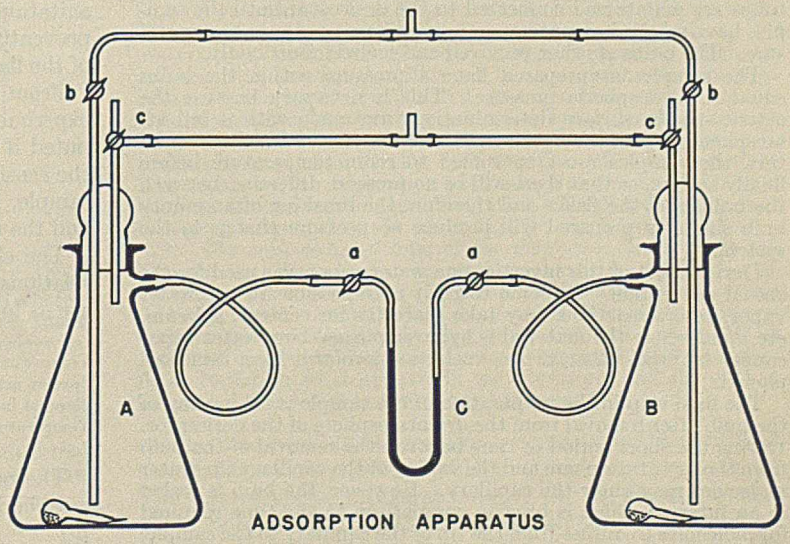

FIGURE 1

All connections for the introduction of gas or vapor, for drying, and for evacuation are made at *b* and *c*.

The differential manometer is of the ordinary inclined type. Its function is to indicate the change in pressure which takes place upon adsorption in either of the flasks, the pressure in the other being maintained constant. Dibutyl phthlate is employed as the manometer liquid because of its low vapor pressure, low specific gravity, and good wetting of the manometer tube. The insolubility of the gases or vapors in the manometer liquid is of importance, and in this respect, too, dibutyl phthalate is a good choice.

The use of each of the flasks for making adsorption measurements on different samples of the same material is only incidental to their original purpose. In an earlier apparatus employing only one flask connected to an open-end manometer, variations in atmospheric pressure caused drifting of the manometer. Originally the second flask was attached to the other end of the manometer to act as a constant reference pressure chamber to eliminate the drift in the manometer. Later this reference pressure chamber was used for adsorption also, giving the symmetrical system as shown in Figure 1.

The gas or vapor can be introduced either by circulation through flasks *A* and *B*, or where pressures less than saturation are required, by partial evacuation of the system and introduction of the gas or vapor to restore any desired pressure. All the present work was done at atmospheric pressure.

This whole system is maintained at a constant temperature in a water bath. Thermoregulator and heating coils are employed, and the bath is stirred continuously.

Sample Preparation

Small thin-walled glass bulbs are made for receiving the sample to be measured, and for sealing the sample from all gases until adsorption is to take place. These bulbs are made small enough to enter the mouths of flasks *A* and *B* easily.

The material, the specific surface of which is to be measured, is given a preliminary drying before weighing the sample in order to eliminate the bulk of the moisture. Samples of the material are then placed in the small glass bulbs and weighed carefully, so that the weight of the sample is known. When the samples have been weighed, the open ends of the bulb are drawn down to fine thin-walled capillaries which are left open. The samples are then ready for the final drying procedure.

Two procedures have been used for the actual drying, equally good results having been obtained by each method. One procedure is to heat the sample bulbs at 185° C. for 18 hours, remove from the oven, and place immediately in a desiccator at room temperature, through which air is circulated after having been dried over calcium chloride and phosphorus pentoxide. When the samples have reached room temperature, they are removed from the desiccator and sealed immediately.

In the second procedure, the bulbs are placed in a Pyrex desiccator and heated at 185° C. for 18 hours with the pressure reduced to 300 mm. of mercury throughout the heating. Air, dried over calcium chloride and phosphorus pentoxide is passed into the desiccator when the heating has been completed and the drying tubes are maintained connected to the desiccator until the samples have reached room temperature and are at atmospheric pressure. The bulbs are then removed and sealed immediately.

The samples as prepared have a pressure within the bulbs equal to atmospheric pressure. This is necessary because the actual specific surface determinations are made with a full atmosphere of pressure in adsorption flasks *A* and *B*. Because of this, the samples must be cooled to room temperature before finally sealing, so that there will be no pressure difference between the bulbs and the flasks and therefore the breaking of an empty bulb similarly prepared will produce no pressure change in the system.

During most of this investigation water vapor was used for the actual adsorption. In some cases it is impossible to use water vapor, where reactions may take place (as for cement, gypsum, etc.) and where the material is hygroscopic. Where water vapor cannot be used, other vapors such as chloroform have been employed.

The final step in the preparation of the sample is the sealing of the bulb after removal from the dry atmosphere of the desiccator. During the short period of time between the removal of the bulb from the dry atmosphere and the sealing of the capillary tip, water molecules may enter the capillary. However, the bulb is sealed in an interval which is brief in comparison to the time required for molecules to diffuse from the tip of the capillary to the sample, so that if water vapor does enter the capillary, any adsorption by the sample will take place after the capillary is sealed.

The introduction of these few water molecules into the sample bulb is equivalent to a corresponding increase in the final number of water molecules available for adsorption in the adsorption flasks. The pressure in the sample bulb, as placed in the adsorption flask, may be somewhat lower than atmospheric pressure, owing to the adsorption of these few water molecules. When the sample bulb is broken in the adsorption flask there will be a small pressure decrease in addition to that produced by the adsorption taking place in the flask. This initial pressure decrease is equal to that which would have resulted from the adsorption of these first few water molecules if they had been adsorbed by the exposed sample in the adsorption flask instead of in the sealed sample bulb. From this point, the remainder of the adsorption proceeds as usual.

The total adsorption by the sample, as indicated by the manometer, is equal to that which would take place in an atmosphere having an added number of water molecules equal to the number sealed into the sample bulb, except that the adsorption would have taken place in two installments to give the same final total adsorption.

Water Vapor Adsorption

Samples of the particulate substance, prepared as outlined above, are placed in flasks *A* and *B*. Air is circulated through the two flasks to eliminate gases or vapors which may be present from any previous measurement. The water vapor is now introduced into *A* and *B* by reducing the pressure to 350 mm. and then restoring the system to atmospheric pressure by the addition of water-saturated air. The system is allowed to come to temperature equilibrium, then the sample in one of the flasks is exposed to the water vapor by shaking the flask to break the bulb. The pressure change is noted when equilibrium has again been attained; then the sample in the other flask is similarly exposed and this second pressure change is noted.

When the sample is first exposed to the water vapor, the pressure decreases very rapidly, owing to adsorption. If the pressure continues to decrease slowly for a considerable period after this initial rapid change, it is assumed to be due to causes other than monomolecular adsorption (condensation in capillaries, etc.) and has been treated as such in evaluating the specific surfaces of the samples.

Up to the instant the sample bulb is broken, the sample is sealed off from the surrounding atmosphere of water vapor. When the bulb is broken, the sample is exposed immediately to the molecules to be adsorbed; no time is required for diffusion from one part of the system to another. The agitation the sample receives facilitates the adsorption by preventing the formation of a mound of material at the bottom of the flask.

From the manometer readings obtained in the adsorption experiment, the specific surface of the sample may be computed if we also know the volume of the adsorption system, the sensitivity of the differential manometer, the mass of the sample, the cross-sectional area of the molecules adsorbed, and the temperature at which the measurements are made.

The characteristics of the apparatus which determine the relationship between specific surface and manometer readings are as follows:

Volume of adsorption flask, liters	1.20
Specific gravity of dibutylphthalate	1.05
Slope of inclined manometer	1/10.9
Temperature of apparatus, ° C.	25

The number of molecules in this system at 25° C. and atmospheric pressure is

$$N = \frac{1.20\ (6.06)10^{23}\ (273)}{22.4 \qquad 298} = 298 \times 10^{20}$$

Each millimeter displacement on the inclined dibutylphthalate manometer is equivalent to

$$\frac{1.05}{13.5\ (10.9)}\ \text{mm. of Hg} = 0.00714\ \text{mm. of Hg}$$

which corresponds to a change in the number of molecules of gas or vapor in the system by

$$\frac{0.00714}{760}\ (298 \times 10^{20}) = 0.279 \times 10^{18}$$

Taking $10A^2$, $(10^{-19}m^2)$, to be a representative figure for the cross-sectional area of the adsorbed water molecule, it follows that each millimeter decrease in pressure as indicated on the inclined manometer corresponds to the coverage of 0.0279 square meter by water molecules adsorbed in a monomolecular layer. Thus, the relationship for specific surface as a function of manometer displacement and mass of sample becomes

$$\text{Specific surface} = 0.0279\ \frac{\Delta p}{m}\ \text{square meter per gram} \qquad (1)$$

for this apparatus.

In a large number of measurements made for various masses of the same particulate substance, it was found that the adsorption followed the Freundlich adsorption isotherm very closely. The decrease in pressure due to the adsorption by the sample is very nearly proportional to the mass of the sample when the pressure change is small. In Figure 2 are some representative curves showing how $\Delta p/m$ becomes nearly constant at small values of m. All the authors' determinations were made with small samples, so that the deviation from the constant relation between specific surface and $\Delta p/m$ was very small.

Average Particle Diameters

In making comparisons of adsorption measurements and microscopical measurements it is well to consider briefly the various average diameters for nonuniform particulate substances (*4*).

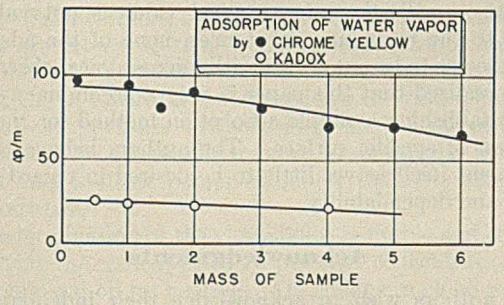

FIGURE 2

The average diameter related to specific surface, S, is d_3. This diameter is equal to $\Sigma nd^3/\Sigma nd^2$, where n is the frequency of particle diameter d. The surface shape factor is σ' and the volume shape factor is ν'. The density of the particle is ρ. The diameter related to average volume is

$$D = (\Sigma nd^3/\Sigma n)^{1/3} \qquad (2)$$

A series of six samples of fine-grained zinc oxide was measured in pairs, taking the average measurement of the pair in each case. The relationship

$$d_3 = \sigma'/\nu'\rho S \qquad (3)$$

was employed to determine d_3 from the specific surface, assuming $\sigma'/\nu' = 6$, which is the same as assuming the particles to be spheres or cubes. This gave the following:

ZnO Sample	Specific Surface	d_3 by Adsorption	d_3 by Microscopy
Grams	*Sq. m./gram*	*Micron*	*Micron*
0.50	5.7	0.19	0.17
1.00	5.7	0.19	0.17
1.50	5.4	0.20	0.17
Average	5.6	0.19	0.17

A list of some of the other materials measured appears in Table I. These materials are merely representatives of the large classes of which they are samples, and the results are not necessarily significant for the whole class. The d_3 by adsorption was determined from specific surface measurements, the microscopic d_3 was determined by the photomicrographic method (*4*), and the D was determined by the slit ultramicroscope.

Accuracy of Method

In the major part of this study, specific surface determinations were checked by making measurements on samples of the same material from each side of the adsorption system. For each determination the samples had been prepared together and therefore received the same treatment. The results of one side of the system checked the other within 2 or 3 per cent, and in a great many cases within 1 per cent, indicating adequate reproducibility for the method.

In the series of measurements made on the fine-grained zinc oxide, all the samples had been prepared together, receiving the same treatment, but specific surface measurements were made at different times. These checked within 1 per cent for two of the groups of samples, and within 5 per cent for a third group. The mass of the samples in these groups varied.

This method of specific surface measurement is an indirect one, and in order to discuss its accuracy, it is necessary to consider the assumptions made. The pressure decreases in the system are measured directly, and from this, specific surface is computed on the following assumptions:

1. *The surface of the material is completely covered by a monolayer of adsorbed molecules.* In the extensive work done by Emmett (*1*) and his co-workers it is shown that the lower pressure end of the linear portion of experimental adsorption isotherms corresponds to monomolecular adsorption.
2. *No condensation takes place on the surface of the material, in any interstices between the particles, or in any pores the material may have.* Conditions of the adsorption have been arranged so that the possibility of condensation has been minimized. The adsorption is carried out in an atmosphere having a vapor pressure of water lower than 50 per cent of the saturation pressure. The sample is intentionally small, so that no mound of exposed material will be formed when the bulb is broken, and therefore condensation between particles will be at a minimum. The pressure change in the system is noted as soon as temperature equilibrium is attained, so that any pressure change due to condensation, which takes place much more slowly than adsorption, is practically excluded in the determination of specific surface. The rate at which adsorption proceeds is so much greater than the rate of condensation that the latter will not have progressed to a great extent when the adsorption is completed.
3. *The cross-sectional area of the absorbed molecule is known.* The error in this value for the water molecule is probably in the order of 10 per cent or less. Figures in the literature vary between $9A^2$ and $11A^2$. Computations from water at maximum density and assuming a cubical molecule give $9.6A^2$ for this area. W. D. Harkins, in a private communication, suggested $10A^2$ as a good figure based on what appears in the literature, and the authors have used this value in their computations.

Wherever possible, the results obtained on the basis of these assumptions were checked by the photomicrographic method. The microscopical measurements of the d_3 diameters were made independently by E. F. Fullam, one of the authors' coworkers, and consequently were not influenced by the results obtained by their absorption method. The d_3 diameter is the

TABLE I. SPECIFIC SURFACES AND AVERAGE DIAMETERS OF SOME COMMON PIGMENTS

Sample	Specific Surface	d_3 by Adsorption	d_3 by Microscopy	D by Microscopy
	Sq.m./gram	*Microns*	*Micron*	*Micron*
ZnO (Green Seal)	2.4	0.45	0.44	...
Heated 0.5 hour at 800° F.	2.3	0.47	0.47	...
Heated 0.5 hour at 1100° F.	2.3	0.47	0.50	...
Heated 0.5 hour at 1400° F.	1.8	0.59	0.59	...
Heated 0.5 hour at 1700° F.	1.7	0.63	0.66	...
Gas black 1	330.	0.010	...	0.025
Gas black 2	110.	0.033	...	0.025
Gas black 3	150.	0.023	...	0.040
Gas black 4	95.	0.036	...	0.050
Gas black 5	110.	0.031	...	0.050
Gas black 6	34.	0.10	...	0.060
Gas black 7	59.	0.058	...	0.060
Pigment black 8	12.	0.29	0.26	0.200
Whiting	2.6	0.85	...	...
Asbestine	4.1	0.51	...	...
Lithopone	2.3	0.61	0.43	...
Blanc fixe	2.2	0.62	...	...
Barytes	0.59	2.3	...	...
Titanium dioxide	8.2	0.19	0.43	...
Antimony oxide	0.61	1.7	0.61	...
Basic carbonate white lead	1.1	0.80	0.66	...
Eosine toner	41.	0.073	...	...
Viridine green lake	69.	0.035	...	...
Hansa yellow toner	2.6	1.5	0.21	...
Rhodamine toner	12.	0.18	...	...
Iron blue	26.	0.13	...	...
Orange mineral	0.76	1.3	0.43	...
Chrome yellow medium	3.9	0.26	0.32	...
Madder lake	53.	0.071	...	...
Red lake C barium	15.	0.23	...	...
Lithol red	36.	0.083	...	...
Ultramarine blue	13.	0.20	0.59	...
Peacock blue	83.	0.036	...	...
Lithol rubine	26.	0.12	...	...

average of a great many measured "horizontal diameters" (*3*) and is obtained by the equation given above. The close agreement between the microscopic measurements and the adsorption measurements made on particles which the authors believe to be nonporous indicates that these assumptions are well made in most cases. On the basis of complete correctness of these assumptions, the specific surface values would deviate about 3 per cent from the actual surface available for the adsorption of water molecules owing to apparatus calibration errors. This error is in addition to any introduced by incomplete correctness of the assumptions made.

Comparison Data

The values of the D diameters were given by the Binney and Smith Co., which also supplied the eight samples of blacks. These D diameters were obtained by counting the number of particles in a given volume of suspension in which the total volume of solids was known. From such data the volume of the particle of average volume was calculated. It is invariably assumed in this type of measurement, with the slit ultramicroscope, that the particles are spheres; then $\pi D^3/6$ becomes the average particle volume and ν' equals $\pi/6$.

It is obvious from what has preceded that no definite relation can exist between the d_3 and D diameters reported here. If D had been calculated from the equation $D = (\Sigma nd^3/\Sigma n)^{1/3}$, where d is the horizontal diameter, it would follow that the authors' d_3 would always be greater than D (*4*); but under the present circumstances the D diameters were obtained by a different method, so that no conclusion can be made as to the relative values of d_3 and D.

In checking the results of the adsorption method with the microscope one has the choice of comparing either the specific surfaces or the average particle sizes. In either case an assumption has to be made that the particles are spheres or some other definite form. The adsorption method measures specific surfaces; the microscopic method measures d_3 and D. Assuming the particles to be spheres, d_3 can be converted to specific surface by the expression

$$S = 6/\rho d_3 \qquad (4)$$

D, however, bears no relation to specific surface and therefore cannot be employed except as an approximate value for d_3. Again, if particle sizes are to be compared, the specific surface obtained from the adsorption method is then used for calculating d_3 from Equation 3, particles still assumed to be spheres.

Since "particle size" is referred to more generally than "specific surface", the latter method was used in securing the data for Table I.

Discussion of Results

An examination of the tabulated measurements shows that the zinc oxides gave the best results, checking remarkably well with the microscope. The blacks also checked satisfactorily, considering the fact that the d_3 and D diameters are not strictly comparable. The first noticeable discrepancy appears to be with titanium dioxide. There can be no question here that a possible error in the microscopical measurement accounts for the difference between 0.19 and 0.43 micron. With a material as uniform as the titanium dioxide particle appears to be, a microscopical error of 0.24 micron is out of the question. The fact that the adsorption method gives a particle size that seems to be too small cannot be accounted for by an insufficiently dried surface, for that would give results of an opposite nature. The large specific surface which is inconsistent with microscopical measurement is explained on the basis of photographs taken with the electron microscope, which show the "smooth particle" as measured by light microscopy to be a firm aggregate of small tetragonal crystals irregularly arranged. This firm aggregate, which the light microscope shows as the ultimate particle, cannot be broken down by any commercial grinding process. This structure has a considerably larger surface than is indicated by the light microscope.

In the cases of antimony oxide and orange mineral, the specific surfaces are undoubtedly too small; this is probably due to the lack of a sufficiently dried surface before adsorption takes place. In the case of ultramarine, the assumption that the particles are spherical could readily be far enough from the truth to account for the discrepancy found here. The hansa yellow particles grow rapidly at elevated temperatures, and have actually grown during the drying process, so that comparison with the microscopical value is not valid. In regard to the remaining substances, none of the adsorption figures seems to be out of line with microscopical observation.

It is realized that this paper is only a preliminary step toward establishing a simple adsorption method for the determination of specific surface. The authors believe that the instrument itself leaves little to be desired in regard to simplicity and dependability.

Acknowledgments

The authors wish to acknowledge their indebtedness to Interchemical Corporation for making this investigation possible; to W. D. Harkins for many valuable suggestions; and to James Massarene, C. W. Jerome, and E. F. Fullam of the research staff for their helpful assistance in carrying out this work.

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The Cloud Point of Varnish Resins in Drying Oils

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The temperature at which a heated mixture of varnish resin and a selected mineral oil clouds on cooling is a useful index of the relative solubility of the resin. The solubility achieved by heating mixtures of resin and drying oil may be measured by mixing the drying oil and resin with a suitable mineral oil and determining the temperature at which the mixture clouds. The changes observed on heating ester-type resins are believed to be due to an ester interchange of the resin and the drying oil, and to the poorer solvent power of the bodied oil. With pure phenolic and some hydrocarbon resins, the effect of the decreased solvent power of the bodied drying oil is the controlling factor.

SEVERAL tests have been developed to measure the solvent power of volatile thinners—for instance, the aniline point and the kauri butanol value (*3*)—but no test has been suggested which will accurately compare the solubility of various varnish resins, or measure the degree of solubility which is attained on heating the resins with drying oils.

The concentration at which a resin clouds in solution on dilution with a volatile solvent has been used as an index of its molecular size (*1, 8*). This method has been used to measure the comparative strength of nitrocellulose solvents (*3*), but as a measure of solubility of a resin it has not been standardized. Such methods have been investigated in this study but they are subject to some qualifications, inasmuch as this cloud point may be hard to duplicate and when the precipitation occurs at low resin concentrations, the values obtained do not always measure the solubility characteristics of the resin accurately. A small amount of relatively insoluble material may indicate poorer solubility than the resin as a whole possesses.

The determination of the cloud point is simple and consists in dissolving the resin at 50 to 70 per cent concentration in a suitable mineral oil by heating. On cooling, a cloud will develop which can usually be duplicated, on reheating and cooling, within one degree. In some cases greater accuracy can be obtained.

The mineral oil used should be chosen for the type of resin to be examined. The aniline point of a mineral oil is a useful index of its solvent power, those with high aniline point having poor solvent power, reflected in high cloud points with a resin, while oils with low aniline point are much better solvents.

While a wide variety of mineral oils have been and may be used, only two mineral oils have been used in this study. One is a viscous paraffinic mineral oil of high (137° C. maximum) aniline point. The other is a white mineral oil (Nujol) of 104° (maximum) aniline point. Various samples of Nujol may vary a degree or two in aniline point and the cloud points will vary accordingly. In general, two mineral oils of the same aniline point will give the same cloud point with a given resin.

Solubility of Rosin–Phenolic Resin Condensates

WW wood rosin was heated with stirring and various amounts of six commercial "heat reactive" phenolic resins, designated as A to F, were added at 125° C. Heating and stirring were continued over a period of one hour when the temperature had reached 250° C. The resin was then poured. In this study a highly paraffinic mineral oil of 137° C. aniline point (maximum) was used as the reference oil.

Determination of Cloud Point

Five to 7 grams of resin are weighed in a 150-cc. beaker to the nearest decigram and the mineral oil is added to bring the total weight to 10 grams. The mixture is then warmed on a hot plate with constant stirring until the resin is thoroughly dissolved. Local overheating should be avoided and the final temperature should not exceed 200° C. The mixture is then transferred to a 15-mm. test tube, immersed in a stirred water bath which has been heated to 10° above the expected cloud point. A glycerol bath may be used for temperatures over 100°. The temperature is allowed to drop at not over 2° per minute. A definite cloud of the oil-resin mixture will develop, and the temperature of the bath at this point is recorded as the cloud point. A slight haze may develop in the case of some resins, but this should not be confused with the true cloud point, which should be read when the mixture becomes opaque.

The determination may be repeated by raising the temperature of the bath 10°, holding until the sample is clear, and repeating the determination.

Figure 1 shows the cloud point of the rosin–phenolic resin condensates with various amounts of phenolic resin. It will be noted that some phenolic resins give much higher cloud point condensates than do others, and that the cloud point increases as the content of phenolic resin increases. Some commercial resins gave condensates which did not cloud at room temperature in the 137° aniline point mineral oil used.

Molecular weight, melting point, and viscosity of a 60 per cent solution in toluene of these condensates were also determined. It was found that the viscosity, molecular weight, and melting point of the condensates which did not cloud at room temperature were ap-

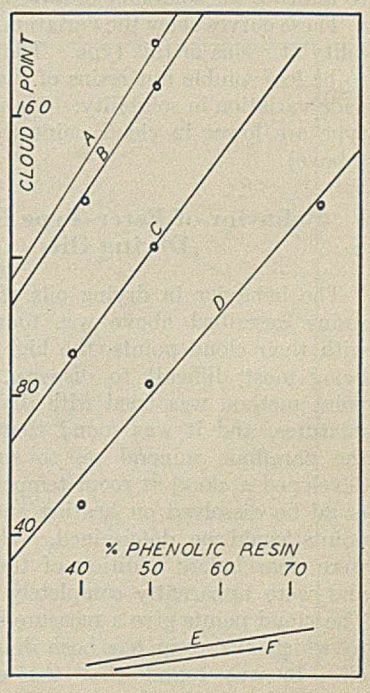

FIGURE 1. CLOUD POINT OF PHENOLIC-ROSIN CONDENSATES
50 per cent resin, 50 per cent mineral oil

preciably lower than the other condensates at the same phenolic content. In the case of the condensates that did cloud, however, the cloud point at the same phenolic resin content did not vary with viscosity, molecular weight, nor melting point, but was a relatively independent property, depending largely on the type of phenolic resin employed.

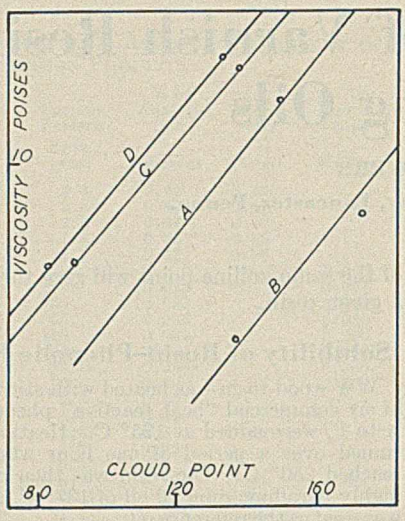

FIGURE 2. VISCOSITY AND CLOUD POINT OF PHENOLIC-ROSIN CONDENSATES

Figure 2 shows the correlation of the viscosity of a 60 per cent solution of the condensates in toluene with cloud point. With a given resin the cloud point increases as the logarithm of the viscosity, but the relationship varies greatly, depending on the phenolic resin used. This difference in solubility is at least in part caused by the nature and size of the alkyl groups in the phenolic compound from which the resin was made.

CLOUD POINT OF ROSIN–PHENOLIC RESINS. The cloud point of nine commercial rosin-modified phenolic resins, designated as G to P, was determined (Figure 3). Resins G to N were difficultly dispersible in the 137° aniline point paraffinic mineral oil, and in these cases a white mineral oil of 104° aniline point (maximum) was used. The cloud point in these cases was taken at several concentrations to determine the variation of cloud point with resin concentration. Usually determination at one concentration (50 per cent) is sufficient to indicate the solubility of a resin.

These curves show the variation that may be found in solubility of resins of this type. The higher melting resins tend to be less soluble but resins of the same melting point show wide variation in solubility. Many commercial resins of this type are lower in cloud point than the resin in Figure 3 (above).

Behavior of Ester-Type Resin in Drying Oils

The behavior in drying oils of the various resins described above was found to agree with their cloud points, the high cloud resins being most difficult to disperse. The cloud point method was tried with drying oil–resin mixtures, and it was found that addition of the paraffinic mineral oil to such mixtures developed a cloud at room temperature which could be dissolved on heating and that cloud points could be determined. These vehicles were clear before addition of the mineral oil and were apparently completely "dispersed". The cloud points give a measure of the extent to which the resin has been dissolved in the oil. It was found that the cloud points decreased as the heating of the oil-resin mixture continued when glyceride resins were used.

In some cases a minimum was reached and the cloud point increased on further heating. The cloud points with a given resin were lower as the ratio of drying oil to resin was increased. This was believed to be caused by an ester interchange between the resin glyceride and the drying oil. Since no simple method was available to measure the ester interchange, the acid interchange between rosin and an acid rosin phenolic resin was measured. All the cloud points of oil-resin mixtures determined in this paper were made on mixtures without volatile thinners. The cloud point may be determined on vehicles containing volatile thinners, but precautions should be used to prevent their loss by volatilization under conditions of the test. The presence of volatile thinners will change the cloud point, depending on the amount and solvent power of these materials.

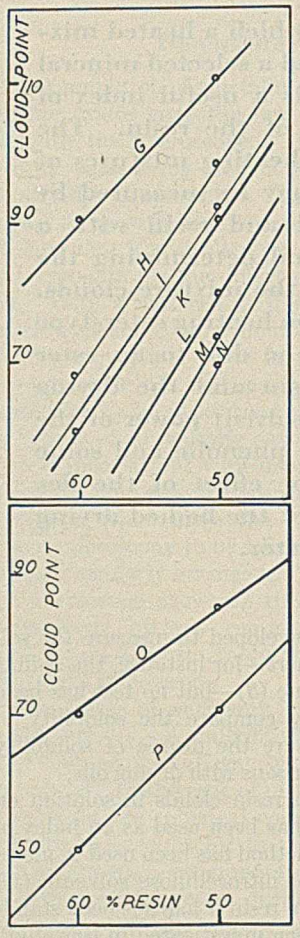

FIGURE 3. CLOUD POINTS OF ROSIN–MODIFIED PHENOLIC RESINS

Above, in white mineral oil
Below, in paraffinic mineral oil

Acid Interchange between Rosin and Oil

Mixtures of WW wood rosin and alkali-refined linseed oil were heated with stirring at 275° and samples taken at intervals. A commercial rosin–phenolic resin condensate was also heated with linseed oil at 275°; in one case the heated mixture was protected by an atmosphere of carbon dioxide.

The free rosin acid was determined by the method of McNicoll (*5*) by determining the decrease in acidity on refluxing the sample with methanol containing *p*-toluenesulfonic acid.

TABLE I. ACID INTERCHANGE OF ROSIN AND ACID PHENOLIC ROSIN RESIN WITH LINSEED OIL

Parts Rosin	Parts Acid Phenolic Resin	Parts Linseed Oil	Time, Hours	Acid No.	Reflux Acid No.	Cloud Point 50% 137° Aniline Point Oil	Remarks
1	...	1	0	85.5	80.6	<0	
1	...	1	0.5	81.8	66.4	<0	
1	...	1	1.0	79.2	58.0	<0	
1	...	1	2.0	78.0	46.3	<0	
...	1	1	0	68.4	56.9	>230	
...	1	1	0.5	53.5	38.5	>230	
...	1	1	1.0	51.9	31.0	>180	
...	1	1	2.0	47.6	24.1	98	
...	1	2	0	41.6	33.5	>230	
...	1	2	0.5	42.0	26.4	84	
...	1	2	1.0	40.0	18.8	0	
...	1	2	2.0	38.0	12.9	0	
...	1	1	0	64.3	55.2	>230	Protected by CO_2
...	1	1	0.5	52.7	38.2	100	Protected by CO_2
...	1	1	1.0	48.8	29.1	52	Protected by CO_2
...	1	1	2.0	42.0	19.4	70	Protected by CO_2

The cloud point was determined as with the resins, except that 10 parts of the oil-resin mixture and 5 parts of 137° aniline point mineral oil were used. Heating was required to bring the mixture into solution.

The results are shown in Table I. The acid number of the rosin–linseed oil mixtures drops slightly on heating but the acid number after refluxing drops considerably, indicating the combination of the rosin acids with the liberation of an equivalent amount of fatty acids. This has been previously suggested (7), but apparently the degree of interchange has not previously been measured.

In the case of the rosin–phenolic resin mixture, the drop of the acid number on heating is larger than with rosin. This may be due to loss of "apparent" acidity of the phenolic resin on heating. The "reflux" acid numbers show that the rosin–phenolic resin has combined with the drying oil with the liberation of drying oil acids.

The cloud points in Table I show that rosin is entirely soluble even at the start of the heating. It will be noted that the phenolic resin clouds at progressively lower temperatures as the heating continues. Where the cloud point exceeds 200°, the resin visibly separates from the linseed oil on cooling without the addition of mineral oil.

The cloud points, when the batch was protected by carbon dioxide, are lower, although the amount of interchange is much the same. This is believed to be due to the poorer solvent power of the bodied oil which is formed more rapidly when oxygen is present. This effect is also shown in cases where the cloud point increases on further heating. The cloud point and reflux acid numbers are lower as the amount of drying oil is increased. The excess of drying oil promotes the interchange with the acid resin.

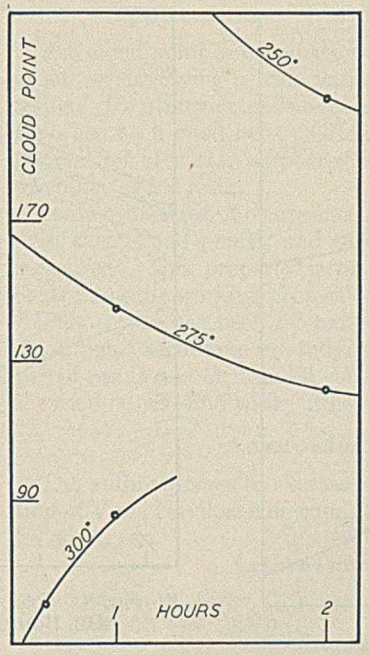

FIGURE 5. CLOUD POINT OF MALEIC RESINS IN LINSEED OIL

TABLE II. CLOUD POINT OF ROSIN PHENOLIC GLYCERIDE IN LINSEED OIL

Concentration of Resin % by wt.	Cloud Point ° C.
1.5	22
1.75	70
2.00	118
2.25	165

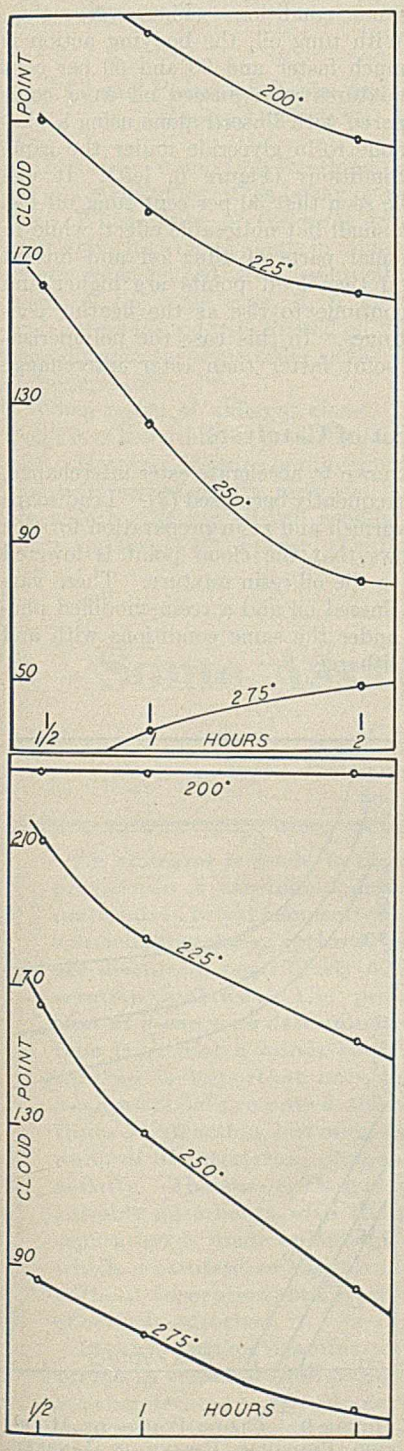

FIGURE 4. CLOUD POINTS
Above, 1 part of resin, 2 parts of oil
Below, equal parts of resin and oil

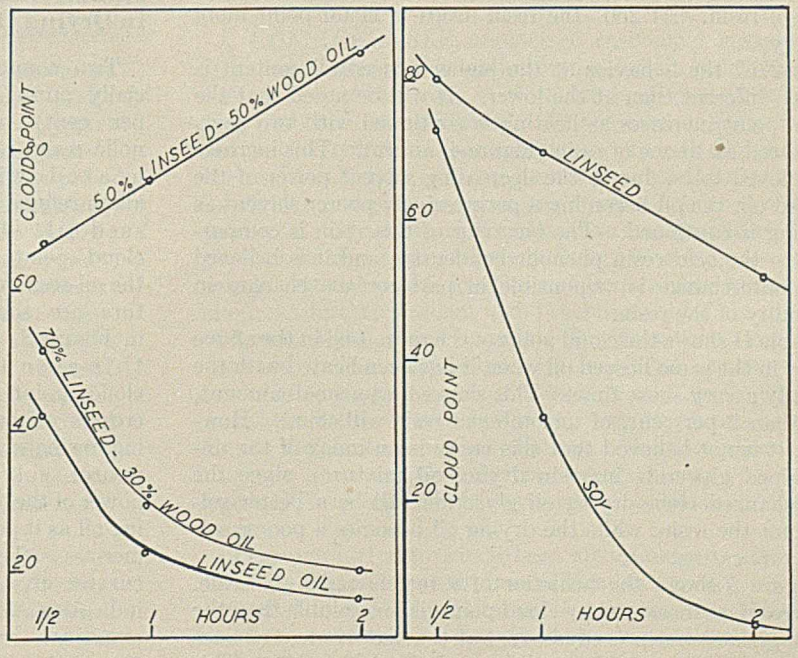

FIGURE 6. CLOUD POINTS
Left, linseed–tung oil mixtures
Right, linseed–soybean oil mixtures

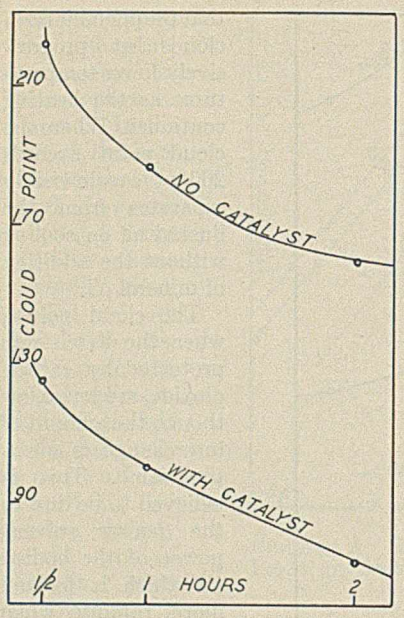

FIGURE 7. EFFECT OF CATALYST

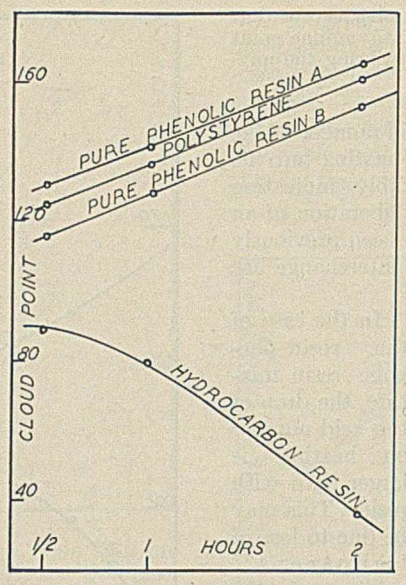

FIGURE 8. CLOUD POINTS IN LINSEED OIL BODIED AT 275° C.

The cloud point results indicate clearly a definite chemical combination between the phenolic resin and rosin. If the phenolic resin were not combined chemically with the rosin, no change in solubility would be expected when interchange with the drying oil acids occurs. It will be shown that pure phenolic resins do not become more soluble on heating with oil.

Varnish Resins and Drying Oils

When the less soluble rosin-modified phenolic resins are heated with drying oils, the changes in solubility may be measured by the cloud point. Figure 4 shows the cloud points of a rosin-modified phenolic glyceride resin heated at various temperatures with nonbreak linseed oil. At 200° C. little change occurs, except when 2 parts of drying oil are used to 1 of resin. At 250° the resin drops in cloud point more rapidly.

At 275° the behavior at the higher linseed oil content is rather different than at the lower. It will be noted that the cloud point increases as heating is continued with two parts of linseed oil to one of resin (Figure 4, above). This increase is believed to be due to the decreasing solvent power of the bodied oil, the oil becoming a progressively poorer solvent as heating is continued. The behavior of this resin is comparable to the acid rosin phenolic condensate and it is believed ester interchange is responsible in part for the changes in solubility of the resin.

Table II shows the cloud point of the resin used in the above study in the same linseed oil when it has been heated with the oil only a very short time. This shows that a small amount, less than 2 per cent, of uncombined resin will cloud. However, it is not believed that this curve is an index of the uncombined glyceride in resin-drying oil mixtures, since the interchanged resin-drying oil glyceride will be a better solvent for the resin, while the drying oil becomes a poorer solvent on heating.

Figure 5 shows the behavior of a maleic resin glyceride. This resin, as measured by cloud point, is less soluble than the rosin-phenolic resin used above, but the high content of ester groups makes it possible to disperse it in drying oils. This shows that cloud point of resins of different types cannot be used in comparing their solubility in drying oils.

Cloud Points in Soybean, Linseed, and Linseed-Tung Oil Mixtures

As noted above, polymerized oils are poorer solvents than unbodied oils. Hence, it might be expected that oils which polymerize more readily will show higher cloud points with the same resin. This has been found to be the case, since soybean oil gives lower cloud points with the same resin than does linseed oil under the same conditions (Figure 6, right). With tung oil, the bodying action is much faster and 30 and 50 per cent mixtures with linseed oil were compared with linseed alone using a phenolic rosin glyceride under the same conditions (Figure 6, left). It will be seen that 30 per cent tung oil has a small but noticeable effect, while in equal parts of tung oil and linseed oil the cloud points are higher and continue to rise as the heating continues. In this case the polymerization raises the cloud point faster than ester interchange lowers it.

Effect of Catalysts

Many materials are known to accelerate ester interchange, and acid catalysts have frequently been used (*2*). Lead soaps are frequently used in varnish and resin preparation for this purpose. Figure 7 shows that the cloud point is lowered when litharge is added to the oil-resin mixture. These varnishes were made from linseed oil and a rosin-modified phenolic resin and heated under the same conditions with and without the addition of litharge.

Pure Phenolic and Hydrocarbon Resins in Drying Oils

Two commercially pure (100 per cent) phenolic resins were heated with alkali-refined linseed oil. The cloud points of the oil-resin mixture are shown in Figure 8. In this case the cloud point increases on heating, owing to the poorer solvent power of the drying oil as it polymerizes. These curves give no indication of a chemical reaction with the drying oil, as has been claimed

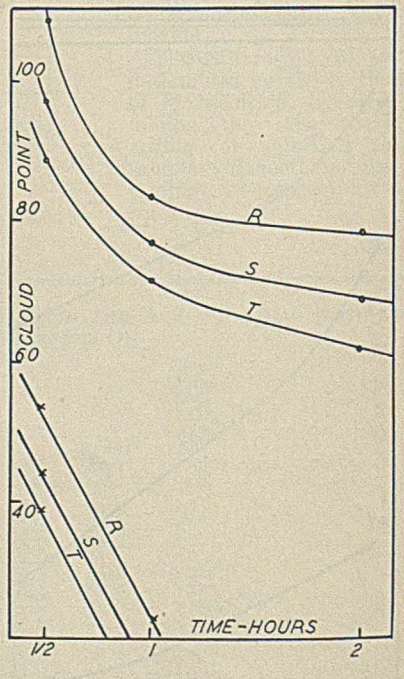

FIGURE 9. CLOUD POINTS OF MODIFIED PHENOLIC RESINS IN LINSEED OIL

○. 2 parts of resin, 1 part of oil
×. 1 part of resin, 2 parts of oil

(4), since there is no time where the cloud point decreases, which would be expected if chemical combination with the oil occurred.

Two hydrocarbon resins, a low molecular weight polystyrene and a coal-tar resin, were heated with linseed oil. The cloud points at various times are shown in Figure 8. The polystyrene–drying oil mixture increases in cloud point in very much the same manner as do the pure phenolic resins. The coal-tar resin decreases in cloud point as heating is continued. This is not believed to be the result of chemical combination with the oil, but rather depolymerization of the larger polymers in the resin. It is known that resins of this type depolymerize at temperatures employed in this experiment (275° C.).

Cloud Point of Resin and Cloud Point of Resin–Drying Oil Mixtures

It has been found that the cloud point of a resin in mineral oil is a good index of its behavior when heated with drying oils. When resins of the same type are compared, resins which give high cloud points in mineral oil will give high cloud points when bodied with drying oils. Figure 9 shows three resins of the same type, R, S, and T (modified phenolic), which were heated with linseed oil. The cloud points in drying oil are in the same order as the cloud points of the resins in mineral oil.

When resins of different classes are compared, the cloud point is of less utility in predicting their cloud points in drying oils. However, if resins of different classes of the same cloud point are compared, the resin which has the greatest reactivity with the drying oil will usually have the greatest solubility and lowest cloud point in the drying oil.

Application of Cloud Point Method

The cloud point determination has been found exceedingly useful in classifying a wide variety of synthetic and natural resins. Its utility extends over a much wider range of resins than has been cited here. Often it is necessary to choose a mineral oil of better or poorer solvent power to match the solubility of the resin.

Some properties of oleoresinous vehicles may be correlated with their cloud points, and the degree of "meta-stability" measured. This property is of considerable importance in controlling the working properties of paints (6).

This report has been offered to suggest the utility of the cloud point technique in studying the solubility characteristics of resins and of resin-oil combinations. It is hoped that the method will find wider utility.

Acknowledgment

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Some Assays of Provitamin A Carotenoids

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An endeavor is made to present the problem of provitamin A carotenoid determination in plant materials in broad perspective. Results on carrots, spinach, tomatoes, apricots, peaches, and prunes are discussed, particularly with reference to preservation practices. The problem is essentially that of β-carotene determination, because α-carotene is at best a minor contributor, and cryptoxanthin is important only in special instances. Each material presents a different problem, sometimes of extraction, but more frequently in effective removal of interfering pigments without vitamin activity. The answer then is found in selection of suitable adsorbents with the proper solvent, and separation is made on small Tswett columns over which β-carotene or cryptoxanthin may be passed without adsorption, but where other pigments are effectively adsorbed.

Because data on vitamins are so frequently interpreted in terms of human needs, and much effort is being devoted to vitamin surveys at the behest of state nutrition committees, the significance of the various findings is briefly evaluated in terms of bioassay and nutrition.

A CONSIDERABLE number of carotenoid assays have been made in this laboratory on certain plant products for a variety of reasons. With carrots and spinach, dehydration problems were involved. In the case of apricots and peaches, dehydration does not yield a product acceptable to the trade, unless the fruit has been exposed to the sun, usually for twelve hours or so, after sulfuring. The nonirradiated product is dull and opaque, rather than translucent, and with peaches in particular, a pale yellow. In cling peaches for canning, an acceptable grading test for maturity was also desired. A further problem with peaches involved the presence of lycopene (23), found in certain European varieties but not here (10). Tomato products were included because of the presence of large amounts of lycopene, relative to carotene, and the authors were interested in attempting a rapid evaluation of the carotene content of juices. Finally, analyses were made on prune samples, thereby including some of the more staple dietary sources of provitamin A carotenoids of plant origin.

These various reasons have been subordinated in assembling the data in an attempt to place in broad perspective the problem of determining provitamin A carotenoids. Difficulties fall into two categories, first, those involving the natural complexity of the pigment mixture, and second, possible changes which the mixture may undergo, either by formation of additional chromophores, as in silage (5), or by bleaching of ex-

TABLE I. EFFECT OF DRYING TEMPERATURE ON RETENTION OF CAROTENE IN DEHYDRATED SPINACH

Drying Temperature		Drying Time	Retention of Carotene	
			Blanched	Not blanched
° F.	° C.	Hours	%	%
180	82	3.00	89.4	85.9
180	82	3.00	88.7	85.0
180	82	3.25	....	81.7
160	71	4.00	73.1	77.3
150	65.5	4.75	70.2	71.4
140	60	5.75	62.8	57.2
120	49	8.00	....	66.6
120	49	8.00	69.5	65.5

tracts, as in certain legumes (*21*, *25*). Such sources of error may be found in many other products, though normally in less aggravated form.

The materials considered here may be expected to contribute, as normally consumed, one tenth or more of the estimated daily adult requirement of about 5000 international units, 3 mg. of β-carotene. It has seemed worth while to discuss these data briefly in relation to known bioassays, for their nutritional significance.

So far as is known, three carotenoids supply virtually all the vitamin A that terrestrial mammals derive, directly or indirectly, from plant sources—namely, β-carotene, α-carotene (a minor contributor), and cryptoxanthin (important only in special cases). Little is known of requirements for insects (*2*) or other forms of animal life. The mussel is an example of other possibilities (*18*). The other carotenoids of known vitamin A potency for mammals either are of limited and local distribution or they occur in amounts inadequate for dietary significance, and in general it may be said that the problem is essentially one of determining β-carotene except where yellow corn meal is a staple food, and in certain fruits such as the peach.

General Considerations

As recognized by Fraps, Kemmerer, and Greenberg (*4*), no one method is applicable to all cases. A sound procedure demands that the nature of the pigment complex be understood in each product examined. For the most part, as Peterson (*17*) points out, the general problems of extraction, isolation, and determination have not changed greatly. The so-called invert soaps may be used more widely and be placed on a quantitative basis. Solutions of alkyl benzyl ammonium salts have been used by Kuhn, Bielig, and Dann (*8*) to isolate carotenoids from chloroplastin and carotene from carrots.

When an analysis is reported for nutritional use, the extent of absorption and utilization of carotene as distinct from vitamin A (*24*) must be considered, and in such cases a high degree of refinement in method is meaningless. This includes differentiation of α- and β-carotenes in mixtures which rarely contain more than 5 to 15 per cent of the α-component. If such analysis be warranted, it should be accomplished spectrophotometrically, setting up the necessary simultaneous equations for a 2-component mixture on the basis of Beer's law. Measurements should not be made on the individual α- and β-carotenes separated by adsorption because the percentage recovery of the more weakly adsorbed component is usually substantially greater than of the one more strongly held.

Experimental

Extraction procedures have been developed with a view to obtaining suitable extracts for spectroscopic examination without use of unwieldy quantities of solvents, the health menace of which is often overlooked. Although various improvements have been suggested (*1*, *12*, *16*, *25*), the authors have for the most part retained the older procedures. As the principles are well known, brief notes will suffice in some cases.

SPINACH. The most reproducible results for fresh spinach are obtained by brief immersion of the 5-gram sample in boiling water. Dehydrated spinach should be thoroughly soaked prior to grinding with sand and acetone. A 0.5-gram sample may thus be extracted with four 25-ml. portions of acetone, transferred to approximately 30 ml. of petroleum ether, and saponified, and xanthophylls removed in the usual way. The petroleum ether is then washed, dried with anhydrous sodium sulfate, made to volume (50 ml.), and filtered in a closed system to obtain a brilliantly clear solution. The concentration of β-carotene is determined by measuring the transmission of the unknown at 480 mμ with a Bausch & Lomb visual spectrophotometer, and a similar measurement is made for a standard β-carotene sample in the same solvent.

The influence of soaking and sample size is clearly shown in the following results, expressed as per cent of β-carotene on a moisture-free basis (*3*). (Results in this paper are reported on a moisture-free basis except for carrot juice and tomato juice, which are more conveniently reported in mg. per 100 ml.) Sample not soaked, 0.5 gram, 0.026; soaked, 2.5 grams, 0.023; 1.5 grams, 0.026; 1.0 gram, 0.029; 0.5 gram, 0.036, 0.036. These compare with values on the same lot, fresh, of 0.038. The fresh samples, ten in number, taken from a local market, varied from 0.027 to 0.045 per cent on the same basis. The temperature of dehydration affected retention considerably. The shorter drying times, at higher temperatures, showed retention of 80 to 90 per cent of the total carotene; the lower temperatures required up to 8 hours, with retention of about 65 per cent. A preliminary steam blanching for 1 minute was also slightly beneficial. Results are shown in Table I. Losses on storage after 2 months at 0° were negligible, at room temperature from 5 to 12 per cent, and at 30° from 40 to 50 per cent. Blanching did not significantly affect losses on storage, though this step was very significant with carrots. Several of the final extracts ready for spectrophotometric analysis were checked by adsorption on magnesium oxide and magnesium carbonate columns for additional chromophores, but none were found.

CARROTS. Analyses were made on fresh and dehydrated carrots (the latter containing 2 to 4 per cent moisture) and on canned carrot juice.

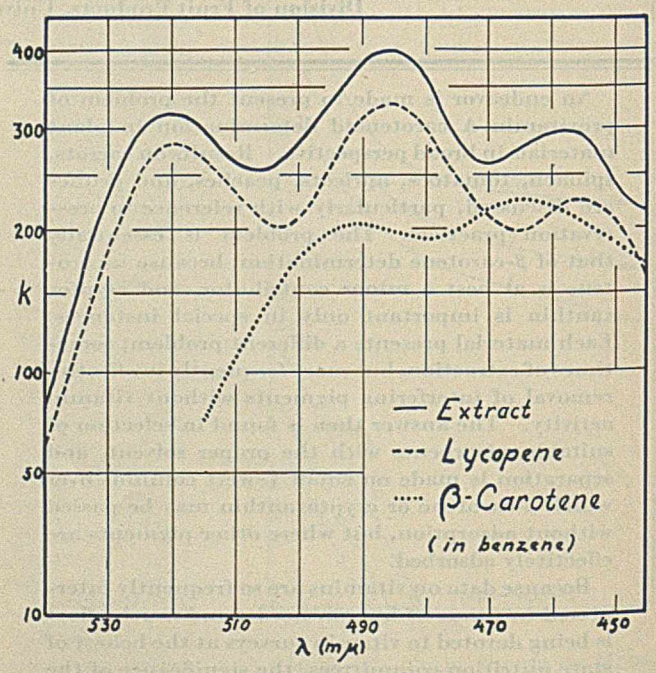

FIGURE 1. ABSORPTION CONSTANTS FOR LYCOPENE, β-CAROTENE, AND TOMATO EXTRACT IN BENZENE

Absorption coefficients, k, are given for pure pigments, plot for extract is in terms of E—i. e., kc.

Blanching for 3 minutes in boiling water prior to dehydration was beneficial to carotene retention. The dehydrated material, originally sliced uniformly in disks 3 mm. thick, is not easily extracted without soaking.

Five grams of fresh, or 0.5 gram of dried, carrots are immersed in 5 ml. of boiling water for 3 minutes, and the residue is extracted as with spinach. In 4 extractions with a total of 75 ml. of solvent the residue is colorless. The extracts are partitioned successively with 50 and 25 ml. of petroleum ether. The second extraction is purely precautionary. The combined petroleum ether layers are washed with water, then 3 times with 85 per cent methanol, in 50-ml. portions. This is followed by water, 3 times (in 50-ml. portions), and the solution is dried with 2 grams of anhydrous sodium sulfate, and made to volume (100 ml.). Five milliliters of canned carrot juice are refluxed for a few minutes with 20 ml. of saturated potassium hydroxide in methanol. The alkali apparently aids disintegration of the finely comminuted particles present. Three 25-ml. portions of acetone suffice for extraction.

It is seen (Table II) that no difficulty is presented in obtaining representative samples from 5 or 6 carrots, total weight about 500 grams.

Table II. Carotene in Carrots, Moisture-Free Basis

	%
Fresh	0.105, 0.104, 0.110, 0.099
Dehydrated, unblanched	0.067, 0.067
Dehydrated, blanched[a]	
Initial	0.105, 0.106[b]
After 40 days' storage[c]	0.104 (0°), 0.101 (22°), 0.079 (30°)

[a] Blanching involves 3 minutes' immersion in boiling water, prior to dehydration.
[b] On basis including solids lost in blanching, this value becomes 0.086.
[c] After 4 months the unblanched samples at room temperature have fallen to 0.055%, the blanched to 0.084% carotene.

Some carotene values in certain laboratory-canned carrot juice samples are as follows: 13.3, 12.8; 11.9, 11.8; 10.6, and 10.5 mg. per 100 ml. of juice. Three commercial brands analyzed gave 9.0, 5.6, and 2.5. The last-mentioned had been strained and, as would be expected, this removed most of the carotene in the pulp. Assuming an average of about 15 per cent solids in these juices, we find the carotene comparable with that of the fresh carrot in most cases. Adsorption tests again revealed the absence of additional chromophores in the final preparations.

Tomatoes. In Figure 1 is shown the absorption spectrum of lycopene, β-carotene, and a tomato extract in benzene solution. It is evident that the lycopene spectrum effectively masks the presence of other pigments. Owing to traces of other carotenoids, the extract cannot be treated as a 2-component system involving only lycopene and β-carotene. Even if this were the case, it is clear by inspection that at least 95 per cent of the absorption is due to lycopene and an error of 1 per cent in the estimation of this component means an error of 20 per cent in the carotene. In the case of the fresh tomato, on which some analyses had been made for another purpose (*6*), considerable difficulty was encountered in securing a representative sample. Wherever possible, the sample should be withdrawn from not less than several hundred grams of macerated well-mixed pulp, and a 50-gram sample is not too small. This requires considerable solvent for satisfactory extraction. In juices, the sampling error is negligible.

Samples of a commercial brand (10, 20, and 30 ml.), well shaken, were extracted exhaustively with acetone (total required about 100 ml. per 10 ml. of juice) and the extracts were transferred to 25 ml. of benzene. The benzene was then thoroughly washed (5 times, 50-ml. water portions), dried with anhydrous sodium sulfate, and adsorbed on magnesium oxide and silica (1 to 1 by weight). The column was 1.5 cm. in diameter and 6 cm. long. The β-carotene appeared in the eluate, and was virtually unadsorbed, and the column was washed with more benzene until this fraction had passed completely into the eluate. This was then made to volume, and the transmission at 490 mμ measured. The results were 0.535, 0.538, and 0.539 mg. per 100 ml. of juice.

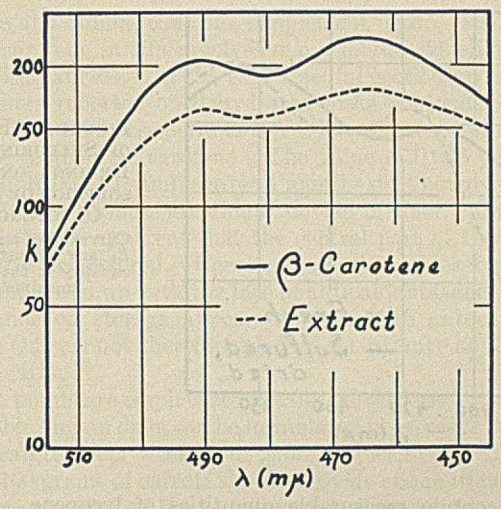

Figure 2. Absorption Constants for β-Carotene and Apricot Extract in Benzene

Results on four commercial brands of juice were 0.535, 0.52; 0.43, 0.43; 0.60, 0.60; and 0.575, 0.60 in mg. of β-carotene per 100 ml. of juice.

On whole tomatoes, results are more variable. A single lot, purchased on the market, may be considered. Sample 1 from 2 halves of a tomato irregularly ripened gave values of 0.0107, 0.004 per cent; two other samples, as homogeneous as practicable, gave 0.0075, 0.0089; and 0.0059, 0.0051 per cent, on a moisture-free basis. Three tomato pastes (of known solid content) gave values of 0.0137, 0.0135, and 0.0127 per cent, dry weight basis.

Apricots. In Figure 2 are given absorption curves for an apricot extract and for β-carotene in benzene. The xanthophylls, lycopene, and γ-carotene do not amount to 10 per cent of the total carotenoid, but again the mixture is too complex for simple spectroscopic assay, though errors in the β-carotene would be small in comparison with a similar assay on tomatoes.

The apricots, fresh or dry, are passed through a fine food chopper, and 10 grams of fresh or 1.0 gram of dried are extracted as for tomatoes. The β-carotene in the eluate is made to volume and estimated as before.

Table III. Carotene in Apricots, Moisture-Free Basis

	%
Fresh (Royals)	0.0212, 0.0200
Sun-dried	0.0168, 0.0164
Dehydrated	0.0165, 0.0160
3 hours ultraviolet then dehydrated	0.0198, 0.0194, 0.0195, 0.0190
Sun-dried, unsulfured[a]	0.0138, 0.0132

[a] From a different lot of apricots. All other dried samples were sulfured.

The effect of light is not to be simply explained. Whether the apricots are sun-dried or dehydrated, carotene losses are comparable (Table III), regardless of the acceptability of the color of the final product to the trade. The ultraviolet source conceivably exerts its effect by rapid inactivation of enzymes, but this phase will not be discussed here.

Peaches. Carotenoids of the following varieties have now been examined: (freestone) Elberta, Muir, Lovell, Foster, Late Crawford, (cling) Phillips, and Halford. The first three have been examined after dehydrating and also sun-drying, and Elbertas after canning. In previous work, carotenoids were isolated from vacuum-dried peach powder (*10*) and owing to low yields only a small quantity of fresh Lovells were included for comparison. Varieties grown here apparently

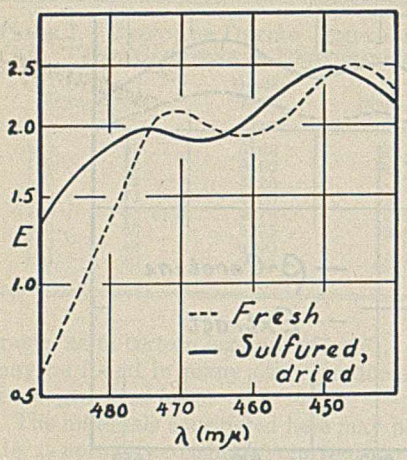

FIGURE 3. EFFECT OF SULFURING AND DRYING ON ABSORPTION OF PEACH CAROTENOIDS

Curves in terms of *E* are adjusted for equality at their main maxima.

do not contain measurable quantities of lycopene. When added to the extent of 3 per cent of the pigment mixture, it can be detected, but it cannot comprise 9 or 10 per cent of the mixture, as observed in European varieties (*23*). As a result of the more extensive work, certain modification of previous findings is necessary. In the first place, mature fresh and canned fruit yields an extract with lutein- or xanthophyll-type absorption maxima. This is also true of fruit sun-dried in the absence of sulfur, but the dried sulfured product is relatively much richer in β-carotene and similar components, as shown by a shift in the absorption maxima (Figure 3). In drying, the brunt of the loss is borne by the xanthophyll fraction.

Secondly, cryptoxanthin is not present in the amount previously indicated (*10*) in all of the above varieties. The carotenoids fractionated from fresh Fosters, for example, gave essentially the same chromatogram, but the supposed cryptoxanthin gave absorption maxima about 3 mμ too far to the blue, and it was more weakly adsorbed than genuine cryptoxanthin on a test column of magnesium oxide in benzene. Finally, as a result of studies on immature fruit, it became apparent that a labile component was present, having absorption characteristics similar in some respects to neoxanthin (*21*)—i. e., with maxima further to the blue than those of lutein. With inclusion of this labile fraction, the β-carotene fraction is less than 10 per cent of the total carotenoid in the fresh peach, and about 20 per cent in the sulfured dried peach, where much of the xanthophyll has been destroyed, including all the very labile fraction. Excluding this latter fraction, an approximate estimate of total carotenoid may be made within 10 to 15 per cent by the following procedure:

A petroleum ether extract is prepared from 10 grams of fresh, or 3 to 5 grams of dried peaches, in the same way that the benzene solutions were prepared from tomatoes and apricots. The absorption maximum for the band at the longer wave length is between 472 and 481 mμ, depending upon whether xanthophylls predominate. The total concentration is estimated by assuming an average specific absorption coefficient of 220, in liters per gram cm., at the individually located maximum for each extract. In actuality, after transmission measurements had been made, the solution was adsorbed on a column of magnesium carbonate (Merck), and the β-carotene was collected in the eluate very rapidly as it is unadsorbed. The details are thus in essence identical with those for carotene in apricots, except for solvent and adsorbent differences. Although these data are available, it is thought of greater interest to report the total carotenoid, based on the assumption just noted, because the effect of treatment causing heavy losses in total pigment is not reflected nearly so strikingly in the carotene itself. The β-carotene may be sufficiently precisely estimated as 10 per cent of the total for fresh, and 20 per cent for the sulfured dried, from the values in Table IV.

Results with fresh, green, borderline, and dead ripe Halford and Phillips peaches gave values of the same order of magnitude as for the fresh freestone varieties, but were 20 to 40 per cent lower for green fruit, where chlorophyll was readily detected.

PRUNES. Trade-dried prunes with 18 per cent moisture or less provide a peculiar extraction problem. The fresh Imperial prune is as readily extracted as peaches or apricots. Nor is any difficulty experienced with the processed dried prune, *ca.* 30 per cent moisture, but prune powder *ca.* 3 per cent, and dried prunes from the yard *ca.* 18 per cent moisture or less yield no coloring matter to acetone or petroleum ether. A yellow solution is obtained with 95 per cent ethanol but the coloring matter is not extracted with petroleum ether. A similar difficulty in preparing extracts from prunes was found in the Department of Home Economics and was obviated by soaking in water. A brief immersion of the sample for 1 to 2 minutes in boiling water is sufficient.

The prune was not investigated in great detail, but it was clear from the absorption curve for the total carotenoid extract in petroleum ether that the mixture approximated the same general picture as in peaches. Prunes are dried whole, usually after a brief lye dip. Dehydration is more common than in other fruits because the crop is harvested later in the season and there is in general poorer drying weather. Normally they are not sulfured.

Analyses reported here are based on the procedure for peaches, with samples from 2 to 8 grams; the total carotenoid in petroleum ether is passed over a short column (1.5 × 5 cm.) of magnesium carbonate, and the β-carotene in the eluate is made to volume and estimated. Analyses on a sample of fresh Imperial prunes gave 0.0041, 0.0037 per cent β-carotene on a moisture-free basis. Two commercial brands (processed) gave 0.0010, 0.0011, 0.00105; 0.0015. Another sample of the second brand gave 0.0025, 0.0030. The former were evidently older packages, and the flesh was definitely browner than in the second sample.

TABLE IV. ESTIMATED TOTAL CAROTENOID IN PEACHES, MOISTURE-FREE BASIS

	Elberta	Muir	Lovell
	%	%	%
Fresh[a]	0.0154	0.0171	0.025
	0.0185	0.0189	0.024
Sun-dried, unsulfured[b]	0.0078	0.0079	...
Sun-dried, sulfured[c]	0.0053	0.0050	0.0058
Dehydrated, sulfured[c]	0.0055	0.0040	0.0058

[a] Approximate ratios of xanthophylls to carotene: [a] 10, [b] 6, [c] 5, to 1.

Discussion

An important source of error lies in the labile nature of carotene. It not only involves the reference standard, but it also impairs the value of a checking procedure where known amounts are added to an unknown sample. The spectroscopic constants for carotene, melting point 182–3°, can be reproduced within 1 to 2 per cent at the maxima.

Little need be added with respect to methods. Where adsorption is necessary, the authors have preferred to use a readily available adsorbent (Micron brand magnesium oxide, with Hyflo-Supercel as diluent, and Merck's magnesium carbonate) in actual Tswett columns of small dimensions, and to employ a solvent which in the individual cases permits the desired separation and allows the fraction in which they are interested—the β-carotene—to pass through unadsorbed.

The underlying assumption is that there is no loss of the completely unadsorbed pigment. Pure β-carotene in benzene on magnesium oxide and in petroleum ether on very dry magnesium carbonate is in actuality slightly adsorbed. Recoveries range from 75 to 95 per cent, when known quantities are added to test columns, depending on the amount added and the rapidity of elution. There is no adsorption, however, of this pigment from crude extracts under otherwise comparable conditions, owing to the presence of colorless impurities which affect the development of the chromatogram. Where

β-carotene was added, not in excess of that present in the aliquot of the unknown, recoveries of 95 to 97 per cent were obtained. If more than double the amount in the unknown was added, between 80 and 92 per cent was recovered, and seemingly the value was determined in part by the additional length of time the zone was in contact with the adsorbent.

It is generally recognized that adsorbents are highly variable in property, and the magnesium carbonate, for example, will work satisfactorily only for relatively small quantities of pigment, per unit weight of adsorbent. The authors suspect that contamination with traces of ethanol or similar solvents is, however, the most frequent cause for unsatisfactory results. They can only emphasize that these adsorbents have served their purpose, and for general applicability, they feel the technique to be more desirable than that involved in adding adsorbent directly to the solution (*4*). Where it has been positively identified, cryptoxanthin may be readily washed off a column of magnesium carbonate by changing the eluting solvent from petroleum ether to benzene. In view of the differences in this fraction in different peach varieties, they have not included data so far obtained. Some preliminary experiments with corn meal and with genuine cryptoxanthin indicate that there should be no serious difficulty in applying the procedure to the peach and prune.

By courtesy of A. F. Morgan, the authors have compared their data with bioassays on similar fruit made during the last decade (*13*, *14*, *15*, *19*). There are no serious discrepancies, though losses of carotene in the authors' dried apricots are much less severe. In large measure this appears to be due to small scale operations, to a short time of storage (one week), and particularly to prompt sulfuring after cutting and pitting. In all yellow peaches tested by bioassay, the differences in carotene content are within 30 per cent of each other. In a tomato paste from the "pear tomato", there was only an 8 per cent difference in the carotene. The authors have therefore not been greatly interested in varietal differences. The gene concerned with plastid pigments affects chiefly the lycopene, according to LeRosen, Went, and Zechmeister (*9*).

A more serious but well-known discrepancy lies in the relative inefficiency of yellow vegetables, notably carrots, when compared with green sources (*20*)[1]. It is consequently not evident that varietal studies designed to increase the carotene content will have utilitarian value. Within a given class of foods—e. g., dried fruits or green vegetables—there seems to be satisfactory agreement between data such as the authors' and bioassays, which is lessened when different classes are compared.

It may be deduced from the arguments of Wald, Carroll, and Sciarra (*24*) that a daily adult requirement of 5000 units—i. e., 3 mg. of β-carotene—is based on a wrong unit of time, because the daily diet is too variable; a weekly basis is more stable. Also, the excretion of single large doses ordinarily begins within 1 to 2 days and is at a maximum from 3 to 5 days, ceasing after 5 to 7. In any event, absorption of carotene must be distinguished as a process not necessarily involving utilization, and even the addition of bile salts (*7*) cannot be assumed to ensure this.

It is unfortunate that bioassays on a given fruit are usually made with A-deficient rats. The results are in practice interpreted as bioassays for man or livestock rarely at such an advanced stage of depletion, and no allowance is made for differences in the respective digestive tracts and their capacities for making the carotene available. It has been pointed out to the authors that in the poultry industry there has been a rather precise standardization of poultry needs based on β-carotene and alfalfa, the chief supplement used. The retention of carotene *in situ* involves many factors which are not easily disentangled. In the experimental section on carrots, the authors reported on dehydrated disks originally 3 mm. thick. This thickness permits rapid dehydration, but a thinner slice retains less carotene. The value of 0.055 per cent for the unblanched sample after 4 months does not give a true picture. The surfaces are almost devoid of color. On soaking, it becomes apparent that the central part of the carrot disk is still well colored. Carotene retention, at least in some instances, is tied up with the fate of antioxidants, and its disappearance on storage appears to follow an autocatalytic curve. One cannot therefore place great reliance on a single determination.

These points are emphasized because of the ease with which reasonable human diets can be formulated in excess of 20 to 25 mg. of carotene per week from plant sources alone. For example, 100 grams of carrots should provide about 10 mg., 100 grams of spinach 3 to 5 mg., 100 grams of prunes 1 to 3 mg., 500 ml. of tomato juice or its equivalent 2 to 3 mg., etc., but these figures do not have necessarily the same nutritional significance. There are omitted from this survey many good sources such as sweet potatoes (*11*), pumpkins, etc., more intermittently consumed, and green vegetables except spinach. Minor sources such as orange juice (*22*) may in the aggregate represent a considerable fraction of the requirement.

The problem of greater urgency therefore is physiological in nature, and that for the food technologist is at present relatively simple, that he retain in a processed food a reasonably high proportion of its original endowment of carotene. Here the broad underlying principles are well appreciated: in canning, exclusion of air, and in dehydration, blanching and storage at low temperature or in inert gases.

Acknowledgment

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Adsorption of Vapors by Crystalline Solid Surfaces

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The important problem of the determination of the surface area of a finely divided powder has been considered by the intercomparison of values obtained from the adsorption of water, propyl alcohol, and benzene vapors at 25° C., and the adsorption of nitrogen gas at −195.5° C. The solids utilized were the common pigments, titanium dioxide, stannic oxide, zinc oxide, and pulverized quartz.

It is concluded that reliable values of the surface area of a crystalline powder may be obtained from vapor adsorption data at room temperature. Thus the area per gram of one of the titanium dioxide powders obtained from the adsorption of nitrogen at −195.5° C. was 8.3 square meters; the adsorption of water vapor at 25° C. gave 9.1, propyl alcohol 8.9, and benzene 9.3 square meters, respectively. A preliminary value from the electron microscope for this powder gave 10.7 square meters per gram.

NUMEROUS reports of vapor phase adsorption on porous solid structures such as the charcoals and silica gel exist in the literature (*6*, *8*). Studies of adsorption on plane crystalline surfaces have been generally avoided, however, owing to the extremely small adsorption that is observed when powders prepared by ordinary methods are utilized. As a consequence of recent industrial developments, nonporous crystalline powders of high specific area have become available, and the need for suitable methods to measure surface areas of such materials has arisen. In 1931, the study reported in this paper was begun with this purpose in view. An experimental investigation of the adsorption of water, propyl alcohol, and benzene vapors by crystalline titanium dioxide, quartz, zinc, and stannic oxides was completed. Langmuir isotherms were observed for the adsorption of propyl alcohol on all these solids up to three-fourths saturation pressure of the vapor. The adsorption of water, however, gave a sigmoid-shaped curve to which the simple Langmuir form could not be fitted.

More recently other workers (*1*, *9*, *10*, *11*) have found an S-shape for the isotherm for water, and sometimes other vapors. This same type of isotherm was also found in the important researches of Brunauer and Emmett (*3*) for the low-temperature adsorption of nitrogen, oxygen, argon, carbon monoxide, carbon dioxide, and butane on many crystalline solids. An adequate theory of this type of isotherm has been given (*4*). The sigmoid form is assumed to result from the building up of a polymolecular film by the adsorption of molecules held by essentially van der Waals forces. Langmuir's kinetic condensation-evaporation mechanism is generalized, and a variety of analytic expressions result. The theory has been extended (*2*) to include other species of adsorption isotherms, including those types observed for adsorption on porous solids. A valuable result of this work has been that a simple and reliable procedure for the determination of the surface areas of powders has been established.

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The theory of Brunauer, Emmett, and Teller (*4*) has been applied to the authors' data and surface areas have been computed. Striking agreement between values obtained from the van der Waals adsorption of nitrogen gas at −195.5° C., and values from the adsorption of water, propyl alcohol, and benzene vapors at about 25° C. has been found. A quantitative measurement of the adsorption of water vapor at room temperatures may afford a convenient procedure for the evaluation of surface area of powders.

Experimental

The adsorption of vapors on powders of metallic oxides was measured in the apparatus shown in Figure 1.

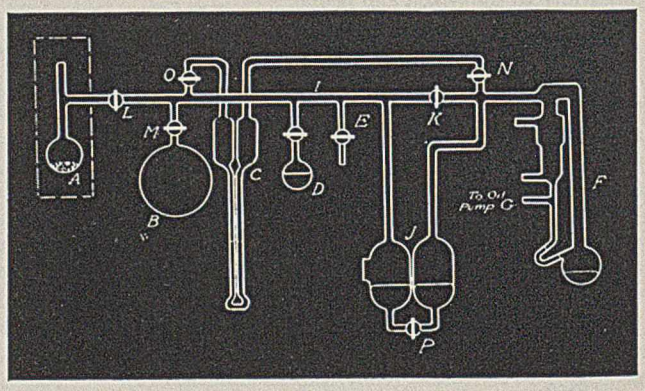

FIGURE 1. APPARATUS FOR STUDYING ADSORPTION OF VAPORS ON CRYSTALLINE NONPOROUS POWDERS

Pressure difference constitutes the measuring scale. The volume of flask *B* was determined directly; from this the volume of the remainder of the apparatus, except that of the adsorbing flask, *A*, was determined by measuring the new pressure of a little air which was allowed to expand from *B*, at a known low pressure, into the apparatus. Boyle's law was used to calculate the new volume. The volume of *A* was measured directly by filling with water at a known temperature and determining the weight difference. Pressures of water vapor and air were measured with a *n*-dibutyl phthalate manometer, *C*. Stanolax, instead of phthalate, was used to measure the pressures of vapors of *n*-propyl alcohol and benzene. Vapor was admitted to the apparatus from flask *D*. A mercury vapor pump, *F*, and a Hyvac oil pump at *G* were used to evacuate the apparatus.

After a series of experiments with these vapors was completed, the optical monometer, *J*, of the type described by Carver (*5*) was attached to the apparatus to study the vapor of butyric acid. Only a few preliminary experiments were done with this vapor.

The titanic oxide was supplied by the Titanium Pigment Co., Inc. The silica was made by pulverizing quartz. The zinc oxide and stannic oxide were reagents of C. P. quality. Each powder was heated in a high vacuum to temperatures which ranged from 350° to 450° C., depending on the substance, for about 10 hours (usually overnight) preparatory to vapor adsorption. Permanent gases in the liquids were pumped off before each experiment was started. The liquids used were ordinary distilled water, *n*-propyl alcohol of C. P. quality which was redistilled, and recrystallized benzene of C. P. quality. Experiments were run in duplicate.

Vapor was admitted to the apparatus while stopcocks *K* and *L* were closed. After the pressure was read, *L* was opened and adsorption was allowed to proceed until equilibrium was reached. The system was considered to have reached equilibrium when the manometer level remained constant for 15 minutes. *L* was then

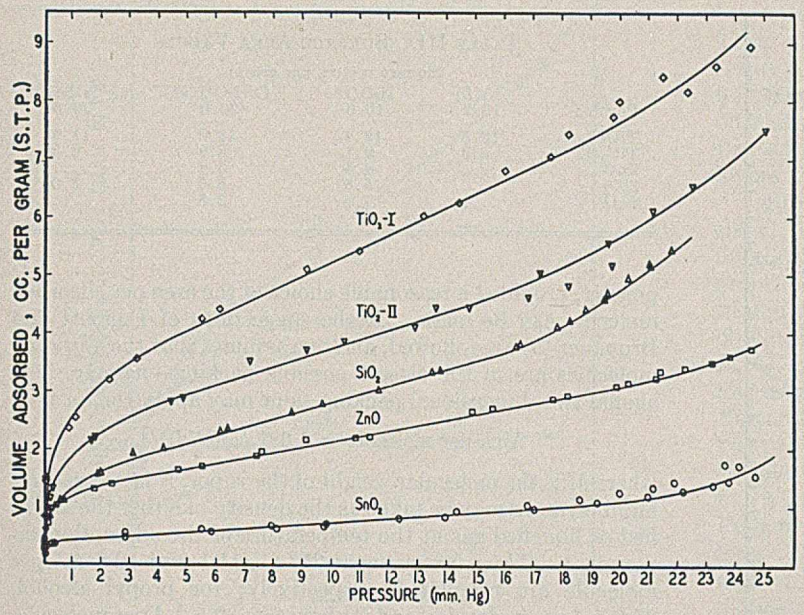

FIGURE 2. ISOTHERMS FOR ADSORPTION OF WATER VAPOR ON CRYSTALLINE POWDERS AT 25° C.

TABLE I. ADSORPTION OF WATER ON TITANIC OXIDE

(t = 31° C.)

P, mm. Hg	$n \times 10^3$ per gram	v, cc. at S. T. P.
0.007	1.5	0.037
0.023	3.6	0.90
0.13	6.6	1.65
0.84	10.4	2.60
2.21	14.1	3.52
5.44	18.8	4.70
9.08	22.8	5.70
13.12	26.8	6.70
16.00	30.2	7.55
18.20	33.1	8.28
19.95	35.6	8.90
21.48	37.6	9.40
33.38 = p_0		

closed and the process was repeated. The calculations are based on the perfect gas law, $PV = nRT$, which is deemed sufficiently accurate for vapors at these temperatures as long as one works appreciably below the vapor tension of the liquid. At the highest pressure attained, $p/p_0 = 0.80$, unquestionable accuracy cannot be expected.

The number of moles of vapor adsorbed per gram of powder and the volume of adsorbed vapor under standard conditions were calculated with due regard to the quantity of vapor present initially and finally in each section of the apparatus. A typical set of results is given in Table I.

Blank experiments were performed to show that no observable decrease in pressure resulted from absorption of any vapors by the manometer fluid or the stopcock grease. Curves of duplicate experiments are identical at low pressures. In some cases they diverge at higher pressures, in part, no doubt, because errors are additive under the method adopted. There is no divergence below a relative pressure (p/p_0) of 0.25. Desorption points were obtained on each isotherm, and agreed satisfactorily with those determined in adsorption. Desorption points are not shown in the figures, however.

Results

The adsorption of water vapor on five powders is shown in Figure 2. Typical S-shaped isotherms have been obtained, the low-pressure portion of the isotherm being concave to the pressure axis, the higher pressure region convex to the pressure axis, and the intermediate region approximately linear with respect to pressure. The data have been fitted to Equation A (*4*).

$$p/v(p_0 - p) = 1/v_m c + (c - 1/v_m c)p/p_0 \quad \text{(A)}$$

where, for the authors' data, p = pressure in mm. of mercury, p_0 = saturation pressure of the vapor, v = volume of vapor adsorbed in cc. at standard temperature and pressure, v_m = volume of vapor adsorbed when the entire adsorbent surface is covered with a complete monomolecular layer, $c = (a_1 b_2/b_1 a_2)\ \exp.\ (E_1 - E_L)/RT$, where a_1, b_1, a_2, and b_2 are constants, E_1 = heat of adsorption in the first layer, and E_L = heat of liquefaction of the vapor in cal. mole^{-1}. A plot of Equation A for water vapor on the adsorbents is shown in Figure 3. Between relative pressures of 0.05 and over 0.35 the plots are closely linear. From them the values of v_m and c were obtained by the methods of least squares. From c approximate values for $E_1 - E_L$ were calculated (Table II).

The adsorption of propyl alcohol vapor on the same crystalline powders is shown in Figure 4, and in Figure 5 are shown the data for the adsorption of benzene vapor on titanium dioxide I, titanium dioxide II, and quartz. These isotherms resemble the well-known Langmuir type over most of their course. Increased adsorptions at high relative pressures (benzene), however, may suggest the beginning of a polymolecular film. The data, when plotted according to Equation C (*4*),

$$p/v = p_0/v_m c + p/v_m \quad \text{(C)}$$

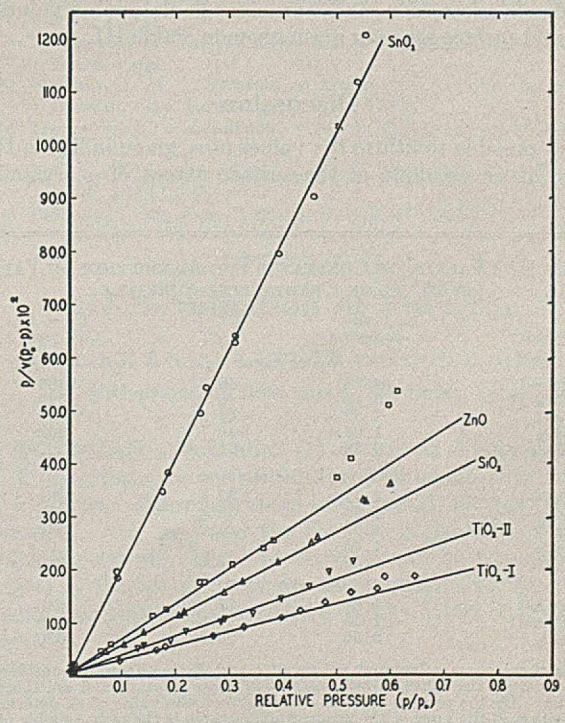

FIGURE 3. ADSORPTION DATA FOR WATER VAPOR PLOTTED ACCORDING TO EQUATION A

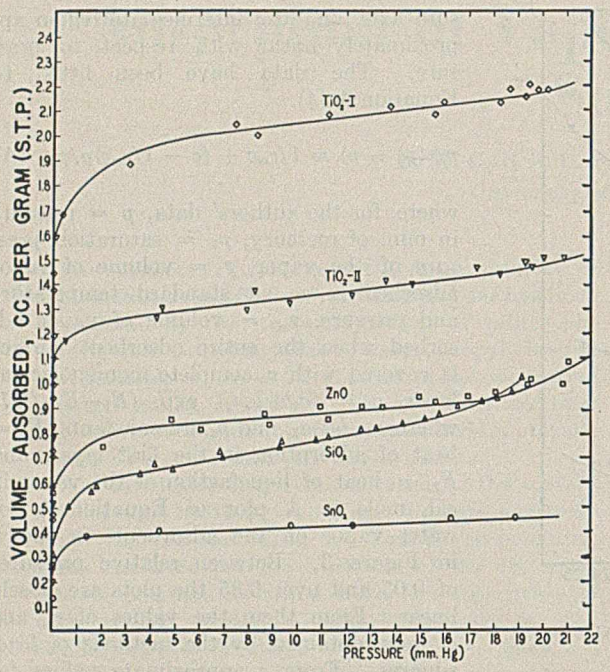

FIGURE 4. ISOTHERMS FOR ADSORPTION OF PROPYL ALCOHOL VAPOR ON CRYSTALLINE POWDERS AT 25° C.

gave good straight lines. Values of c and v_m calculated by least squares are listed in Table II.

In addition to the vapor adsorptions, isotherms for the adsorption of nitrogen gas at −195.5° C. were determined for both samples of titanic oxide. The apparatus in which these experiments were conducted was patterned closely after that described by Emmett and Brunauer (*3*), and their suggested technique was followed. Temperatures were accurately determined by a calibrated copper-constantan thermocouple in conjunction with a White double potentiometer and sensitive galvanometer. The curves obtained were typical of van der Waals adsorption, and were used to determine values of the total surface area per gram given in Table III.

Discussion

It is possible to utilize the values of v_m given in Table II to arrive at an estimate of the surface extent of a crystalline

TABLE II. VALUES OF CONSTANTS FOR ADSORPTION OF VAPORS AT 25° C. ON CRYSTALLINE SURFACES

Powder	v_m Cc./gram	c	$E_1 - E_L$ Cal. mole^{-1}
	Water vapor		
TiO_2-I	4.28	48.6	2400
TiO_2-II	3.17	48.9	2380
ZnO	1.52	64.3	2500
SiO_2	1.96	46.7	2300
SnO_2	0.48	....[a]	....[a]
	Propyl alcohol vapor		
TiO_2-I	2.41	32.1	2080
TiO_2-II	1.65	32.3	2080
ZnO	1.07	23.0	1840
SiO_2	1.18	....[a]	....[a]
SnO_2	0.52	25.5	1940
	Benzene vapor		
TiO_2-I	1.44	77.0	2640
TiO_2-II	1.13	56.4	2450
SiO_2	0.36	33.4	2330

[a] In these cases calculated values of c and $E_1 - E_L$ were considerably different from the other agreeing values in these columns and are therefore omitted. By the present method of calculation the value of v_m determined by position of the knee of the adsorption isotherm is the most reliable of the three, while c and $E_1 - E_L$ are very sensitive to slight changes in the slope of the isotherm beyond its knee.

TABLE III. SURFACE AREA VALUES

(Square meters per gram)

Powder	$N_2(L)$	$H_2O(L)$	$C_3H_7OH(M)$	$C_6H_6(L)$
	16.2	10.6	20.0	30.5
TiO_2-I	13.5	12.4	13.0	11.9
TiO_2-II	8.3	9.1	8.9	9.3
ZnO	....	4.4	5.8	....
SiO_2	....	5.6	6.3	3.0
SnO_2	....	1.4	2.8	....

powder, provided a reasonable choice of the area per adsorbed molecule can be made. If the suggestions of Emmett and Brunauer (*3*) are followed, and one assumes that the adsorbed molecules are in the closest possible packing—namely, hexagonal two-dimensional packing—one may apply the formula

$$\text{Area per molecule} = 1.091\ (M/Nd)^{2/3}$$

where M is the molecular weight of the vapor, N is Avogadro's number, and d may be taken as the density of either the solidified or liquefied gas at the temperature of the adsorption experiment. Thus, for water at 25° C., 11.1 and 10.6 Å.^{2} per molecule are calculated, respectively; for propyl alcohol, 27.0 Å.^{2} per molecule; and for benzene, 30.5 Å.^{2} per molecule. These values, with the exception of propyl alcohol, have been

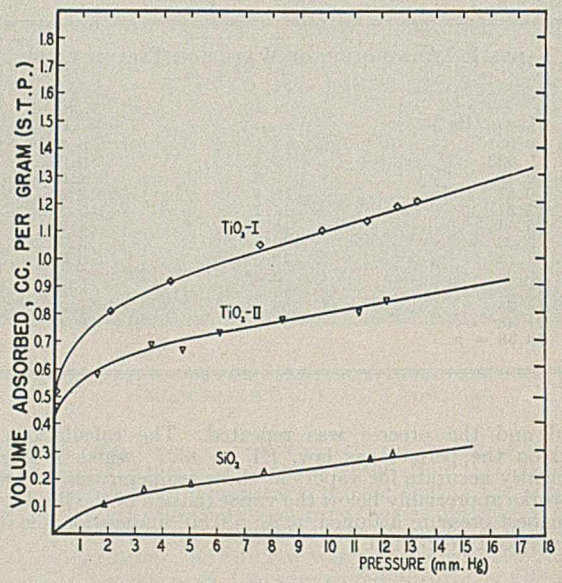

FIGURE 5. ISOTHERMS FOR ADSORPTION OF BENZENE VAPOR ON CRYSTALLINE POWDERS AT 25° C.

used in the construction of Table III. In the case of the alcohol, this value appears high, and the limiting area for close-packed hydrocarbon chains vertically oriented as obtained from insoluble films of long-chain alcohols has been used instead. This fact is indicated by M (M = monolayer); L denotes that the density of the liquefied vapor has been used.

The concordance for the surface areas in the case of the titanium dioxides is as satisfactory as is to be expected, considering the uncertainty involved in the somewhat arbitrary manner in which the area per adsorbed molecule was chosen. Table III seems to indicate that for specific areas greater than 5 square meters the method of vapor adsorption is of approximately the same degree of reliability as low-temperature adsorption. More recent work (*7*) wherein a basically different technique for the determination of the amount adsorbed is used, supports this contention.

Owing to the relatively small size of the water molecule, it is evident that a larger number of them is necessary to form a

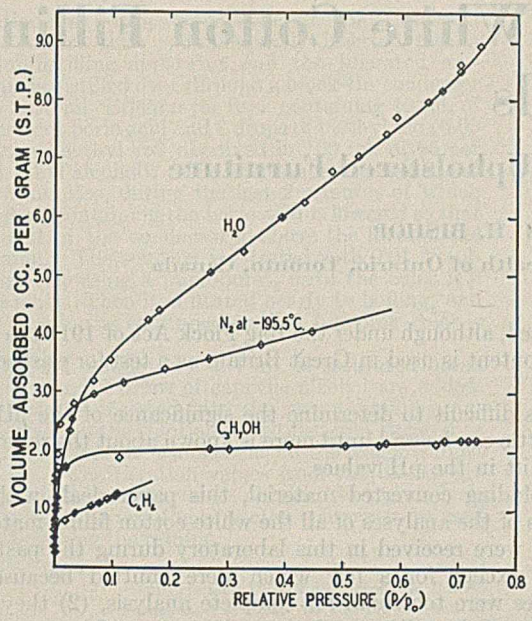

FIGURE 6. COMPARISON OF ADSORPTION ISOTHERMS OF VAPORS ON CRYSTALLINE TITANIUM DIOXIDE (ANATASE, TiO_2-I)

Curves for adsorption of water, propyl alcohol, and benzene vapors at 25° C.

monomolecular layer on a unit surface; hence, greater volumes of water vapor than nitrogen gas are observed to be adsorbed on the same solid surface. This fact should serve to make for greater accuracy in surface area determinations when water vapor is employed if the smaller equilibrium pressures may be read without increased percentage error.

It is of interest to speculate on the cause of the great difference between the character of the adsorption of propyl alcohol, and of water and benzene vapors. The distinction is illustrated by Figure 6 where the abscissa is taken as the relative pressure, p/p_0, in order to bring the isotherms to a common basis. The flatness of the curve for propyl alcohol might be interpreted as signifying that adsorption to form second and higher layers does not occur until pressures near saturation are reached. Since it is probable that the alcohol molecule is nearly vertically oriented on the polar solid surface with its hydroxyl group down, the uppermost surface of an adsorbed monolayer would present an oil-like or hydrocarbon surface on the average. On such a surface, the condition for condensation to form multilayers, $E_2 \cong E_L$, might not be satisfied; rather, it may be that $E_2 < E_L$, since the configuration of the molecules in the first layer differs strongly from the structure of a unimolecular liquid layer. In the classification suggested by Brunauer, Deming, Deming, and Teller (*2*), the adsorption isotherm for propyl alcohol on anatase is a combination of their Types I and III.

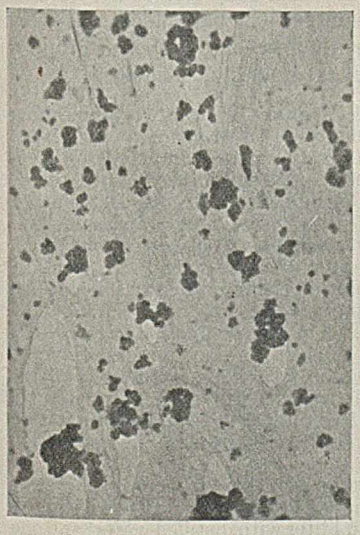

FIGURE 7. ELECTRON MICROSCOPE PHOTOGRAPH OF TiO_2-I (× 14,600)

Figures 7 and 8 show the electron microscope photographs of the two titanium dioxides, obtained with the R. C. A. electron microscope of the Research Laboratories of Interchemical Corporation by E. F. Fullam, who calculates the specific area of 20.9 square meters per gram for titanium dioxide I and 10.7 for titanium dioxide II for the particles as spheres from the statistical determination of d_3 which is $\Sigma nd^3/\Sigma nd^2$. The value for titanium dioxide II is in as good agreement as may be expected with the values of Table III; that for titanium dioxide I is not in as good agreement, and leaves a discrepancy to be clarified by further work.

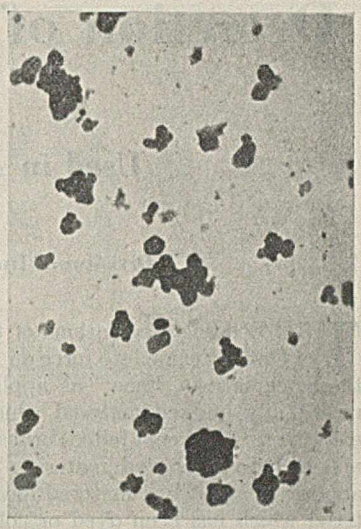

FIGURE 8. ELECTRON MICROSCOPE PHOTOGRAPH OF TiO_2-II (× 14,600)

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Determination of Fluorine

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HOFFMAN and Lundell (*1*) as well as Specht (*2*) have described procedures for the determination of fluorine, in which lead chlorofluoride is precipitated, filtered, and dissolved in nitric acid and the chloride is determined by the Volhard method. These procedures may be somewhat improved by the use of fritted Gooch-type glass-disk crucibles of medium porosity (Corning Glass Works 32960-30M) for the filtration.

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(1) Hoffman, J. I., and Lundell, G. E. F., *Bur. Standards J. Research*, 3, 589 (1929); 20, 619 (1938).
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Detection of Second-Hand White Cotton Filling Materials

Used in Articles of Bedding and Upholstered Furniture

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THE Bedding Regulations of the Department of Health of Ontario came into force on December 28, 1938. Under these regulations, "new" as applied to any filling material means any undyed material which has not been previously manufactured (except felted) or used for any purpose but does not include converted material. "Converted material" as applied to any filling material means any otherwise new material which has been dyed or colored or spun into yarn or knit or woven into fabric but not further manufactured than cut up, torn up, broken up, shredded, or felted. "Second-hand" as applied to any filling material means any material which is neither new nor converted.

For administrative purposes it is necessary to be able to detect the presence of second-hand filling materials. A review of the literature shows that little information has been published in this field.

In 1932, Wynne and Donovan (*5*) of the Maryland State Department of Health reported that new and second-hand white cotton could be distinguished by the difference in the color of the fluorescence exhibited by these materials under a suitable source of ultraviolet light. They concluded that this test was quick and simple, could readily be demonstrated, and in the hands of an experienced person gave fairly accurate results in a large majority of cases. In 1933, Burke and Kane (*1*) of the Bedding Division of the New York State Department of Labor developed a chemical method to differentiate new from second-hand cotton fillings. They found that new cotton fillings had a water-soluble sulfate content of less than 0.25 per cent expressed as sodium sulfate and that second-hand cotton fillings were characterized by a sulfate content in excess of this amount. As they found that new cotton did not contain sufficient sulfate in any form to account for this increase, they concluded that the additional sulfate found in second-hand cotton fillings must have come from an outside source.

In 1935, Moskowitz, Landes, and Himmelfarb (*3*) of the Bedding Division of the New York State Department of Labor published data on the ammonia, urea, and sodium sulfate content of new and second-hand cotton filling materials. They reported that unused cotton materials had both an ammonia and urea content of less than 0.030 and 0.010 per cent, respectively, whereas used cotton fillings had in most cases an ammonia content above 0.030 per cent, a urea content above 0.010 per cent, or both. Racicot of the Massachusetts Department of Public Health developed qualitative methods for the detection of urea and creatinine (*4*). Later Racicot and Lythgoe (*4*) determined the ash, oil, ammonia, and urea content of white cotton wastes; they stated that the urea content of new white cotton wastes was very unlikely to exceed 0.0028 per cent. In 1940, Fetherolf (*2*) of the Pennsylvania Bedding Law Division published data on the pH values of the aqueous extracts of white cotton filling materials. He reported that new undyed cotton materials had in no single instance exhibited a pH value of less than 6.0, whereas samples removed from known second-hand mattresses had not approached 6.0 in any one case.

The various tests which have been devised to recognize second-hand white cotton filling materials may be divided into two groups: those which show alteration in the cotton itself, such as the fluorescent, staining, and reduction tests, and those which detect contamination from body excretions, such as ammonia, urea, sulfate, and creatinine determinations. It is believed that second-hand white cotton filling materials have been analyzed for phosphates and chlorides, but to the knowledge of the authors, no study of this subject has been reported, although under the Rag Flock Act of 1911 the chlorine content is used in Great Britain as a test for washed material.

It is difficult to determine the significance of the pH test and little can be said until more is known about the reason for the shift in the pH values.

Excluding converted material, this paper deals with the results of the analyses of all the white cotton filling materials which were received in this laboratory during the past two years, except for a few which were omitted because (1) samples were too small for complete analysis, (2) they contained sufficient dirt or oil to mask the true fluorescence, or (3) the fluorescence was not sufficiently uniform to be properly classified. Included in this report are the remaining 400 samples, among which are specimens from the United States, Brazil, Egypt, Russia, India, China, and Java.

Methods

FLUORESCENT TEST. The equipment consisted of a 400-watt Hanovia analytic model quartz mercury vapor lamp installed in a dark room. The lamp was fitted originally with a red-purple Corex A No. 986 molded glass filter 16.25 cm. (6.5 inches) square and 5 mm. thick. This filter was broken and was replaced by one of the same type ground and polished to a thickness of 3 mm. Although these two filters transmitted rays of the same wave lengths (2480 to 4359 Å.), the thinner filter transmitted a higher percentage of the total radiation.

The first filter did not necessitate protection for the eyes but the second demanded the use of goggles. Nine different types made by the Willson Products, Inc., were tried. Willsonite goggles (shade C) were chosen as the most suitable as, under ultraviolet light, it was found that the difference in appearance of the samples tested was accentuated by their use. It was readily possible to separate samples of white cotton into at least five groups, depending upon the intensity of their violet fluorescence. This is difficult if not impossible without the use of goggles.

In practice, each sample of white cotton is placed in one of five groups which are numbered from 4 to 0. In group 4 are placed those samples which exhibit a deep violet fluorescence. Groups 3, 2, and 1 contain samples which show progressively decreasing amounts of violet fluorescence, and into group 0 are put samples which exhibit no violet fluorescence.

pH TEST. Two and one-half grams of white cotton filling material are immersed in 50 ml. of distilled water and kneaded with a thick glass rod until thoroughly wetted and the hydrogen-ion concentration of the aqueous extract is determined to the nearest 0.1 pH by means of a Coleman Model 3A pH electrometer.

CHEMICAL TEST. *Ammonia and Urea Determinations.* Five grams of white cotton filling material are placed in a 400-ml. beaker, 150 ml. of distilled water are added, and the whole is boiled for 20 minutes. The solution is filtered by suction through a Büchner funnel and the sample is boiled again with 150 ml. more of distilled water. One milliliter of 0.1 *N* hydrochloric acid is added to the pooled filtrate, which is then evaporated down to about 5 ml; 1 ml. of 0.1 *N* sodium hydroxide is added, and the solution is washed into a 15-ml. graduated Pyrex centrifuge tube, the volume being made up to 12.5 ml. with distilled water. Two aliquots of 5 ml. each are pipetted into Pyrex culture-tubes (250 by 25 mm.).

To aliquot No. 1, 5 ml. of buffer solution (6.0 grams of potassium dihydrogen phosphate and 9.1 grams of anhydrous disodium hydrogen phosphate made up to 1 liter with distilled water) and one eighth of a crushed Squibb urease tablet are added. After standing 20 minutes at room temperature, 7 ml. of saturated borax

solution and 7 drops of caprylic alcohol are added. The culture tube is immediately inserted into a steam-distilling apparatus and the liberated ammonia is distilled over through a block-tin condenser into a 50-ml. Erlenmeyer flask containing 10 ml. of 2 per cent boric acid and 3 drops of methyl red (0.05 gram of methyl red dissolved in 100 ml. of 95 per cent ethyl alcohol). The solution is steam-distilled for 5 minutes, during the last 2 minutes of which the flask containing the boric acid is lowered so that the end of the condenser is above the level of the solution. The solution is titrated with 0.01 *N* sulfuric acid, using a microburet, until the color is a pale pink. Then it is heated nearly to boiling, and the titration is continued until a full pink color is reached.

To 5-ml. aliquot No. 2, 7 ml. of saturated borax solution and 7 drops of caprylic alcohol are added. The tube is inserted into the steam-distilling apparatus, and the solution is distilled and titrated as above. Blank determinations on the reagents must be run. The titration values from the blanks are subtracted from the values obtained in the analysis of the sample to get the corrected volumes to be used in calculating the results.

CALCULATIONS. Percentage of ammonia in sample = ml. of sulfuric acid (No. 2 corrected) × 0.0085

Percentage of urea in sample = ml. of sulfuric acid (No. 1 corrected minus No. 2 corrected) × 0.015

TABLE I. CORRELATION BETWEEN AMMONIA CONTENT AND INTENSITY OF VIOLET FLUORESCENCE

No. of Samples	Intensity of Violet Fluorescence	Ammonia Content: Highest % found	Ammonia Content: Average % found	Ammonia Content: Lowest % found	Below 0.0125%: No. of samples	Below 0.0125%: % of samples	Above 0.0125%: No. of samples	Above 0.0125%: % of samples
61	4	0.011	0.004	0.000	61	100.0	0	0.0
158	3	0.017	0.005	0.000	155	98.1	3	1.9
25	2	0.032	0.013	0.003	14	56.0	11	44.0
111	1	0.091	0.031	0.012	1	0.9	110	99.1
45	0	0.094	0.043	0.015	0	0.0	45	100.0

TABLE II. CORRELATION BETWEEN UREA CONTENT AND INTENSITY OF VIOLET FLUORESCENCE

No. of Samples	Intensity of Violet Fluorescence	Urea Content: Highest % found	Urea Content: Average % found	Urea Content: Lowest % found	Below 0.0105%: No. of samples	Below 0.0105%: % of samples	Above 0.0105%: No. of samples	Above 0.0105%: % of samples
61	4	0.010	0.003	0.000	61	100.0	0	0.0
158	3	0.015	0.004	0.000	154	97.5	4	2.5
25	2	0.035	0.008	0.001	19	76.0	6	24.0
111	1	0.080	0.011	0.002	69	62.2	42	37.8
45	0	0.071	0.015	0.001	19	42.2	26	57.8

Sulfate Determination. The residual solution from the steam-distillation in the ammonia determination (No. 2 above) is transferred from the culture tube to a 250-ml. beaker and diluted with 100 ml. of distilled water. The solution is acidified with hydrochloric acid heated to boiling and treated with 5 ml. of 5 per cent barium chloride solution. The solution is allowed to stand overnight; the precipitate is filtered off, washed, and ignited in the usual way. The water-soluble sulfates are expressed as sodium sulfate.

CALCULATION. Percentage of sodium sulfate = mg. of barium sulfate × 0.0304

Chloride Determination. Five grams of white cotton filling material are extracted twice with 150 ml. of boiling distilled water as in the determination for ammonia. The filtrate is concentrated to about 100 ml. and 6 ml. of concentrated nitric acid (density 1.42) are added. The solution is further concentrated until it becomes clear and light yellow in color. It is then filtered into a 250-ml. volumetric flask and the precipitate washed with distilled water. Ten milliliters of 0.1 *N* silver nitrate are added and the solution is allowed to stand at room temperature, with frequent shakings, for at least 20 minutes, then made up to 250 ml., and filtered. The first 50 ml. of the filtrate are discarded. If the filtrate is not perfectly clear the solution is refiltered through the same filter paper, as finely divided silver chloride will act like silver nitrate in the titration with potassium thiocyanate. A 100-ml. aliquot of the filtrate is titrated with 0.1 *N* potassium thiocyanate solution, using a microburet. Ten milliliters of a 2 per cent solution of ferric ammonium sulfate are added to act as an internal indicator. The end point is taken as the first appearance of a permanent red color. The soluble chlorides extracted from the cotton are expressed as sodium chloride.

CALCULATION. Percentage of sodium chloride = (4.00 minus ml. of 0.1 *N* potassium thiocyanate) × 0.292

Phosphate Determination. The initial steps are the same as those in the chloride determination, except that 15 instead of 6 ml. of concentrated nitric acid are used, no silver nitrate is added, and the solution in the 250-ml. flask is not filtered before taking the 100-ml. aliquot. The 100-ml. aliquot is heated to 70° C. and 25 ml. of ammonium molybdate solution are slowly added with constant stirring. (The ammonium molybdate solution is prepared by dissolving 100 grams of pure molybdic acid in a mixture of 400 ml. of water and 80 ml. of strong ammonia, density 0.90. When the molybdic acid is dissolved the solution is slowly poured with constant stirring into a mixture of 400 ml. of strong nitric acid, density 1.42, and 600 ml. of distilled water.) It is then allowed to stand on a hot plate at a temperature of 40° to 60° C. for 2 hours with occasional stirring, and then at room temperature for an hour longer. The solution is filtered, and the precipitate is washed with 1 per cent potassium nitrate until the washings are neutral to litmus. The filter paper and precipitate are transferred to a beaker containing 25 ml. of 0.1 *N* sodium hydroxide. When the precipitate has been completely dissolved the solution is diluted with distilled water and titrated with 0.1 *N* nitric acid, using phenolphthalein as the indicator.

CALCULATION. Percentage of P_2O_5 = (25.00 minus ml. of 0.1 *N* nitric acid used) × 0.0062

Results

As mentioned above, the samples were separated into five groups (numbered from 4 to 0), depending upon the intensity of their violet fluorescence. A representative sample from each group was chosen and kept as a reference standard, and the intensity of the violet fluorescence of any individual sample was determined by comparison with the fluorescence of these standards. It has been shown, by others, that new white cotton has the power of exhibiting a violet fluorescence under ultraviolet light and that the loss of this property is associated with second-hand material.

The results of the ammonia determinations are summarized in Table I and include the high, average, and low amounts for each of the five groups. The concentration of ammonia steadily increases as the intensity of the violet fluorescence decreases. The highest amount of ammonia found in groups 4 and 3 was 0.017 per cent and the lowest amount found in groups 1 and 0 was 0.012 per cent. A study of the individual results led to the adoption of 0.0125 per cent as the standard for the ammonia determination.

As shown in Table I, 216 out of 219 or 98.6 per cent of the samples in groups 4 and 3 had substandard concentrations, whereas 155 out of 156 or 99.4 per cent of the samples in groups 1 and 0 had an ammonia content greater than 0.0125 per cent. Samples in group 2 were not compared with the ammonia or other determinations for reasons discussed below.

Table II gives the results of the urea determinations. It will be observed that the urea content on the average gradually rises as the intensity of the violet fluorescence falls. However, the results are not consistent, as both high and low values have been encountered in the samples of the last two groups. It has been suggested that the low concentrations might occur as a result of the slow conversion of urea into ammonia in the cotton by enzyme action.

The largest quantity found in groups 4 and 3 was 0.015 per cent, which is considerably in excess of the amounts occurring in many samples in groups 1 and 0. Thus the choosing of a standard for urea presents some difficulty and a figure of 0.0105 per cent was finally adopted. A study of Table II

shows that 98.2 per cent of the samples in groups 4 and 3 and 56.4 per cent of the samples in groups 1 and 0 have a substandard urea content. Thus less than one half of the samples, in groups 1 and 0, contain excess amounts of urea.

Table III reports the results of the sodium sulfate determinations. A decrease in the intensity of the violet fluorescence is accompanied by an increase in the percentage of sodium sulfate. It has been pointed out that some foreign cottons, before shipment to this continent, are treated with hygroscopic sulfates to prevent loss of moisture in transit; thus an occasional sample of foreign cotton containing a relatively high percentage of sodium sulfate might be expected. In the authors' experiments, however, the greatest amount found in groups 4 and 3 was 0.32 per cent, which is considerably less than the quantity obtained from many of the samples in groups 1 and 0. As the majority of the samples in the first two groups had a sodium sulfate content below 0.255 per cent and as most of the samples in the last two groups had a content above 0.255 per cent, this figure was chosen as the standard.

As shown in Table III, 213 out of 219 or 97.3 per cent of the samples in groups 4 and 3 had substandard concentrations, whereas 151 out of 156 or 96.8 per cent of the samples in groups 1 and 0 were above the standard amount.

Table IV reports the results of the sodium chloride determinations. Although from group 4 to group 0 there is a gradual rise on the average in the amount of sodium chloride found, some samples in the first two groups showed relatively high values, mainly among the samples of foreign cottons. It is possible that some of these materials had been treated with hygroscopic chlorides prior to their shipment to this continent and this factor was taken into consideration in choosing the standard. The figure finally adopted was 0.135 per cent. As shown in Table IV, 95.4 per cent of the samples in groups 4 and 3 had a substandard content, whereas, in groups 1 and 0 only about two-thirds of the samples contained more than 0.135 per cent.

Table V gives the results of the phosphate determinations. It is apparent that there is no significant difference between the amounts of phosphate found in the samples showing a deep violet and those exhibiting a weak violet fluorescence. For this reason the phosphate test was abandoned after one hundred determinations had been made.

Table VI gives the results on the one hundred pH analyses which have been made. Although at the present time, the information available is only sufficient to permit the presentation of a preliminary report, it appears that in most cases a decrease in the intensity of the violet fluorescence is accompanied by an increase in the acidity of the aqueous extracts. As 6.1 was the lowest pH value found in groups 4 and 3, 6.05 was chosen as the tentative standard. Thus all samples in groups 4 and 3 had pH values greater than 6.05 and over 90 per cent of those in groups 1 and 0 had values below 6.05.

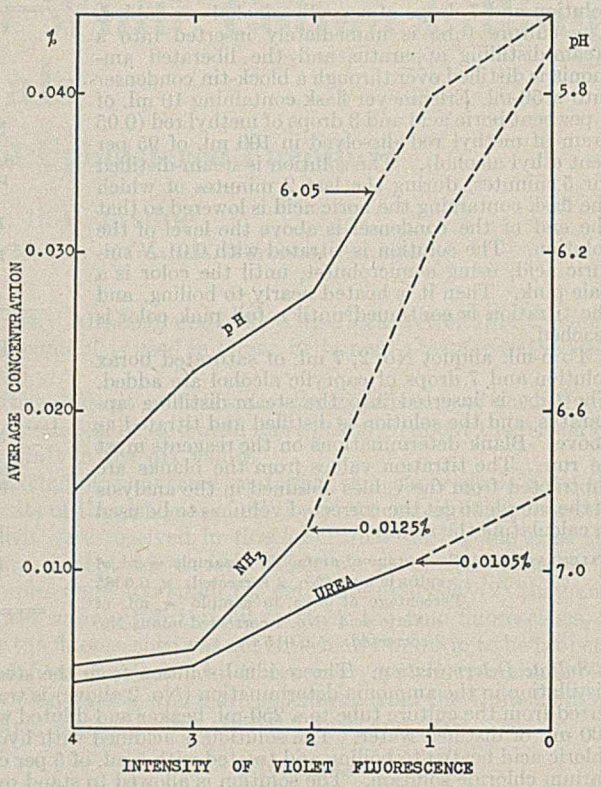

FIGURE 1

Discussion

In previous papers dealing with the detection of second-hand white cotton filling materials, attempts have been made, in most cases, to determine at the time of collection the new or second-hand nature of the samples to be analyzed. Prior to any experimental work, samples were classified, in part, according to information supplied by manufacturers, wholesale dealers, supply houses, etc., or according to the opinion of an independent expert.

Grading samples as new or second-hand on information obtained from manufacturers, wholesale dealers, supply houses, etc., has already been severely criticized by others. It has been pointed out that the trade is likely, in some instances, to submit material far different from what is expected. The second method, that of placing absolute reliance on the opinion of an expert, is also open to question, as it places too much faith in the ability of anyone to grade correctly all doubtful samples by visual inspection alone. Such information should be treated with reserve, as any error would tend to invalidate the interpretation of the results.

In this paper, the samples were collected by a bedding inspector from numerous retailers, manufacturers, felters, and supply houses. In most

TABLE III. CORRELATION BETWEEN SODIUM SULFATE CONTENT AND INTENSITY OF VIOLET FLUORESCENCE

No. of Samples	Intensity of Violet Fluorescence	Sodium Sulfate Content Highest % found	Average % found	Lowest % found	Below 0.255% No. of samples	Below 0.255% % of samples	Above 0.255% No. of samples	Above 0.255% % of samples
61	4	0.27	0.14	0.01	60	98.4	1	1.6
158	3	0.32	0.17	0.02	153	96.8	5	3.2
25	2	0.37	0.24	0.03	14	56.0	11	44.0
111	1	0.84	0.36	0.20	4	3.6	107	96.4
45	0	0.71	0.44	0.20	1	2.2	44	97.8

TABLE IV. CORRELATION BETWEEN SODIUM CHLORIDE CONTENT AND INTENSITY OF VIOLET FLUORESCENCE

No. of Samples	Intensity of Violet Fluorescence	Sodium Chloride Content Highest % found	Average % found	Lowest % found	Below 0.135% No. of samples	Below 0.135% % of samples	Above 0.135% No. of samples	Above 0.135% % of samples
61	4	0.17	0.07	0.01	60	98.4	1	1.6
158	3	0.23	0.09	0.02	149	94.3	9	5.7
25	2	0.20	0.11	0.04	19	76.0	6	24.0
111	1	0.30	0.16	0.06	43	38.7	68	61.3
45	0	0.58	0.18	0.11	11	24.4	34	75.6

TABLE V. CORRELATION BETWEEN PHOSPHATE CONTENT AND INTENSITY OF VIOLET FLUORESCENCE

No. of Samples	Intensity of Violet Fluorescence	Phosphate Content (P_2O_5) Highest % found	Average % found	Lowest % found
16	4	0.10	0.06	0.04
36	3	0.14	0.07	0.05
9	2	0.08	0.06	0.05
25	1	0.10	0.07	0.05
14	0	0.10	0.08	0.06

TABLE VI. CORRELATION BETWEEN pH VALUE AND INTENSITY OF VIOLET FLUORESCENCE

No. of Samples	Intensity of Violet Fluorescence	pH Values Highest found	Average found	Lowest found	Above 6.05 No. of samples	Above 6.05 % of samples	Below 6.05 No. of samples	Below 6.05 % of samples
15	4	7.1	6.8	6.4	15	100.0	0	0.0
30	3	7.0	6.5	6.1	30	100.0	0	0.0
5	2	6.9	6.3	5.7	3	60.0	2	40.0
25	1	6.7	5.8	5.2	3	12.0	22	88.0
25	0	6.1	5.6	5.2	1	4.0	24	96.0

cases, opinions concerning the new or second-hand nature of the materials were obtained by the inspector at the time of collection. To eliminate any possible error, all information received from outside sources was disregarded (except data concerning the geographical origin of the foreign cottons), as was the personal opinion of any member of the staff connected with the enforcement of the bedding regulations. Thus the authors were not influenced by any preconceived ideas about the new or second-hand nature of the samples which were tested but were able to base their conclusions directly on the experimental results.

It is of the utmost importance to be able to tell whether a sample of white cotton contains second-hand material. Three methods of testing are available. The first is the chemical test to detect contamination from body excretions, the second is the use of ultraviolet light to detect some alteration in the cotton itself, and the third is the pH test.

CHEMICAL TEST. An ideal single chemical determination, to distinguish between a sample of new and second-hand material, would be one which fulfills the following conditions: the results for new cotton should always be negative, and the results for second-hand cotton should always be positive and sufficiently high to make the evidence conclusive. So far none of the methods available meets the first requirement.

In assessing the relative merits of the chemical determinations, reported in this paper, it is evident that they are not all of equal value. Figures 1 and 2 show the average percentage concentration of ammonia, urea, sodium sulfate, sodium chloride, and phosphate plotted against the intensity of the violet fluorescence.

The authors consider that the ammonia determination is the best of the chemical methods which were investigated, as it most closely approaches the ideal conditions mentioned above. It will be seen from Figure 1 that, as the intensity of the violet fluorescence decreases, there is an initial gradual rise followed by a sharp increase in the concentration of ammonia. Next in importance is the sodium sulfate determination. As shown in Figure 2, there is a steep rise in the sodium sulfate curve which is similar to the one for ammonia except that the initial slope is somewhat greater.

The urea and sodium chloride determinations are of less value as, on the average, the concentrations of these compounds vary within narrower limits. This is apparent from a study of the curves in Figures 1 and 2. As stated above, the phosphate determination was abandoned after one hundred analyses had been made.

The standards adopted for ammonia, urea, sodium sulfate, and sodium chloride were 0.0125, 0.0105, 0.255, and 0.135 per cent, respectively. In some samples none of these standards were exceeded, while in others excess quantities of one or more of these compounds were found. The results are shown in Table VII. In group 4, 100 per cent and in group 3, 98.8 per cent of the samples had substandard values in all or all but one of the four chemical determinations, while in group 1, 97.3 per cent and in group 0, 100 per cent of the samples exceeded two or more of these standards. Thus when none or only one of the standards is exceeded, the sample is not considered to contain second-hand material by the chemical test.

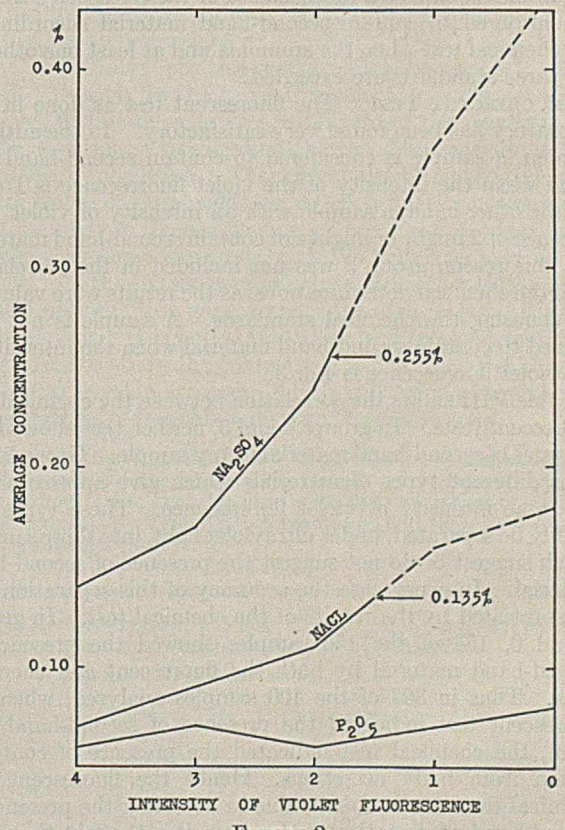

FIGURE 2

TABLE VII. CORRELATION BETWEEN NUMBER OF CHEMICAL STANDARDS EXCEEDED AND INTENSITY OF VIOLET FLUORESCENCE

Samples Which Exceed None, One Only, Two Only, Three Only, and All Four of Ammonia, Urea, Sodium Sulfate, and Sodium Chloride Standards

No. of Samples	Intensity of Violet Fluorescence	None No. of samples	None % of samples	One Only No. of samples	One Only % of samples	Two Only No. of samples	Two Only % of samples	Three Only No. of samples	Three Only % of samples	All Four No. of samples	All Four % of samples
61	4	59	96.7	2	3.3	0	0.0	0	0.0	0	0.0
158	3	139	88.0	17	10.8	1	0.6	1	0.6	0	0.0
25	2	12	48.0	3	12.0	1	4.0	7	28.0	2	8.0
111	1	1	0.9	2	1.8	32	28.8	42	37.9	34	30.6
45	0	0	0.0	0	0.0	6	13.3	18	40.0	21	46.7

TABLE VIII. CORRELATION BETWEEN CHEMICAL AND FLUORESCENT TESTS

No. of Samples	Intensity of Violet Fluorescence	Classification by Chemical Test[a] New: No. of samples	New: % of samples	Second-Hand: No. of samples	Second-Hand: % of samples
61	4	61	100.0	0	0.0
158	3	158	100.0	0	0.0
25	2	15	60.0	10	40.0
111	1	3	2.7	108	97.3
45	0	0	0.0	45	100.0

[a] Based on results of ammonia, urea, sodium sulfate, and sodium chloride determinations.

As has been pointed out, new foreign cottons may have been treated with hygroscopic sulfates and/or chlorides. If a sample of cotton has been treated with both these chemicals, excess amounts of both sodium sulfate and sodium chloride will be found and the sample classed as containing second-hand material by the chemical test. Thus, to say that a sample contains second-hand material when any two of the four standards are exceeded might be open to some criticism. In Table VII, in group 3 one of the 158 samples exceeded "any two" standards and one sample exceeded "any three" standards. In both these samples the ammonia content was below 0.0125 per cent, which means that in neither case was the ammonia standard exceeded. For these reasons a sample is considered to contain second-hand material according to the chemical test when the ammonia and at least one other of the three standards are exceeded.

FLUORESCENT TEST. The fluorescent test as done in this laboratory has been found very satisfactory. In the authors' opinion, a sample is considered to contain second-hand material when the intensity of the violet fluorescence is 1 or 0. On the other hand, a sample with an intensity of violet fluorescence of 2 might or might not contain second-hand material. For this reason group 2 was not included in the correlation with the chemical determinations, as the results were valueless for choosing the chemical standards. A sample is not considered to contain second-hand material when the intensity of the violet fluorescence is 4 or 3.

Table VIII shows the correlation between the chemical and fluorescent tests. In groups 4 and 3, neither test showed the presence of second-hand material in any sample. Group 2 contains different types of materials which give approximately the same intensity of violet fluorescence. These types can readily be separated, under ultraviolet light, into those samples which suggest or do not suggest the presence of second-hand material. In every case the accuracy of this separation was substantiated by the results of the chemical test. In groups 1 and 0, 153 of the 156 samples showed the presence of second-hand material by both the fluorescent and chemical tests. Thus in 397 of the 400 samples analyzed, when the fluorescent test indicated the presence of second-hand material, the chemical test indicated the presence of contamination from body excretions. Hence the fluorescent and chemical tests are of equal value in detecting the presence of second-hand white cotton. However, it is thought that both these tests should be made before a sample is graded.

From the results of the experiments on the 400 samples which were tested, the authors consider that a sample of white cotton contains second-hand material when the following three conditions are fulfilled:

1. The intensity of the violet fluorescence is of the order of 2 or less.
2. The concentration of ammonia exceeds 0.0125 per cent.
3. Either the concentration of urea exceeds 0.0105 per cent, the concentration of sodium sulfate exceeds 0.255 per cent, or the concentration of sodium chloride exceeds 0.135 per cent.

pH TEST. This test is thought to be worthy of further investigation. Unfortunately, the hydrogen-ion concentration of the distilled water used varied markedly and, as yet, the effect of this factor has not been studied. In Figure 1, the average pH values are plotted against the intensity of the violet fluorescence.

One hundred per cent and 92 per cent of the samples which were not considered to contain second-hand material by the fluorescent and chemical tests, respectively, had pH values greater than 6.05. On the other hand, 90 and 97 per cent of the samples considered to contain second-hand material by the fluorescent and chemical tests, respectively, had pH values below 6.05. Thus, at present, a pH value less than 6.05 might be used as confirmatory evidence of second-hand material.

Summary

The chemical, fluorescent, and pH tests to detect the presence of second-hand white cotton filling materials found in articles of bedding and upholstered furniture have been investigated.

The chemical test is based on the determination of the ammonia, urea, sulfate, and chloride content to detect contamination of the material from body excretions. From the results of the experiments on 400 samples of white cotton, the following standards were chosen: ammonia 0.0125 per cent, urea 0.0105 per cent, sodium sulfate 0.255 per cent, and sodium chloride 0.135 per cent. Phosphate determinations were also made on 100 of the above samples, but no standard was chosen, as the results were not significant.

The fluorescent test is a measure of the intensity of the violet fluorescence under ultraviolet light. The 400 samples were divided into 5 groups numbered from 4 to 0, depending upon the intensity of the violet fluorescence exhibited.

The pH test is based on the determination of the hydrogen-ion concentration of the aqueous extract of a sample of white cotton. From the results of the experiments on 100 samples, a pH value of 6.05 was chosen as the tentative standard.

For the detection of second-hand material in a sample of white cotton, the chemical and fluorescent tests were found very satisfactory. From the preliminary investigation which has been made on the pH test, it appears that this test might be of value.

A sample of white cotton is considered to contain second-hand material when the intensity of the violet fluorescence is of the order of 2 or less; the concentration of ammonia exceeds 0.0125 per cent; and either the concentration of urea exceeds 0.0105 per cent, the concentration of sodium sulfate exceeds 0.255 per cent, or the concentration of sodium chloride exceeds 0.135 per cent.

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CORRECTION. In the paper by Steigman, Birnbaum, and Edmonds entitled "Ruthenium Dipyridyl—A New Oxidimetric Indicator" [IND. ENG. CHEM., ANAL. ED., 14, 30 (1942)] reference should have been made to the paper by Smith and Getz [*Ibid.*, 10, 191, 308 (1938)]. The conditions for using the indicator—in 2*M* perchloric acid—were those described by Smith and Getz for titrations with 0.1 *M* ceric nitrate.

SYLVAN M. EDMONDS

A Supersensitive Schiff's Aldehyde Reagent

Demonstration of a Free Aldehyde Group in Certain Aldoses

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OF RECENT years, cyclic formulas have been generally adopted as best representing many of the known properties of carbohydrates. At the same time, it is generally recognized that certain carbohydrates possess many properties which indicate that in solution a certain proportion of the molecules exist in a straight- or open-chain configuration. In the case of aldoses, this implies the presence of a certain proportion of free aldehyde groups. In the present paper, a very sensitive form of Schiff's reagent is described which can be used for a direct demonstration of aldehydic groups in simple aldoses, and which, conversely, gives a negative reaction with ketoses, glycosides, or polyhydroxy alcohols. Monosaccharide aldoses usually react strongly, and the disaccharide aldoses react rather weakly, so that the test in its present form is of limited value for the latter type of compounds.

Ordinary Schiff's reagent is known to give a positive aldehyde reaction with certain aldoses, but the pink color obtained is usually very weak, owing to the presence of considerably more sulfur dioxide than is necessary to decolorize the fuchsin, and is often obscured by the brown or yellowish impurities which are almost invariably found in samples of commercial basic fuchsin.

Preparation of Reagent

Add 0.500 gram of basic fuchsin to 500 ml. of water, and pass in sulfur dioxide until the weight has increased by 1.0 gram. The solution may retain a considerable portion of its red color, but this will disappear upon standing overnight in a stoppered container, so that the last of the suspended fuchsin dissolves. Make to a volume of 1 liter. The solution will have a brown discoloration due to traces of impurities which are always present in commercial fuchsin. To remove the discoloration, add 1.0 gram of decolorizing carbon, agitate, and filter by gravity, keeping the filter covered with a watch glass to prevent undue contact with the air. If a suitable decolorizing carbon has been used, the filtrate will be completely colorless. Satisfactory brands of fuchsin and carbon are considered under "Reproducibility of the Reagent". A less sensitive form of the colorless reagent, containing 5.0 grams of sulfur dioxide per liter and adapted for the determination of aldehydes in distilled alcoholic liquors, has been previously reported (*8*, *9*).

Determination of Free Aldehyde Groups in Simple Aldoses

Before the reagent is used for testing unknowns, control tests should be run against pure samples of glucose (dextrose) and sucrose. To 0.2-gram portions of the carbohydrates in 7.5-cm. (3-inch) test tubes, add either 1.0 or 2.0 ml. of the reagent. The reagent should be pipetted accurately, and the carbohydrates weighed on a centigram balance or a sensitive decigram balance. Let the tubes stand at room temperature with occasional swirling to dissolve the carbohydrates. After one hour, the tubes containing dextrose show a light but distinct pink color, while those containing sucrose remain completely colorless. If desired, the test may be allowed to run for only 15 or 30 minutes, but in this case positive reactions (pink color) are correspondingly weaker. The colors continue to darken for about 24 hours, but after this time a pink color appears in the sucrose controls.

The regular determination is run in exactly the same fashion. One milliliter of reagent generally gives a more intense pink color with any given aldose than do 2 ml., since there is only half as much sulfurous acid present in proportion to the small amount of free aldehyde present. However, the use of 2 ml. is probably preferable, since it diminishes the weak positive tests obtained with carbohydrates other than simple aldoses, and permits *d*-galactose to dissolve completely. Certain compounds are relatively insoluble in both 1 and 2 ml. of the reagent, but since their reactions are essentially negative, this is of minor importance. If 1 ml. is used, it is desirable to run a test on glucose each time the test is made and to regard pink colors weaker than that obtained with the glucose as "negative" as regards the presence of simple aldoses.

Reactions of Carbohydrates and Glucosides

The results in Table I show that simple aldoses give a distinct positive reaction (pink color) with the reagent. Aldopentoses give a stronger color than aldohexoses. Glucose gives the weakest reaction of any of the simple aldoses tested. The intensities of the pink colors developed are approximately proportional to the concentrations of free aldehyde groups in solutions of the simple aldoses determined polarographically by reduction at a dropping mercury electrode by Cantor and Peniston (*1*). Both the polarographic determinations and the tests with Schiff's reagent indicate that the concentration of free aldehyde is considerably lower in glucose solutions than in equivalent solutions of other common simple aldoses. On this basis, a test with a pure unknown giving a color weaker than that obtained with glucose should be regarded as a negative test for simple aldose.

The weak "false" positive reactions may be variously interpreted. Possibly in the case of certain aldose disaccharides (maltose, melibiose, cellobiose, and lactose) the reactions are due to very low concentrations of the disaccharides in the free-aldehyde condition. They might also be due to the presence of traces of simple aldoses, either primarily present as a slight impurity in the compounds, or produced secondarily by hydrolysis of the compounds with the sulfurous acid of the reagent. Of the sixteen compounds tested (other than simple aldoses) only maltose gave a strong enough false positive to be misleading. It is hoped that there will be opportunity at a later date to study the compounds which react weakly and possibly to develop a reagent which will react to even lower concentrations of free aldehyde than the present one.

Except for the *i*-inositol monohydrate which was manufactured by Pfanstiehl, all the compounds tested were prepared by the Difco Laboratories, Inc., for use in the differentiation of various microorganisms by fermentation reactions, and therefore were of high commercial purity.

TABLE I. INTENSITY OF PINK COLOR PRODUCED BY SUPERSENSITIVE SCHIFF'S ALDEHYDE REAGENT

(After one hour at room temperature, 25° to 30° C.)

Compound	Chemical Type	Intensity of Pink Color 1 ml. of reagent	2 ml. of reagent
l-Arabinose	Aldopentose	Intense	Very strong
l-Xylose	Aldopentose	Intense	Strong
Rhamnose	Methylaldopentose	Very strong	Light
d-Mannose	Aldohexose	Very strong	Light
d-Galactose	Aldohexose	Strong[a]	Light
d-Glucose	Aldohexose	Light	Faint
Maltose	Disaccharide	Faint	Faint
Melibiose	Disaccharide	Faint[a]	Very faint
Cellobiose	Disaccharide	Faint[a]	Very faint[a]
Lactose	Disaccharide	Faint[a]	Very faint[a]
Melezitose	Trisaccharide	Faint	Very faint
d-Sorbitol	Polyalcohol	Very faint	Colorless
Trehalose	Disaccharide	Very faint	Colorless
Salicin	Glucoside	Very faint[a]	Colorless[a]
Dulcitol	Polyalcohol	Very faint[a]	Colorless[a]
Levulose	Ketohexose	Colorless	Colorless
Sucrose	Disaccharide	Colorless	Colorless
Raffinose	Trisaccharide	Colorless[a]	Colorless[a]
Inulin	Polysaccharide	Colorless[a]	Colorless[a]
Esculin	Glucoside	Colorless[a]	Colorless[a]
d-Mannitol	Polyalcohol	Colorless[a]	Colorless
i-Inositol hydrate	Polyalcohol	Colorless[a]	Colorless[a]

[a] Compound wholly or partly insoluble in the amount of reagent used.

TABLE II. RELATIVE INTENSITY OF PINK COLOR OBTAINED WITH BASIC FUCHSIN AND CARBON

(Reaction with 0.2 gram of Digestive Ferments Co. glucose dissolved in 1 ml. of reagent at a room temperature of 24° C.)

Reagent No.	Fuchsin	Grams of SO_2	Carbon	Intensity of Pink
1	National Aniline and Chemical Co., 89% dye content, certification No. NF 31	1.3	Norit A	Moderate
2		1.3	Nuchar	Palest
3		1.0	Norit A	Moderate
4		1.0	Nuchar	Moderate
5		1.0	Eimer & Amend	Moderate
6		1.0	Darco S-51	Moderate
7	National Aniline and Chemical Co., 94% dye content, certification No. NF 29	1.0	Norit A	Moderate
8		1.0	Nuchar	Moderate
9	Merck's medicinal, control No. 30,287	3.4	Norit A	Light
10		3.4	Nuchar	Light
11		1.0	Norit A	Strong
12		1.0	Nuchar	Strongest

Reproducibility of Results

In order to demonstrate the reproducibility of the reagent, twelve different lots were prepared, employing different fuchsins and different decolorizing carbons. The results of tests upon pure glucose (Difco Laboratories, Inc., dextrose, Lot No. 316,282), given in Table II, indicate that various combinations of carbon and fuchsin can be used successfully with but minor variations in the amount of color produced. They also indicate that an increase in the amount of sulfur dioxide tends to reduce the sensitivity of the reagent, although the carbon used may be a secondary factor in modifying the sensitivity.

The Norit A was marketed by the Pfanstiehl Chemical Co. The Nuchar was sold by the Eastman Kodak Co., reagent No. 776. The Eimer and Amend carbon was an 8- to 14-mesh activated coconut charcoal. It was used in an attempt to obtain more rapid filtration and reduce the time of exposure to the air, but the rapidity of filtration was not increased, and the amount of carbon had to be raised to 7 grams to obtain a completely colorless reagent.

Both samples of National Aniline and Chemical Co. basic fuchsin had been certified by the Commission on Standardization of Biological Stains, but nevertheless their solutions showed a considerable brown color after treatment with sulfur dioxide. A fuchsin which would not show this discoloration when reduced to the leuco condition in solution would permit the preparation of a very sensitive Schiff's reagent without the necessity for carbon treatment.

Many other varieties of basic fuchsin and carbon can undoubtedly be employed, although it may be necessary to make slight changes in the amount of sulfur dioxide and carbon. Commercial fuchsins not only vary somewhat in the chemical composition of the dye, but also vary from about 80 per cent to 95 per cent in true dye content as put on the market. Thereafter they frequently slowly deteriorate with age, with the formation of a brownish water-soluble material and a black water-insoluble material (*9*). Therefore each new lot of the reagent should be carefully standardized against glucose and sucrose. It is also a good plan to run a blank without any carbohydrate present. Any development of color in the blank, particularly a deeper pink in the topmost layer than in the deeper portions of the mixture, indicates that the sulfur dioxide content of the reagent is too low and that atmospheric oxygen or possibly evaporation is restoring the color of the fuchsin.

When properly prepared, the reagent is extremely stable. The results in Table I were obtained with a reagent prepared from the National Aniline and Chemical Co., basic fuchsin certification No. NF 29, 94 per cent total dye content, and decolorized with Norit A iron-free decolorizing carbon (Pfanstiehl). It had been standing in a nearly full, colorless, glass-stoppered bottle for 13 months, exposed to diffuse daylight and laboratory temperatures of 20° to 35° C.

Discussion

Schiff's aldehyde reagent has seldom been used in testing carbohydrates for aldehydic groupings, although it has occasionally been employed to determine the presence of oxycellulose in cellulose (*3*, *4*). Some standard textbooks even state that the reagent does not react with glucose or other simple aldoses (*6*, *7*, *10*). This is probably due to the fact that as ordinarily prepared the reagent is relatively insensitive. The more sensitive reagent here described should be useful in routine qualitative testing of carbohydrate unknowns in laboratory instruction, as well as in research. It may also be used in a striking lecture demonstration of the presence of free aldehydic groups in solutions of aldoses, by applying the test to glucose on a large scale, with sucrose as a control.

No originality is claimed for the use of carbon as a decolorizing agent for reagents containing leuco basic fuchsin. It has been so used by other workers (*2*, *5*), but seems not to have attracted general attention.

In its present form, the test is satisfactory for simple aldoses, maltose being the only other common carbohydrate giving a strong enough pink color to interfere. The author hopes later to develop the method further to permit the detection of the very small amounts of free aldehyde in solutions of substituted aldoses in a more definite fashion. Some of the compounds recorded as giving a negative (colorless) reaction or a faint false positive test, in Table I, showed an appreciable pink color in the still undissolved solid shortly after addition of the reagent but lost this color when all the solid had dissolved.

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Stable Sodium Thiosulfate and Starch Solutions

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A 0.1 *N* sodium thiosulfate solution that will retain its original titer approximately 5 months is readily prepared by combining 0.05 per cent sodium hydroxide and 0.1 per cent sodium benzoate as preservatives.

A stable starch solution, essentially the preparation of the Association of Official Agricultural Chemists, modified by the addition of sodium benzoate, has the composition: 0.6 per cent soluble starch, 0.1 per cent sodium hydroxide, 0.3 per cent potassium iodide, and 0.1 per cent sodium benzoate. In an iodometric titration 1 cc. of this solution is sensitive in a volume of 200 cc.

Analytical Classes of Cannabinol Compounds in Marihuana Resin

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IN 1896 Wood, Spivey, and Easterfield isolated from cannabis resin a toxic "red oil", seemingly a definite chemical compound, which they named "cannabinol" (*24*). In 1899 they isolated a pure compound from the red oil (as the crystalline acetate) and transferred to it this name, cannabinol (*25*). Their own work, and that of Cahn in 1931 (*11*) showed that the "crude cannabinol" was not just an impure form of pure cannabinol, but rather a mixture of closely related compounds. Other authors, however, especially before 1931, continued to refer to the complex of related compounds as cannabinol (*11*). Blatt, in reviewing the literature in 1938, used "crude cannabinol" intentionally as a collective name for these compounds (*9*).

In this paper all the compounds of the resin, whether they go into the distilled red oil or not, which are closely related to cannabinol, are referred to collectively as "cannabinol compounds".

In 1940 Adams, Hunt, and Clark reported the isolation of another of these related compounds, naming it "cannabidiol" (*2*). Jacob and Todd have isolated still another, calling it "cannabol" (*17*). Pure cannabinol and cannabidiol are stated not to have the specific narcotic effect of marihuana, but Adams and his co-workers have shown that cannabidiol isomerizes to tetrahydrocannabinol (which dehydrogenation converts to cannabinol), and that this compound, and also hexahydrocannabinol, do have "marihuana activity" (*3, 4, 5*).

Extractive Separations

All the work mentioned above was done on the distilled product. In 1913 a German patent issued to F. Hoffmann–La Roche & Co. gave the division of the resin into parts, distinguished from each other in their poisonous and narcotic properties, by means of an organic solvent immiscible with water and aqueous alkali (*15*). Casparis recognized the division of hashish resin into two parts, obtained by shaking the petroleum ether extract with aqueous sodium carbonate solution, but considered that the sodium carbonate solution removed "acid, inert substances", which constituted "up to 50 per cent" of the resin (*13*). Nickolls, on the other hand (though basing his remarks only on the acid Beam test), writes as if the aqueous alkali should be regarded as containing the active principle (*22*). Cahn tried the separation, but his material contained very little of the alkali-soluble compounds (5 per cent or less) and he used the process only as a preliminary to distillation (*11*).

As a matter of fact both parts contain cannabinol compounds, and the separation is of paramount value. However, except for the water-soluble quebrachitol (*6*, which does not require alkali for its extraction) the substances that have been identified in the red oil would all be in the petroleum ether. The colorless cannabinol compounds soluble in aqueous alkali do not distill as such, for they are readily altered by heat to alkali-insolubility. Hence, the extractive separation has little value as a mere preliminary to distillation; and distillation has little or no value for the study of the alkali-soluble cannabinol compounds.

Myttenaere has done considerable work on extractive separations (*19, 20, 21*). Possibly this has been neglected because he calls that part of the petroleum ether extract insoluble in aqueous alkali "the alcohol", as distinguishing it from the alkali-soluble phenols; and yet the known compounds cannabinol and cannabidiol, which have had their formulas established as phenols, both have the property of insolubility in aqueous alkali. All the cannabinol compounds are phenolic; and if some of them are also alcohols they are the ones soluble in aqueous alkali. Nevertheless Myttenaere's work has great value. He separates the alkali-soluble part primarily into two parts, calling them "phenol I" and "phenol II" (*19*), and gives some information about a "phenol III", apparently an oxidation product of "phenol II" (*20, 21*). Further reference is made below to the Myttenaere separation and nomenclature.

Classification of Colorless Cannabinol Compounds

Solutions of fairly fresh resin in ethyl or isopropyl alcohol, or ethyl acetate, are readily decolorized completely by activated charcoal (*23*), and then still contain the greater part of the cannabinol compounds originally present. The writer here presents a classification of the colorless cannabinol compounds into four distinct kinds. There are also yellow or brown colored cannabinol compounds naturally present in the resin, especially when it is old. These are probably oxidation and perhaps decomposition products of the colorless compounds.

The compounds in question give similar reactions in a number of tests, which in this paper are called "cannabinol reactions". These include the Ghamrawy test (with *p*-dimethylaminobenzaldehyde in sulfuric acid, *16*), the Duquenois and Moustapha test (with vanillin, acetaldehyde, and hydrochloric acid, *14*), the mercuric sulfate test (as modified by the writer, originally suggested by Duquenois and Moustapha, *14*), the writer's diazo tests, and others. Phenols commonly react in such tests as these.

The alkaline Beam test (*7*) is of a different kind from those just mentioned. Some cannabinol compounds give this test and some do not. Cannabidiol is the known compound yielding it (*2*); cannabinol and cannabol do not give it (*17*), nor do the physiologically active tetrahydro- and hexahydrocannabinols (*5*).

Cannabidiol constitutes at least 45 to 50 per cent of the purified red oil from Minnesota wild hemp (*6*). In the writer's experience, individual wild hemp plants invariably give the alkaline Beam test, even as tiny seedlings. Moreover, even with separation of the resin into different parts, the depth of the purple color in a suitable form of this test (such as potassium hydroxide in isopropyl alcohol) usually still correlates closely to the depth of color obtained in a diazo test or other cannabinol reaction on the same portion. Adams, Pease, and Clark isolated some cannabinol from Minnesota resin, but it is certainly a very minor constituent in our wild hemp. The cannabinol compounds present must nearly all be such as give the alkaline Beam test.

On the other hand it is easily demonstrated that in the resin from hemp of Manchurian origin (which is occasionally grown in this district) most of the cannabinol material consists of compounds not yielding the alkaline Beam test. With some samples of marihuana, or some plants of Manchurian hemp, the test has been completely negative, even though the cannabinol reactions were very strong. In most plants of the variety, however, both kinds of cannabinol compounds are present; the alkaline Beam, or at least the related and more

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sensitive Bouquet test (*10*), is positive, but, by comparison with the cannabinol reactions very weak. The resin of our Manchurian hemp is probably of similar average composition to that from which cannabinol was first isolated, and to that of the Egyptian *Cannabis indica* from which Jacob and Todd isolated cannabinol, cannabidiol, and cannabol (*17*).

There are at least two distinct "chemical varieties" of hemp, though neither is a pure race in producing only one type of cannabinol compound. Their differences do not depend upon climate, for both grow in Minnesota. Both the Manchurian and the wild hemps are used by marihuana addicts for smoking.

The two kinds of cannabinol compounds, as differentiated by the alkaline Beam test, in the alkali-soluble part of the resin can be separated more or less successfully by Myttenaere's method. However, the writer did not originally depend on this, but on wild hemp for the reactive compounds, and on selected samples or plants of Manchurian hemp for resin containing the nonreactive compounds virtually free of the reactive.

When a petroleum ether solution of the resin from either type of plant is shaken with aqueous alkali or sodium carbonate solution, it is separated into two parts. By extracting with several portions of alkali, which are in turn shaken with a little fresh petroleum ether, the separation is made complete. The part extracted by alkali consists almost entirely (as far as the writer can determine) of cannabinol compounds, even though no such compound has yet been definitely isolated as a chemical individual and named. The petroleum ether retains cannabinol compounds insoluble in alkali (which includes those that have been isolated from red oil), and also the essential oil, the paraffin hydrocarbon, and (if an undecolorized extract is used) nearly all of the chlorophyll.

Designating the cannabinol compounds soluble in aqueous alkali as I, and those remaining in the petroleum ether as II, this analytical distinction by solubilities is independent of the distinction between the classes of cannabinol compounds reactive (RAB) and nonreactive (NRAB) in the alkaline Beam test. Thus there are four kinds of colorless cannabinol compounds: I-RAB, I-NRAB, II-RAB, and II-NRAB. The II-RAB material reacts more quickly and readily than I-RAB in the alkaline Beam test, but by using the test of potassium hydroxide in isopropyl alcohol just as strong a purple is obtained, more gradually, from a similar amount of I-RAB.

A color test which distinguishes I from II in the cannabinol compounds is made with ferric chloride in methyl alcohol. The colors obtained with I compounds—green, blue, red—are suggestive of *o*-diphenols; but the writer has recently found that the *m*-diphenol resorcinol can be made to give a similar test, though less sensitive and with the red color (on addition of ammonium acetate) much more transitory. The II cannabinol compounds give negligible reactions (if any).

The fourfold classification is doubly confirmed. The distinction between I and II compounds by solubilities is confirmed by a color test; and the distinction by the Beam test between reactive and nonreactive compounds is not merely analytical, but relates to the type of cannabinol compounds predominating in the resin of distinct races of hemp.

Change of Compounds from Solubility to Insolubility in Alkali

The writer discovered that the cannabinol compounds soluble in aqueous alkali change readily to insolubility, if heated or even on long standing at room temperature.

The best laboratory conversion found is by refluxing in absolute methyl alcohol for 12 hours or so. A small quantity of colorless compounds soluble in aqueous alkali, say 0.1 gram, is mostly converted to alkali-insoluble by this procedure, with scarcely any development of color. The ferric chloride test is destroyed, but the cannabinol reactions remain almost unchanged. Laboratory manipulations such as concentration of an extract, or evaporation of a solvent and heating of the residue on the steam bath, always convert some of the I compounds to II.

Purified red oil is distilled at 175° to 190° under 2-mm. pressure (*2*). Even without the reduced pressure, which probably assists the change, conversion takes place gradually at much lower temperatures. The writer has not personally distilled red oil, but there is little prospect of finding any of the alkali-soluble cannabinol compounds in it.

Johns of Iowa State College studied marihuana extracts briefly and on a small scale, at the same time as the writer's first conversion experiments, and he also discovered the change. He separated decolorized resin into two parts with petroleum ether and aqueous alkali, and after acidifying reextracted the latter part with petroleum ether. Thus two residues in similar condition were obtained by evaporating the petroleum ether. The alkali-insoluble compounds distilled readily under a good vacuum, but the alkali-soluble compounds gave off a gas, probably water, making it difficult to maintain the vacuum, and then distilled as alkali-insoluble (*18*).

In fresh or recently air-dried marihuana itself the compounds soluble in aqueous alkali predominate, but after it is several years old the alkali-insoluble compounds predominate. Obviously some, at least, of the alkali-insoluble compounds are not produced as such by the plants, but are formed indirectly from the alkali-soluble compounds. In the writer's opinion all the cannabinol compounds insoluble in alkali have this origin. The essential oil (terpene and sesquiterpene) and paraffin hydrocarbon (chiefly *n*-nonacosane) are, of course, insoluble in aqueous alkali from the start.

Simply by extracting fresh marihuana with alcohol and treating with activated charcoal the writer has obtained decolorized resin, 90 per cent of which was soluble in aqueous alkali, and a substantial part of the remaining 10 per cent was paraffin hydrocarbon. Color tests have indicated only traces of the alkali-insoluble cannabinol compounds in decolorized resin from fresh young plants.

However, the statement that the alkali-soluble compounds predominate in fresh resin relates only to the cannabinol compounds in the two parts. In the whole, undecolorized resin the amount of alkali-soluble compounds may not be greater than the total of alkali-insoluble compounds of all kinds. Activated charcoal does remove relatively more alkali-insoluble material, but it is chiefly *n*-nonacosane, chlorophyll, etc.

In the past the importance and amount of the alkali-soluble compounds have been greatly undervalued, for the following reasons:

1. It has not been realized that the alkali-soluble compounds are cannabinol compounds, closely related to the most important known compounds of the insoluble part.
2. It has not been realized that alkali-soluble material may become insoluble during laboratory manipulations and does slowly become insoluble as the marihuana ages.
3. Paraffin hydrocarbon constitutes a fairly large part, in fresh resin even the greater part, of the alkali-insoluble material; whereas the alkali-soluble part consists of cannabinol compounds comparatively free from unrelated compounds.
4. Petroleum ether has generally been used to extract the resin from the crude drug; but some of the alkali-soluble compounds are already combined with alkali in the plant itself, and so are not extracted directly by petroleum ether, without acidification.

Despite the last, and possibly the second, of the foregoing factors, Casparis noted that up to 50 per cent of the resin was soluble in sodium carbonate solution, and Myttenaere gives an analysis of *charas* which was 65 per cent soluble in aqueous alkali (*20*).

The writer has tried to determine the relative proportion of the alkali-soluble and -insoluble cannabinol compounds in the whole resin of fully mature plants, using the diazo color reaction. The alkali-insoluble cannabinol compounds were separated from most of the paraffin hydrocarbon with 90 per cent methyl alcohol, in which the paraffin is but slightly soluble. Colored compounds made the determination somewhat inaccurate, but as well as could be determined, in resin from freshly cut plants late in the fall the alkali-soluble compounds account for close to 90 per cent of the total cannabinol compounds present.

The resin mostly coats the outside of the upper leaves, twigs, and flowers, so that this much alkali-insoluble may easily be formed from alkali-soluble out in the field, under the heat of the summer sun. The available facts indicate that most, and probably all, of the alkali-insoluble cannabinol compounds are formed only indirectly; that the compounds, soluble in alkali and not found in the distilled red oil, are the cannabinol compounds actually formed by the plants.

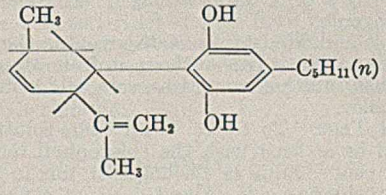
Unless there is some further change on distillation, the extracted II-NRAB must consist largely of cannabinol, and II-RAB mostly of cannabidiol. Then I-NRAB and I-RAB must be largely or even entirely the precursors of cannabinol and cannabidiol, respectively. It is certain that the hemp plants form at least two chemically distinct cannabinol compounds, but whether or not more than two are formed initially has not yet been determined. I-NRAB and I-RAB may each be fundamentally a single compound, with a small part (especially of I-NRAB) combined as a glucoside. (Such a glucoside probably accounts for the original acid Beam test.) However, remembering that the cannabinol compounds of red oil behave in most ways like a single compound and were at first mistaken for a chemical individual, it is possible that both I-NRAB and I-RAB are similarly complex.

Probable Nature of Precursors of Cannabinol and Cannabidiol

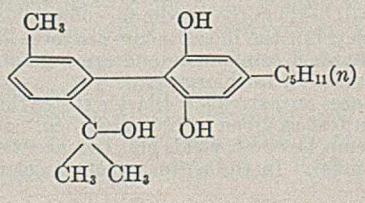
In 1933 Cahn discussed the possibility, suggested by his experiments on synthetic compounds, that a phenolic alcohol may be precursor to cannabinol (*12*). He had observed no formation of water during the distillation of the high-boiling fractions, and concluded therefrom that cannabinol is not formed during this process but had existed in his crude resin. His conclusion was probably correct, but does not follow as to fresh resin. His resin was almost entirely insoluble in alkali (*11*). The change discussed here had already taken place.

Cannabidiol was first formulated as an *o*-diphenol (*2*), but the formula now recognized, (A), has the two phenol groups meta to one another, as in resorcinol (*3*). It is therefore considered much more likely that the precursors of cannabinol and cannabidiol are at the time alcohols and *m*-diphenols, rather than *o*-diphenols. If this is the case, the formula for the precursor of cannabinol is (B). The established formula for cannabinol itself is (C) (*1*).

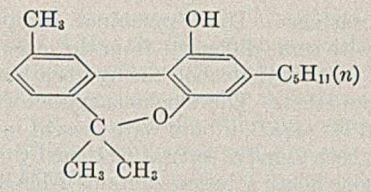
(A)



(B)

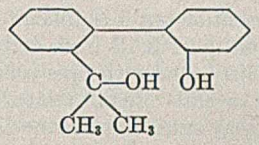


(C)



This accords with Johns' observation on the distillation of compounds soluble in aqueous alkali, and also with Cahn's observation that synthetic phenol-alcohols of type D are partly dehydrated by distillation at 30 mm. (*12*).

(D)



The only point not explained is that cannabidiol, which is certainly a *m*-diphenol, does not give the ferric chloride test. However, this test is linked in some way to solubility in aqueous alkali, and the alkali-insoluble compounds do not have this solubility even though phenol groups are certainly present.

Separations

The resin is best extracted from the marihuana with ethyl or isopropyl alcohol. Unless a quantitative study is desired, it is best to remove the chlorophyll with activated charcoal. With fresh marihuana a colorless solution can thus be obtained. However, in the case of old marihuana the resin itself has turned brown, and the solution cannot be decolorized without the loss of nearly all the resin. Instead of attempting complete decolorization, just enough charcoal should be used to remove the chlorophyll, and the procedure continued with brown resin when necessary.

Dilute the alcoholic extract with two or three times as much water, acidify with hydrochloric acid, and extract with petroleum ether. The concentration of resin in solution can be increased at this step. This is better than trying to obtain a highly concentrated alcoholic extract from the crude drug.

Most of the resin, whether colorless or brown, readily dissolves in the petroleum ether; but a small amount of yellow-brown compounds may not be extracted. Myttenaere distinguished as "phenol III" a small part of the resin relatively insoluble in petroleum ether, but readily soluble in aqueous alkali (*20*). It is probably some of this same material which is more soluble in diluted alcohol than in petroleum ether. Chloroform extracts it readily. In Myttenaere's experiments (*21*) it seemed to be an oxidation product of his "phenol II" here called I-RAB.

Extract the petroleum ether solution with aqueous alkali, using a generous volume of alkali solution, and four or five shake-outs. This easily separates I compounds from II. The writer uses a 2 per cent sodium hydroxide solution, recently boiled to remove oxygen. Sodium sulfite (2 per cent) is also helpful to prevent oxidation. Without these precautions there is likely to be considerable production of the alkaline Beam purple color during the extraction. This color results from a mild oxidation. The purple compound is soluble in aqueous alkali and insoluble in petroleum ether (until acidified), even when formed from II-RAB. Some of the yellow-brown compounds are probably similar oxidation products of the cannabinol compounds.

With old brown resin some material collects at the interface, insoluble in both layers and dark brown in color: compounds that combine with the alkali, but do not thereby become sufficiently soluble in water. This difficulty does not occur with fresh resin.

Remaining in the petroleum ether along with the alkali-insoluble cannabinol compounds are the essential oil and par-

affin hydrocarbons. The cannabinol compounds can be separated, with some difficulty, from the others by extracting with 75 per cent ethyl alcohol, the hydrocarbons remaining in the petroleum ether. This alcoholic extract will contain both II-RAB and II-NRAB, if both were present in the resin. No way has yet been found of separating them by mere extraction.

The aqueous alkali solution contains I-RAB and I-NRAB. By shaking with ethyl ether the I-NRAB goes into the ether, the I-RAB remaining in the alkali. The ether used should be free of peroxides.

This last separation is due to Myttenaere (*19*). I-NRAB corresponds to his "phenol I" and I-RAB to his "phenol II". This is a valid separation but it is difficult to make it absolute. Ether extracts even the I-RAB from carbonate solution, but the separation is fairly good with 2 per cent sodium hydroxide. Possibly there are several different related compounds in both portions, and this may cause some of the difficulty.

Shake the ether extract with dilute hydrochloric acid to free the I-NRAB from its alkali combination. Filter to remove droplets of water and evaporate the solvent without much heating.

If selected marihuana was used, containing only RAB or only NRAB, the step of ether extraction can be omitted. If only NRAB compounds are present they can be extracted like the RAB compounds in the following step.

Acidify the aqueous layer and extract the I-RAB with low-boiling petroleum ether. Filter and evaporate the solvent without much heating. This portion, as well as the mixed II compounds, is, according to Dautrebande, physiologically active (*20*).

Some yellow colored material may remain unextracted by the petroleum ether. It is easily shaken out with chloroform, and is no doubt the same as Myttenaere's "phenol III".

Conversions

Isolates of II-RAB and II-NRAB are best obtained by conversion.

Dissolve I-RAB and I-NRAB separately in absolute methyl alcohol and reflux for about 12 to 20 hours over a small flame. Then dilute the methyl alcohol with water, acidify slightly, and extract with petroleum ether. Shake the petroleum ether solution with aqueous alkali to remove unconverted alkali-soluble compounds, as well as any unrelated compounds extracted by alkali and not converted to insolubility. Wash with a little acidified water, filter, and evaporate the solvent. The writer's experiments have mostly been on a small scale. Conversion of 0.1 to 0.2 gram in 50 cc. of methyl alcohol is almost complete.

By this means II-RAB and II-NRAB can be isolated free from extraneous matter. It seems probable that each is a complex of closely related compounds. This is a controlled conversion that just produces the change from alkali-solubility to alkali-insolubility. Possibly vacuum distillation produces further changes. Presumably, however, II-RAB is mostly cannabidiol and II-NRAB largely cannabinol.

The Color Reactions

Following are specific directions for the color tests as made by the writer, with mention of the results given by the separated classes of cannabinol compounds.

Ghamrawy Test (Modified). Evaporate the solvent from a little of the resin in a casserole. Remove from the steam bath and treat with 1 cc. of Wasicky's reagent, 3 grams of *p*-dimethylaminobenzaldehyde in 100 cc. of 87 per cent sulfuric acid (85 volumes of concentrated sulfuric acid to 15 volumes of water). A color more or less orange is produced, quickly becoming intense scarlet, then gradually intense purplish red. Let stand about 30 minutes. Dilution with water changes the color to pure deep blue, which gradually fades on standing. Dilution may be made with only 3 cc. of water, and the color extracted with 1 cc. or more of benzyl alcohol, or instead of water, 9 or 10 cc. of concentrated ammonium acetate solution (75 grams per 100 cc. of water) may be added to the sulfuric acid solution. The color is changed through blue to green. Shake with 1 to 1.5 cc. of amyl alcohol. The solvent becomes an intense deep green.

The Ghamrawy test is very sensitive. It succeeds with the brown cannabinol compounds in addition to the colorless, and shows little variation with the different classes of colorless compounds.

Diazo Test A. To 1 cc. of ethyl or isopropyl alcohol solution of the resin add 5 cc. of sulfanilic acid solution (1.5 grams of pure sulfanilic acid in 490 cc. of water and 10 cc. of concentrated hydrochloric acid). Note the amount of resin thrown out by the dilution (when the concentration is not otherwise known). Add a small pinch of granulated sodium nitrite and shake. Then add sodium bicarbonate in excess. An intense yellow color is at once produced, quickly becoming orange to orange-red when much cannabinol material is present. (The dye formed is capable of coloring strongly a large volume of solution.) Acidify strongly with (1 to 3) sulfuric acid, adding about 1 cc. in excess. The color becomes bright red to muddy reddish brown. Shake with 1 cc. (or more) of benzyl alcohol. This extracts all or nearly all the color, and becomes deep dark red.

In this test RAB compounds give upon acidification a bright red solution; NRAB compounds give reddish brown insoluble material. In the writer's opinion this is the best test known for tracing the distribution of cannabinol compounds in extractions, determining the completeness of extraction, etc.

Diazo Test B. To 0.7 cc. of stronger sulfanilic acid solution (7 grams of sulfanilic acid in 475 cc. of water and 25 cc. of concentrated hydrochloric acid), in a test tube, add a few grains of sodium nitrite, then an excess of sodium bicarbonate. Pour into this 3 or 4 cc. of an ethyl or isopropyl alcohol solution of the resin. An intense yellow color is instantly produced, which gradually becomes more or less orange. After some standing add 10 cc. of water and pour through a folded filter if full of sediment. The solution is orange. Shake with 1 cc. (or more) of amyl alcohol. Typically, the amyl alcohol becomes orange to bright red, leaving the aqueous layer bright yellow.

The diazo tests succeed with the brown cannabinol compounds, as well as with the colorless. Complete decolorization of the resin is therefore unnecessary, but it is best to remove chlorophyll from the solvent solution with activated charcoal.

Mercuric Sulfate Test. To 3 cc. of a methyl alcohol solution of the resin (decolorized or at least with chlorophyll removed) add 2 cc. of Denigès' reagent (5 grams of mercuric oxide in 20 cc. of sulfuric acid and 100 cc. of water). Either at once, or on standing a short time, both color and precipitation are produced. The color is salmon or pink to scarlet, brick red, or rose-red. The precipitation may begin as a whitish cloudiness, before any color develops, or may not develop until some time after the color. The precipitate formed is of similar color to that produced in the solution, and is wholly or partly crystalline.

In this test the RAB compounds precipitate more readily than the NRAB, and the II compounds develop more color than the I.

I-RAB	Most precipitation; amorphous material and branching thread crystals. Least color; salmon
II-RAB	Precipitation somewhat less but similar to I-RAB; color stronger, tending to scarlet
I-NRAB	Precipitate forms more slowly than with RAB compounds, wholly crystalline, plates. Color pink
II-NRAB	Precipitation as with I-NRAB. Most reactive of the four classes as to color; rose-red

Duquenois and Moustapha Test (Modified). To 1 cc. of alcoholic solution of the resin add 0.5 cc. of 8 per cent vanillin solution (in alcohol), and 0.5 cc. of 2 per cent aldehyde solution (2.8 per cent aldehyde-ammonia in alcohol); then mix with 3 cc. of concentrated hydrochloric acid. An intense purple or violet color soon develops; it may begin either as pink (passing through magenta-red to purple) or as green (passing through blue, or slate and indigo), depending on the kind of resin present. The distinction is more clear if the aldehyde is omitted (vanillin test). The aldehyde makes the test a little more sensitive and uniform. In interpreting a weak result it may be advisable to try a blank with the reagents.

Duquenois and Moustapha state green as the initial color in this test (*14*). This is true of the resin from Manchurian and related varieties, but that from Minnesota wild hemp yields pink as the initial color.

Vanillin Test. To 1.5 cc. of alcoholic solution of the resin (decolorized or at least with the chlorophyll removed) add 0.5 cc. of 8 per cent vanillin in alcohol, then mix in 2 cc. of concentrated hydrochloric acid. About 4 cc. of concentrated hydrochloric acid will develop the maximum color, which then tends to purple or violet, but the initial colors are most significant of the different cannabinol compounds and are best observed with no more than 2 cc. of hydrochloric acid.

The differences are extreme. Blackie states the color produced in his vanillin test as green or blue-green (*8*). Resin from Minnesota wild hemp, however, yields pink to magenta-red (reaction of RAB compounds). In the writer's tests decolorized resin from

fairly fresh Manchurian hemp yielded light green changing to blue, and these colors were stronger and greener with resin from old Manchurian hemp (brown resin). The green or blue with decolorized resin was found to be due to the II-NRAB portion. Yellow-brown cannabinol compounds also yield green. Results on the separated portions have not been entirely consistent, but the purest colorless isolates obtained by the writer (in small quantity) gave initial colors as follows:

I-RAB	Pink	II-RAB	Pinkish lavender
I-NRAB	Pink	II-NRAB	Greenish, then blue

Alkaline Beam Test. Evaporate the solvent from a little of the decolorized resin in a white dish, and treat the residue with a few drops of colorless 2 per cent potassium hydroxide in alcohol. RAB compounds yield a purple color. II-RAB responds much more promptly and readily than I-RAB.

Modified Bouquet Test. To 0.5 gram of marihuana add 0.1 gram of activated charcoal and 6 or 7 cc. of 2 per cent potassium hydroxide in ethyl alcohol. Let stand 5 or 10 minutes, then pour through a filter which will retain the charcoal. With RAB compounds the filtrate is violet. If it is very dark brown, add a little more charcoal and refilter. If light brown or nearly colorless, mix 4 or 5 cc. of filtrate with 1.5 cc. of amyl alcohol and 15 cc. of water in an 18-mm. test tube. The amyl alcohol extracts a purple color if any RAB compounds were present.

This procedure can be used with brown resin to determine if any of the colorless RAB compounds are present. In the writer's experience this is by far the most sensitive variation of the alkaline Beam test.

Peroxide Test. Dilute 2 or 3 cc. of isopropyl (or ethyl) alcohol solution of decolorized resin with 10 cc. of water; add 1 to 3 drops of 3 per cent hydrogen peroxide and 1 drop of 1 to 1000 copper solution (1 to 250 copper sulfate pentahydrate); mix, and add 0.5 cc. of 10 per cent aqueous sodium hydroxide. With RAB compounds a purple color rapidly develops. Amyl alcohol extracts the color.

This is also a variation of the alkaline Beam reaction.

Potassium Hydroxide in Isopropyl Alcohol. To about 3 cc. of isopropyl alcohol solution of decolorized resin add 2 small pellets of potassium hydroxide. The color is produced in other solvents, but isopropyl alcohol, 98 to 99 per cent grade, is much the best for this simple form of the test. With RAB compounds a purple color soon develops, gradually becoming deeper and stronger up to the limit of the reactive compounds present. II-RAB yields the purple color at once, and maximum intensity quickly. With I-RAB the purple develops rather slowly, but in time just as strongly as with the II-RAB. This is the best variation of the alkaline Beam test for obtaining the full reaction with I-RAB.

A yellow color may be produced by the I-NRAB portion. Perhaps this is due to a glucoside of the phenol, or even to some unrelated compound accompanying it throughout the extraction. The I-RAB portion also occasionally yields a yellow before the purple color develops.

Ferric Chloride Test. To 5 cc. of solution of the decolorized resin in absolute methyl alcohol add gradually not over 0.1 cc. at a time, a fresh 1 per cent solution of ferric chloride in absolute methyl alcohol. The reagent in excess produces a green to dark green color. (If sufficient reactive substance is present, a blue or even violet color is produced with the first reagent.) With ordinary marihuana extracts about 0.4 to 0.5 cc. of reagent is usually most satisfactory; seldom is more than 1 cc. required. (*a*) To the green solution (5 to 6 cc.) add 1 cc. of water, and mix. The color is changed to dark blue. (*b*) In another test, to the green solution (5 to 6 cc.) add 1 cc. of ammonium acetate solution (1 per cent in absolute methyl alcohol). The color changes to a beautiful deep purplish red, which soon fades by degrees, the solution finally becoming brownish yellow.

The positive test described is a reaction of the I cannabinol compounds only. Both I-RAB and I-NRAB respond, but the II compounds do not. With solvents other than methanol the reaction either does not occur, or (as seems to be the case in ethyl alcohol solution) the reactive compounds are immediately oxidized by the ferric chloride.

Summary

The term "cannabinol compounds" is used as a collective name for all the compounds of the hemp resin closely related to cannabinol and cannabidiol. These have a number of "cannabinol reactions" in common.

Hemp plants form two kinds of cannabinol compounds, colorless or virtually so; one kind reactive in the alkaline Beam test, the other not (designated as RAB and NRAB). The former always predominate in Minnesota wild hemp, the latter in hemp of Manchurian origin grown in Minnesota. Some plants produce exclusively only one kind or the other.

Some of the cannabinol compounds are extracted by aqueous alkali from petroleum ether solution; some cannot be so extracted. (These compounds are designated as I and II.) This classification is confirmed by a color test with ferric chloride in methyl alcohol, to which only the compounds soluble in aqueous alkali respond.

The two classifications, by the alkaline Beam test and by solubilities, are independent, so that there are four kinds of colorless cannabinol compounds.

The compounds soluble in aqueous alkali readily become changed by heat, or even by long standing at room temperature, to alkali-insoluble compounds. In the writer's opinion all the alkali-insoluble cannabinol compounds have this origin. By decolorizing an alcoholic extract of fresh marihuana from young plants with activated charcoal, resin is obtained with as high as 90 per cent solubility in aqueous alkali. The essential oil and paraffin hydrocarbons are insoluble in aqueous alkali from the start; but referring only to the cannabinol compounds in the whole undecolorized resin, compounds soluble in aqueous alkali greatly predominate in fresh resin, even late in the fall.

Table I. Distinguishing Reactions

Cannabinol Compounds	Alkaline Beam Test	In Aqueous Alkali	Ferric Chloride	Extraction[a]
I-RAB	Purple	Soluble	Positive	AA from PE and from EE
I-NRAB	O or yellow	Soluble	Positive	AA from PE, EE from AA
II-RAB	Purple	Insoluble	Negative	PE from AA
II-NRAB	O	Insoluble	Negative	PE from AA

[a] AA = aqueous alkali, EE = ethyl ether, PE = petroleum ether.

Extractive methods and avoidance of many laboratory manipulations are necessary for study of the alkali-soluble compounds. With distilled red oil the known cannabinol compounds that have been isolated are insoluble in aqueous alkali. Unless some additional change occurs with vacuum distillation, the extracted II-RAB must consist mostly of cannabidiol and II-NRAB largely of cannabinol.

The precursors of cannabinol and cannabidiol are very likely at the same time alcohols and *m*-diphenols.

General directions are given for separations. The alkali-soluble compounds are extracted from petroleum ether by aqueous alkali. I-NRAB can be separated from I-RAB by ethyl ether and aqueous alkali.

The laboratory conversion of alkali-soluble compounds to alkali-insoluble is effected by refluxing for some 12 to 20 hours in absolute methyl alcohol. A small quantity, about 0.1 to 0.2 gram, is almost all converted by this procedure. Isolates II-RAB and II-NRAB are best obtained by separately converting I-RAB and I-NRAB and re-extracting.

Directions are given for performing the color tests, and the effects described. The separated classes show some differences even in their cannabinol reactions, those in the mercuric sulfate and vanillin tests being especially noteworthy.

Table I gives the reactions distinguishing the four kinds of colorless cannabinol compounds.

Acknowledgment

The writer is indebted to Roger Adams and his associates in marihuana research at the University of Illinois for a small amount of pure crystalline cannabidiol. It was verified on this pure substance that its reactions are in all essentials like those described above for the II-RAB portion of the resin, including the responses in the cannabinol reactions, non-extraction by aqueous alkali when shaken with the petroleum

ether solution, and absence of any observable ferric chloride test.

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Amperometric Titrations

Amperometric Determination of Phosphate with Uranyl Acetate

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SINCE phosphate is not reducible at the dropping mercury electrode, its polarographic determination or amperometric titration has to be carried out indirectly. A reagent which forms a precipitate with phosphate and yields a well-defined diffusion current must be used for its determination with the dropping mercury electrode.

A polarographic determination of phosphate by precipitation with an excess of molybdate has been described by Uhl (*21*), and an amperometric titration by means of precipitation with bismuth oxyperchlorate was attempted by Neuberger (*16*). Both methods have the advantage that the precipitation is performed in strongly acid medium in which interference by coprecipitation of alkaline earth metals, etc., does not occur. The precipitation with molybdate has also the very favorable ratio of 1 to 12 between phosphate and molybdate. Both methods, however, involve several disadvantages. The molybdate wave is badly defined and is much affected by changes in the acidity and composition of the solution. The precipitation procedure is complicated and requires heating to boiling, standing overnight, and filtering. Chloride, if present, has to be removed. The titration with bismuth oxyperchlorate as described by Neuberger yields useful results only with relatively concentrated phosphate solutions (lower limit 0.035 *M* in phosphate). The end point does not correspond to the stoichiometric composition of the precipitate and is not easily located. Moreover, all anions except perchlorate must be absent because they form complexes with bismuthyl ions (*17*).

In this paper a simple amperometric titration of phosphate with uranyl acetate is described[1].

Attempts were made to titrate phosphate with lead solutions in weakly acid medium from which alkaline earth phosphates are not precipitated. Although quantitative precipitation could be obtained, the method had serious limitations and cannot be recommended. Uranyl acetate is a well-known reagent for the gravimetric (*7*, *14*) and volumetric (potentiometric) (*1*, *2*, *9*, *13*, *18*) determination of phosphate. According to Chrétien and Kraft (*3*) uranyl acetate forms with solutions of orthophosphates precipitates of the composition UO_2MPO_4 (M = Na, K, NH_4, 1/2 Ca) which are insoluble in acetic acid but freely soluble in mineral acids. Analogous compounds are formed with arsenate (*15*) and vanadate (*14*), which also have been recommended for analytical purposes.

[1] After this paper had been submitted for publication, an important paper on the polarographic determination of phosphate was published by Stern (*20*).

Polarographic Behavior of Uranyl Acetate

The reduction wave of uranyl ion at the dropping mercury electrode was found to be greatly affected by the composition of the medium (*11*). Since the phosphate titration had to be carried out in weakly acid solution, the effect of acetic acid and buffer solutions upon the current voltage curve of uranyl acetate was studied first. These measurements do not involve an exhaustive study of the polarography of uranyl solutions, which is being carried out at present in this laboratory. The experiments described in this paper were carried out with the purpose of finding the potential and concentration range at which uranyl ion gives well-defined diffusion currents in the most suitable titration medium.

Experimental

The manual apparatus, described in previous publications (*10*), was used. The temperature was 25° C., and the solutions were free from air. For the sake of convenience a pool of mercury was usually employed as the anode. Analytically pure chemicals were used. The stock solution was 0.1 *M* in uranyl acetate, and 0.1 to 0.18 *M* in acetic acid, the latter being necessary to prevent hydrolysis. In the literature different statements are found concerning the stability of uranyl acetate solutions. Courtois (*4*) found that both concentrated and dilute solutions were stable in the dark, but that hydrolysis occurred in the presence of light. Although according to Dworzak and Reich-Rohrwig (*6*) a precipitate soon appears in a 0.05 *M* solution, the uranyl-ion concentration is not appreciably diminished, even after several months. Singh and Ahmad (*19*) found uranyl acetate to be 2.13 per cent hydrolyzed in 0.00078 *M* solution at 30° C. In the present work it was found that the solutions were stable only in presence of some free acetic acid. The minimum concentration of acetic acid in 0.1 *M* uranyl acetate solution which yielded quick dissolution of the solid uranyl acetate was 0.1 *M*.

CURRENT-VOLTAGE CURVES OF URANYL ACETATE IN WEAKLY ACID AND IN BUFFERED SOLUTIONS. In order to get constant current readings within a reasonably short time it

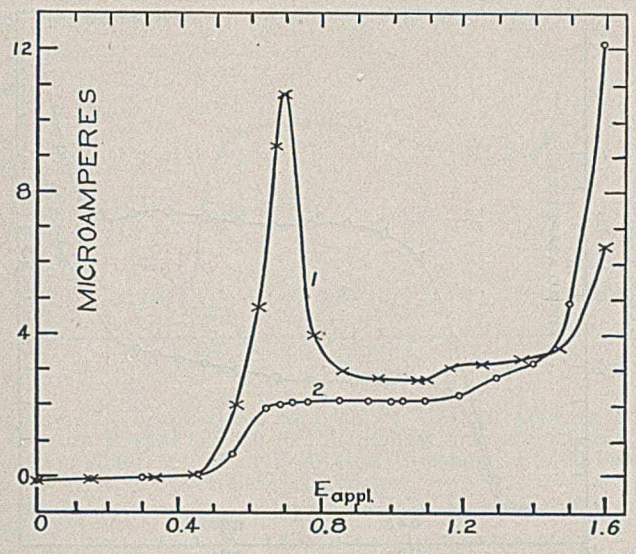

FIGURE 1. POLAROGRAMS OF 0.001 *M* URANYL ACETATE

1. In 0.05 *M* potassium nitrate, 0.04 *M* acetic acid, and 0.005 *M* ammonium acetate. 2. Same in 20% ethanol. Values not corrected for residual current.

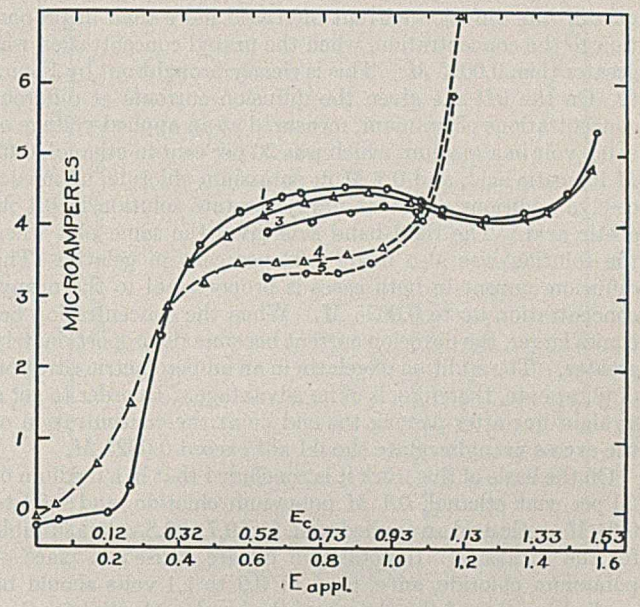

FIGURE 2. POLAROGRAMS OF 0.002 *M* URANYL ACETATE IN 0.002 *M* ACETIC ACID AND 20 PER CENT ETHANOL

1, 2, 3. In 0.1 *M* potassium chloride. 4, 5. In 0.1 *M* potassium chloride and 0.1 *M* potassium nitrate. Curve 1 measured 30 minutes after mixing; curve 2, 30 minutes later; curve 3, 2 hours after curve 2. Curve 4 measured 25 minutes after mixing; curve 5, 25 minutes later. Values not corrected for residual current. Cathode potential refers to saturated calomel electrode.

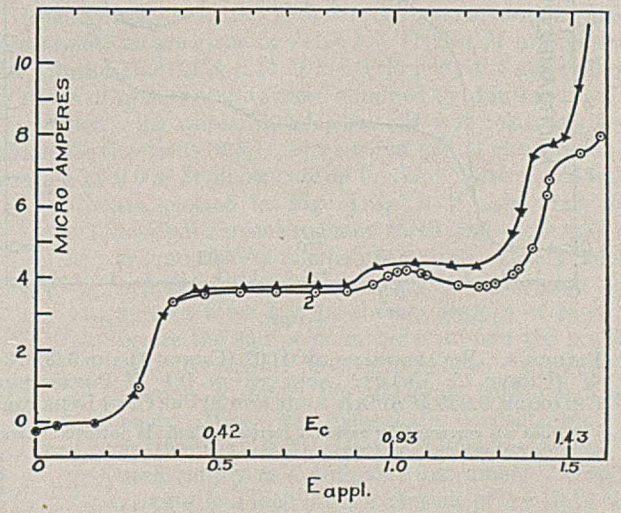

FIGURE 3. POLAROGRAMS OF 0.002 *M* URANYL ACETATE

1. In 0.042 *M* acetic acid, 0.1 *M* potassium chloride, and 20% ethanol. 2. In 0.022 *M* acetic acid, 0.1 *M* potassium chloride, and 20% ethanol. Values not corrected for residual current. Cathode potential refers to saturated calomel electrode.

was found desirable to carry out the titrations in a medium of 20 per cent ethanol. Therefore, most of the current-voltage curves of uranyl acetate were determined in this medium. The presence of alcohol also aids in suppressing or eliminating the maximum of the first uranyl wave, as is evident from Figure 1. The diffusion current of the first wave was constant over an applied voltage range between −0.8 and −1.1 volts.

Solutions which were 0.002 *M* in uranyl acetate, 0.002 *M* in acetic acid, and 0.1 *M* in potassium chloride and 20 per cent in ethanol were not stable. The diffusion current was found to decrease with increasing time of standing. The decrease was noticeable even before a precipitate was visible (Figure 2). This hydrolysis does not interfere in titrations because it takes place slowly, and moreover does not occur in the more strongly acid medium which usually was obtained after the precipitation of phosphate. The first wave of the uranyl ion, which is the only one obtained in the above solution, showed a slight flat maximum and a poorly defined diffusion current. At this uranium concentration the maximum was eliminated when the solution was also made 0.1 *M* in potassium nitrate. However, hydrolysis occurred more quickly in the presence of nitrate (Figure 2).

With increasing acetic acid concentration the wave in 20 per cent ethanol and 0.1 *M* potassium chloride became better defined. At higher acidity the first wave did not show a maximum and the diffusion current was constant between an applied e. m. f. of 0.6 and 0.85 volt. A rise in the current started at about −0.9 volt, the curve showing a peculiar maximum at −1.05 volts. The shape of the curve near the maximum was dependent on the amount of acetic acid present. At the higher acidity no hydrolysis occurred and the current-voltage curves were found unchanged after various periods of standing. Figure 3 shows the current-voltage curves obtained in 0.042 *M* and 0.022 *M* acetic acid. After the peculiar maximum the current became nearly constant, but a new wave started at about −1.25 volts. This wave was better defined in 0.042 *M* than in 0.022 *M* acetic acid. The total height at −1.45 volts was about twice the height of the first wave at −0.7 volt.

In the amperometric titration of phosphate we are mainly interested in the diffusion current of the first wave. In 0.042 *M* acetic acid and 0.1 *M* potassium chloride solution the diffusion current of the first wave was hardly affected by the addition of potassium nitrate or sodium sulfate.

In a medium which was 20 per cent in ethanol, 0.1 *M* in potassium chloride, and 0.02 to 0.05 *M* in acetic acid the first diffusion current was found proportional to the uranyl concentration up to concentrations of 0.0025 *M*. At higher concentrations the currents at various potentials were larger than expected. This, in part at least, is due to the occurrence of a maximum, as is evident from Figure 4. Addition of 0.01 to 0.2 per cent of gelatin suppressed the maximum (Figure 5), but the solutions apparently were not stable in the presence of gelatin. The diffusion current increased gradually on standing. Even if the measurements were made in the presence of

gelatin, the diffusion current increased more than in proportion to the concentration, when the uranyl concentration was greater than 0.0025 *M*. This is clearly brought out by Figure 6. On the left are given the diffusion currents at different concentrations of uranium, measured at an applied voltage of −0.8 volt in a medium which was 20 per cent in ethanol, 0.02 *M* in acetic acid, and 0.1 *M* in potassium chloride, upon successive additions of 0.1 *M* uranyl acetate solution in 0.1 *M* acetic acid. The right-hand side gives the same plot when the solution was also made 0.02 per cent in gelatin. The diffusion current in both cases is proportional to the uranyl concentration up to 0.0025 *M*. When the concentration becomes larger, the diffusion current becomes disproportionately greater. The addition of gelatin in an amperometric titration of phosphate, therefore, is of no advantage. In order to get a straight line after passing the end point the concentration of the excess uranyl acetate should not exceed 0.0025 *M*.

On the basis of this work it is concluded that in a medium of 20 per cent ethanol, 0.1 *M* potassium chloride, and 0.02 to 0.04 *M* acetic acid an applied e. m. f. of 0.7 to 0.8 volt is suitable for the titrations. If potassium nitrate is used in place of potassium chloride, an e. m. f. of 0.9 to 1.1 volts should be applied because of the change of the anode potential (pool of mercury).

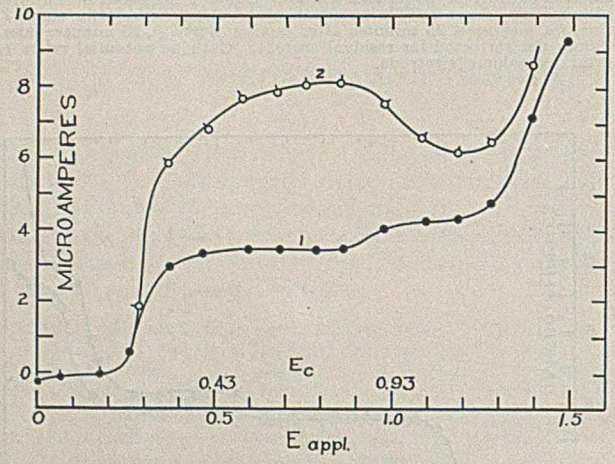

FIGURE 4. POLAROGRAMS OF 0.002 (CURVE 1) AND 0.00373 *M* (CURVE 2) URANYL ACETATE IN 0.1 *M* POTASSIUM CHLORIDE, 0.055 *M* ACETIC ACID, AND 20 PER CENT ETHANOL

Values not corrected for residual current. Cathode potential refers to saturated calomel electrode.

The titration of phosphate has also been investigated in buffers of acetic acid and acetate in a medium of 20 per cent ethanol. Therefore, current-voltage curves of uranyl acetate were also investigated in such buffer solutions. A solution of 0.002 *M* uranyl acetate in 0.2 *M* sodium acetate and 0.022 *M* acetic acid and 0.1 *M* potassium chloride, which had a pH of 5.6, gave a current-voltage curve shown in Figure 7. The curve is similar to the one obtained in 0.002 *M* acetic acid (see Figure 2) except that it was displaced 0.1 volt to more negative potentials. The solution in dilute acetic acid was not stable but on standing deposited a precipitate as a result of hydrolysis. In spite of the much higher pH, the uranyl solution in the buffer was stable and did not form a precipitate on standing. This is explained by complex formation between uranyl and acetate. Dittrich (*5*) found that in more concentrated solutions of sodium acetate the uranium was transported to the anode while in dilute solution it moved to the cathode. The complex formation also accounts for the fact that the wave in the buffer solution occurred at more negative potentials than in dilute acetic acid alone.

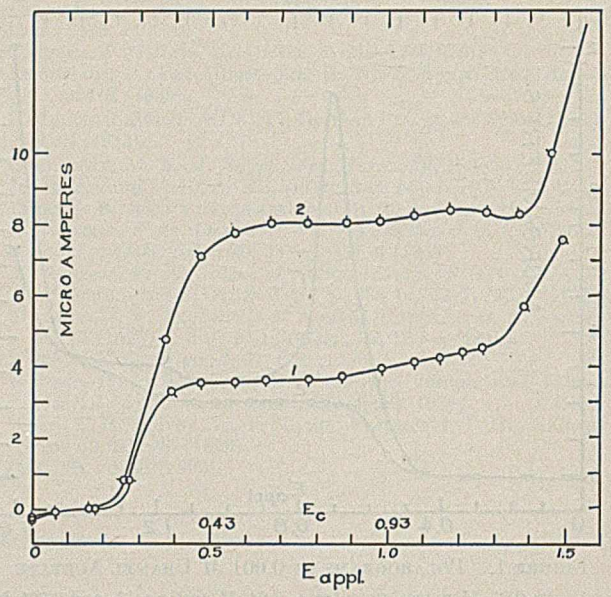

FIGURE 5. POLAROGRAMS OF 0.002 *M* (CURVE 1) AND 0.004 *M* (CURVE 2) URANYL ACETATE IN 0.1 *M* POTASSIUM CHLORIDE AND 20 PER CENT ETHANOL

1 in 0.01% gelatin, 2 in 0.02% gelatin. Values not corrected for residual current. Cathode potential refers to saturated calomel electrode.

Amperometric Titration of Phosphate

After many preliminary experiments, it was decided to carry out the titration in a medium containing 20 per cent ethanol. Alcohol decreases the solubility of the precipitate UO_2MPO_4, and before the end point it considerably reduces the time

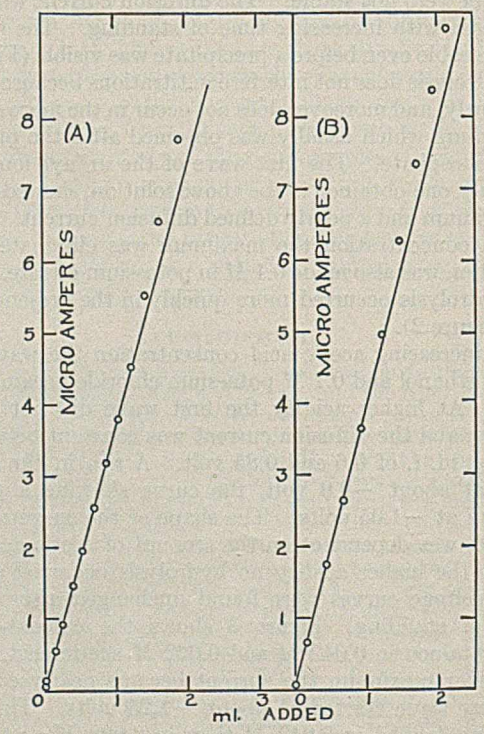

FIGURE 6. DIFFUSION CURRENTS OF INCREASING URANYL CONCENTRATIONS

Successive addition of 0.1 *M* uranyl acetate to 50 ml. of 0.1 *M* potassium chloride and 0.02 *M* acetic acid in 20% ethanol. (*a*) Without gelatin, $E_{appl.}$ 0.8 volt; (*b*) with 0.02% gelatin, $E_{appl.}$ 0.7 volt. Correction applied for dilution.

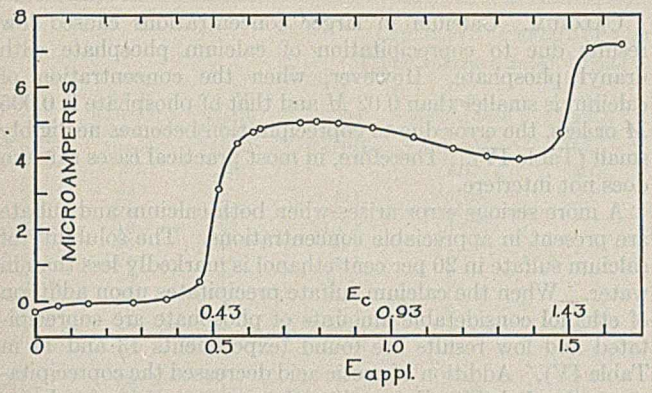

FIGURE 7. POLAROGRAM OF 0.002 *M* URANYL ACETATE IN ACETATE BUFFER pH 5.6 (0.2 *M* SODIUM ACETATE, 0.022 *M* ACETIC ACID), 0.1 *M* IN POTASSIUM CHLORIDE, AND 20 PER CENT IN ETHANOL

Values not corrected for residual current. Cathode potential refers to saturated calomel electrode.

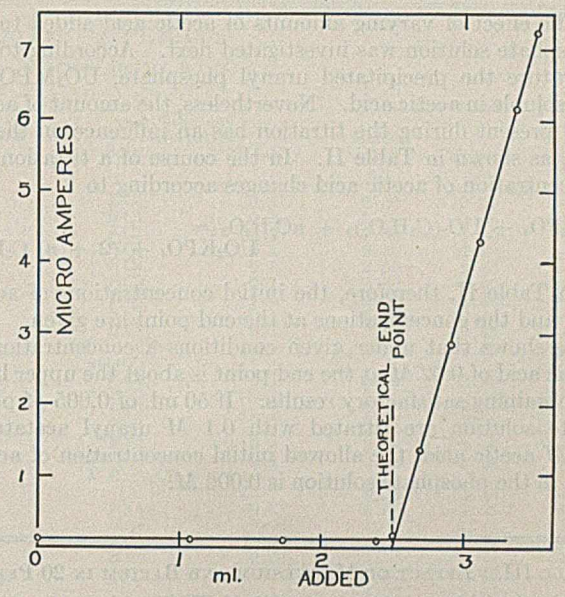

FIGURE 8. TITRATION OF 50 ML. OF 0.005 *M* PHOSPHATE SOLUTION IN 0.1 *M* POTASSIUM CHLORIDE AND 20 PER CENT ETHANOL WITH 0.1 *M* URANYL ACETATE

$E_{appl.}$ 0.8 volt. Correction applied for dilution.

necessary for attaining a constant reading of the current after each addition of uranyl acetate. In the following the authors usually titrated 50 ml. of a solution of monopotassium phosphate in 20 per cent ethanol under the conditions specified in the tables.

A layer of mercury was introduced into the cell to serve as an anode. With the pool of mercury as anode the applied e. m. f. was 0.7 to 0.8 volt when the solution was 0.05 to 0.2 *M* in chloride. If chloride was absent and potassium nitrate was used as the supporting electrolyte, the applied e. m. f. was 1.05 ± 0.05 volt. When an outside saturated calomel electrode is used as an anode, the potential applied to the dropping electrode should be −0.7 ± 0.05 volt. The titrations were carried out at room temperature. Nitrogen (or hydrogen) was passed through the solution until the oxygen had been removed. The uranyl acetate was added in successive portions from a microburet graded in 0.01 ml., nitrogen being passed through for 2 to 3 minutes after each addition. The current readings, after correcting for the dilution, were plotted against the volume of reagent added and the end point was found graphically (*12*).

Results obtained in the titration of monopotassium phosphate solutions are given in Table I. The 0.1 *M* uranyl acetate solution was 0.1 or 0.14 *M* in acetic acid; the more dilute solutions of uranyl acetate were obtained by diluting the 0.1 *M* solution with water; hence, the pH was relatively low during the titrations (3.5). The current values measured in a titration of 0.005 *M* phosphate in 0.1 *M* potassium chloride are plotted in Figure 8. It is seen that the residual current remains small and constant (of the order of 0.1 microampere) until the end point. Hence, it is only necessary to determine the current after two or three additions of reagent before the end point in order to find the practically horizontal precipitation line. Thus it is possible to finish the titrations within half an hour. When the phosphate concentration is greater than 0.004 *M*, the galvanometer can be used simply as a nullpoint instrument. Uranyl acetate is added with exclusion of air until the deflection of the galvanometer suddenly increases (Table I). The accuracy of the titrations is very satisfactory. Concentrations of phosphate greater than 0.0003 *M* can be determined with an accuracy of 1 per cent or better. When the concentration gets smaller, the accuracy decreases because these concentrations are near the lower limit of ordinary polarographic work. Titrations 11 and 12 in Table I show, however, that in 0.00008 *M* phosphate solution an accuracy of 5 per cent could be obtained with two titrations. The addition of ammonium salts, usually recommended in order to obtain well crystalline $UO_2NH_4PO_4$, had no influence upon the titrations.

TABLE I. TITRATION OF MONOPOTASSIUM PHOSPHATE WITH URANYL ACETATE

No.	Molarity of KH_2PO_4	Indifferent Electrolyte Salt	Indifferent Electrolyte Molarity	Concentration of Uranyl Acetate *M*	Uranyl Acetate Used Calculated *Ml.*	Uranyl Acetate Used Found *Ml.*	Error %
1	0.005	KNO_3	0.2	0.1	2.50	2.48	−0.8
2	0.005	KCl	0.2	0.1	2.50	2.48	−0.8
3	0.005	KCl	0.1	0.1	2.50	2.48	−0.8
4	0.005	KCl	0.1	0.1	2.50	2.51	+0.4
5	0.005	KCl	0.1	0.1	2.50	2.495	−0.2
6	0.005	KCl + KNO_3	0.1 + 0.05	0.1	2.50	2.49	−0.4
7	0.002086	KCl	0.1	0.05	2.086	2.075	−0.5
8	0.0002	KCl	0.02	0.01	1.00	0.99	−1.0
9	0.0001	KCl	0.02	0.01	0.50	0.47	−6.0
10	0.00010093	KNO_3	0.02	0.005	1.009	1.01	0.0
11	0.00007845	KNO_3	0.02	0.005	0.7845	0.84	+7.1 } +4.6
12	0.00007845	KNO_3	0.02	0.005	0.7845	0.80	+2.0 }
13	0.00005097	KNO_3	0.02	0.005	0.505	0.56	+11

TABLE II. EFFECT OF ACETIC ACID CONCENTRATION UPON TITRATION

No.	Molarity of KH_2PO_4	Indifferent Electrolyte Salt	Indifferent Electrolyte Molarity	Molarity of Uranyl Acetate Solution Used $UO_2(C_2H_3O_2)_2$	Molarity of Uranyl Acetate Solution Used $C_2H_4O_2$	Molarity of Acetic Acid At beginning	Molarity of Acetic Acid At end point	Uranyl Acetate Used Calculated *Ml.*	Uranyl Acetate Used Found *Ml.*	Error %
1	0.005	KCl	0.1	0.1	0.18	0.002	0.02	2.50	2.48	−0.8
2	0.005	KCl	0.1	0.1	0.1	0.02	0.0333	2.50	2.46	−1.6
3	0.005	KCl	0.1	0.1	0.18	0.02	0.0371	2.50	2.44	−2.4
4	0.005	KCl	0.1	0.1	0.1	0.04	0.0524	2.50	2.45	−2.0
5	0.005	KCl + KNO_3	0.1 + 0.05	0.1	0.1	0.04	0.0524	2.50	2.44	−2.4
6	0.005	KCl	0.1	0.1	0.18	0.04	0.0562	2.50	2.43	−2.8
7	0.0001009	KNO_3	0.1	0.005	0.008 + 0.001 ammonium acetate	0.04	0.0401 + 0.00005 ammonium acetate	1.01	1.05	+4

The effect of varying amounts of acetic acid added to the phosphate solution was investigated next. According to the literature the precipitated uranyl phosphate, UO_2MPO_4, is not soluble in acetic acid. Nevertheless, the amount of acetic acid present during the titration has an influence on the result, as shown in Table II. In the course of a titration the concentration of acetic acid changes according to

$$KH_2PO_4 + UO_2(C_2H_3O_2)_2 + nC_2H_4O_2 = UO_2KPO_4 + (2 + n)\ C_2H_4O_2$$

In Table II, therefore, the initial concentrations of acetic acid and the concentrations at the end point are given. The table shows that under given conditions a concentration of acetic acid of 0.02 *M* at the end point is about the upper limit for obtaining satisfactory results. If 50 ml. of 0.005 *M* phosphate solution are titrated with 0.1 *M* uranyl acetate in 0.1 *M* acetic acid, the allowed initial concentration of acetic acid in the phosphate solution is 0.006 *M*.

Table III. Effect of Magnesium and Barium in 20 Per Cent Ethanol

(Total volume titrated, 50 ml. of 0.005 *M* KH_2PO_4 solution; concentration of uranyl acetate, 0.1 *M*)

No.	1 *M* $MeCl_2$ Solution Added	Molar Ratio Me:PO_4	1 *M* Acetic Acid Added before Titrating	Indifferent Electrolyte Salt	Indifferent Electrolyte Molarity	Uranyl Acetate Used Calculated	Uranyl Acetate Used Found	Error
	Ml.		*Ml.*			*Ml.*	*Ml.*	%
1	0.1 $MgCl_2$	1:2.5	..	KCl	0.1	2.50	2.49	−0.4
2	1 $MgCl_2$	4:1	..	KCl	0.1	2.50	2.49	−0.4
3	1 $BaCl_2$	4:1	2	KCl	0.1	2.50	2.48	−0.8
4	2 $BaCl_2$	8:1	1	KCl	0.1	2.50	2.48	−0.8

The titration was also performed in buffered solutions. In citrate and in oxalate solutions uranyl ion obviously forms complexes which delay the precipitation of phosphate and interfere with the titration. Correct results were obtained in acetate buffer of pH 4.7 but the precipitation was slow and therefore impracticable. At a pH between 5 and 6 the titration could be carried out in the usual way, but the precipitation remained slow near the end point. In a medium which was 0.2 *M* in sodium acetate and 0.02 to 0.06 *M* in acetic acid and 20 per cent in ethanol, good results were obtained when the phosphate concentration was 0.005 *M* or greater. In more dilute phosphate solutions the precipitation was too slow for practical purposes. In biphthalate buffers with a pH between 5.6 and 3.8 the precipitation was rapid. After the end point, however, the current did not increase linearly with the concentration of uranyl acetate. Still, the end point can be found satisfactorily when the galvanometer is used as a nullpoint instrument and the titration is carried out until the deflection suddenly increases.

Effect of Other Substances on Titration

Organic Acids. Because of the interference of organic anions like citrate, oxalate, much acetate, and probably many others (tartrate, formate, etc.) which form complexes with uranyl ion, it is recommended that organic matters be decomposed by ashing or by treating with sulfuric acid (and nitric acid) similar to the decomposition according to Kjehldahl.

Magnesium and Barium. Magnesium, when present in not too large amounts, did not interfere with the titration. Relatively large amounts of barium also can be present if the precipitation of barium phosphate in the presence of 20 per cent ethanol is prevented by addition of acetic acid (Table III). In experiment 3 with an initial concentration of 0.04 *M* acetic acid in the solution a correct result was obtained, while in corresponding experiments without barium an error of about −2.5 per cent was found (Table II).

Calcium. Calcium in larger concentrations caused low results due to coprecipitation of calcium phosphate with uranyl phosphate. However, when the concentration of calcium is smaller than 0.02 *M* and that of phosphate is 0.005 *M* or less, the error due to coprecipitation becomes negligibly small (Table IV). Therefore, in most practical cases calcium does not interfere.

A more serious error arises when both calcium and sulfate are present in appreciable concentrations. The solubility of calcium sulfate in 20 per cent ethanol is markedly less than in water. When the calcium sulfate precipitates upon addition of ethanol considerable amounts of phosphate are coprecipitated and low results are found (experiments 13 and 14 in Table IV). Addition of acetic acid decreased the coprecipitation only slightly. Even when the precipitation of calcium sulfate took place from about 0.04 to 0.08 *M* hydrochloric acid, there was a marked coprecipitation of phosphate, although it was less than when the precipitation took place in more weakly acid medium (experiments 17 to 24 in Table IV). Before the addition of alcohol, enough hydrochloric acid was added to make the final concentration of chloride in the titration mixture 0.04 to 0.08 *M*. After addition of the ethanol the solution was neutralized with sodium hydroxide, taking the green color of bromocresol green as the end point.

The error due to coprecipitation of phosphate with calcium sulfate depends upon the calcium, sulfate, and phosphate concentrations, and it is not possible to give minimum concentrations of the three separately at which no error occurs. In a mixture of 25 ml. of 0.01 *M* monopotassium phosphate, 1 ml. of 1 *M* calcium chloride, and 0.5 ml. of 1 *M* sodium sulfate diluted to 50 ml., the results were still correct. When 1 ml. instead of 0.5 ml. of sodium sulfate was added, the error was −1.4 per cent and with 2 ml. of sodium sulfate −5.2 per cent. In general, the error caused by the presence of both calcium and sulfate is negligibly small when upon addition of alcohol little or no precipitate is formed. If the amount of precipitated calcium sulfate is appreciable, a separation has to be made. When organic anions which form complexes with uranyl (citrate, etc.) are absent, the easiest method is by precipitation of the phosphate in ammoniacal solution as calcium phosphate. The precipitate, which need not be washed, is dissolved in hydrochloric acid and the solution is neutralized with sodium hydroxide and bromocresol green as indicator. In this way phosphate determinations were carried out in the presence of a hundredfold molar excess of calcium with an accuracy of 0.5 per cent. When organic matter which has to be decomposed is present, the interference of calcium can be eliminated at the same time. From the data just given regarding the amounts of calcium and sulfate which can be present in 0.005 *M* phosphate solution without causing interference, it can be seen that the concentration limit corresponds approximately to a saturated calcium sulfate solution (about 0.015 *M*). However, even when higher concentrations of calcium and sulfate were present, a precipitate was not formed until alcohol was added. When the presence of organic matter makes necessary a decomposition by treating with strong sulfuric acid (and nitric acid) the remaining concentration of calcium sulfate after the treatment with acid does not interfere in the phosphate determination. Such a treatment is usually also used for the dissolution of insoluble phosphates. For the present purposes the removal of calcium in the presence of organic acids can also be accomplished by ashing with an excess of sodium carbonate.

Iron. Iron, both in the ferrous and ferric states, interferes with the titration. The authors found that the simplest way to remove the iron was by precipitation with cupferron.

TABLE IV. EFFECT OF CALCIUM IN 20 PER CENT ETHANOL

(Total volume titrated, 50 ml.; concentration of uranyl acetate, 0.1 *M*)

No.	0.01 *M* KH_2PO_4 Solution Used	1 *M* $CaCl_2$ Solution Used	Molar Ratio $Ca:PO_4$	1 *M* Acetic Acid Added before Titrating	1 *M* $NaSO_4$ Solution Used	2 *M* HCl Added before Precipitation of $CaSO_4$	Indifferent Electrolyte		Uranyl Acetate Used		
							Salt	Molarity	Calculated	Found	Error
	Ml.	*Ml.*		*Ml.*	*Ml.*	*Ml.*			*Ml.*	*Ml.*	%
1	25	1	4:1	0.05	...	...	KCl	0.1	2.50	2.47	− 1.2
2	25	2	8:1	0.05	...	...	KCl	0.1	2.50	2.46	− 1.6
3	10	1	10:1	0.05	...	...	KCl	0.1	1.00	1.00	0.0
4	25	1	4:1	..	...	...	KCl	0.1	2.50	2.48	− 0.8
5	25	1	4:1	1	...	...	KCl	0.1	2.50	2.45	− 2.0
6	25	1	4:1	1	...	...	KCl	0.1	2.50	2.46	− 1.6
7	25	4	16:1	1	...	...	KCl	0.1	2.50	2.435	− 2.6
8	25	1	4:1	2	...	...	KCl	0.1	2.50	2.43	− 2.8
9	25	2	8:1	2	...	...	KCl	0.1	2.50	2.42	− 3.2
10	10	1	10:1	2	...	...	KCl + 0.02% gelatin	0.1	1.00	1.01	+ 1.0
11	25	1	4:1	0.05	0.5	...	KCl	0.1	2.50	2.50	0.0
12	25	1	4:1	0.05	1	...	KCl	0.1	2.50	2.465	− 1.4
13	25	1	4:1	0.05	2	...	KCl	0.1	2.50	2.37	− 5.2
14	25	1	4:1	0.05	5	...	KCl	0.1	2.50	2.35	− 6.0
15	25	1	4:1	2	5	...	KCl	0.1	2.50	2.40	− 4.0
16	25	1	4:1	2	5	...	KCl + 1 ml. 1 *M* $BaCl_2$	0.1	2.50	2.26	− 9.6
17	25	0.5	2:1	..	5	2	..	..	2.50	2.46	− 1.6
18	25	0.5	2:1	..	5	2	NaAc HAc	0.4[a] 0.04	2.50	2.48	− 0.8
19	25	0.5	2:1	..	5	2	NaAc HAc	0.4[a] 0.04	2.50	2.51	+ 0.4
20	25	1	4:1	..	5	2	..	..	2.50	2.46	− 1.6
21	25	1	4:1	..	5	2	..	..	2.50	2.45	− 2.0
22	25	1	4:1	..	5	4	..	..	2.50	2.44	− 2.4
23	25	2	8:1	0.1	2	2	..	..	2.50	2.30	− 8.0
24	25	5	20:1	..	10	2	NaAc HAc	0.2[b] 0.06	2.50	2.20	−12.0

[a] pH = 5.7. [b] pH = 5.3.

The solution is made 10 per cent in hydrochloric acid and cooled in ice, and the iron is precipitated with about 0.5 *M* cupferron solution. The free phenyl nitrosohydroxylamine and its decomposition products, which are quickly formed in acid medium, yield polarographic waves. Therefore, the iron precipitate with the excess of cupferron and its decomposition products were removed by shaking out with ether. The resulting aqueous phase was freed from ether (by gently heating or bubbling nitrogen through), neutralized to bromocresol green, and titrated after addition of alcohol. As an example, the following analysis is reported.

To a mixture of 25 ml. of 0.01 *M* monopotassium phosphate solution and 1 ml. of 1 *M* ferric chloride solution were added 3 ml. of concentrated hydrochloric acid, and after cooling in ice about 7 ml. of 0.5 *M* cupferron solution. After separation as described above, the aqueous solution was diluted to exactly 100 ml., and 50 ml. were then added to 10 ml. of ethanol and titrated with 0.1 *M* uranyl acetate solution. The error of the titration was −0.8 per cent.

OTHER INTERFERING SUBSTANCES. Interference will occur with all metals which at a pH of about 3.5 precipitate phosphate—e. g., lead, aluminum, and trivalent chromium. Chemical separations might be possible in these cases. Pyrophosphate and other anions, such as arsenate and vanadate, which precipitate with uranyl acetate interfere with the phosphate titration. According to Dworzak and Reich-Rohrwig (*6*), pyrophosphate forms soluble complexes with uranyl: $[UO_2(P_2O_7)_2]^{------}$. Obviously these complexes are not very stable, because when working in a medium of 20 per cent ethanol, pyrophosphate was immediately precipitated with uranyl. It seems possible to eliminate pyrophosphate by precipitation with cadmium according to Hull (*8*).

The precipitation of uranyl by arsenate can also be used for an amperometric titration of arsenate. Preliminary experiments performed by titrating a 0.005 *M* arsenate solution in a medium of 20 per cent ethanol under the same conditions as used in the titration of phosphate gave results accurate to about 1 per cent.

Procedure

On the basis of the various experiments the following general procedure is given.

REAGENTS. *Uranyl Acetate.* A 0.1 *M* solution is prepared by dissolving 42.422 grams of chemically pure uranyl acetate, $UO_2(C_2H_3O_2).2H_2O$, in a 1-liter volumetric flask by shaking with about 300 ml. of water and 6 to 10 ml. of glacial acetic acid. After complete dissolution the flask is filled up to volume. The dissolution can be accelerated by careful heating. The solution can be standardized against standard phosphate solution. For titrations of low phosphate concentrations the uranyl acetate solution is diluted with water.

Standard Phosphate. Merck's preparation "Sörensen" of monopotassium phosphate is dried at 110° C. for 1 hour and a 0.01 *M* solution is prepared by dissolving 1.3614 grams in 1 liter.

Potassium Chloride. A 1 *M* or 2 *M* solution is prepared by dissolving 74.56 or 149.12 grams of potassium chloride of Merck's purest quality in 1 liter.

PROCEDURE. The dissolved sample is diluted, so that in the final volume (all additions included) the concentration of phosphate is not greater than approximately 0.01 *M*, or if calcium (and sulfate) are present, not greater than 0.005 *M*. The concentration of calcium and sulfate must not be greater than 0.02 *M* and 0.01 *M*, respectively. If a previous neutralization is necessary, a few drops of bromocresol green are added to the solution and sodium hydroxide or hydrochloric acid is added until the indicator has a green color. About 0.5 ml. of 0.1 *M* acetic acid is added and enough potassium chloride to make the chloride concentration approximately 0.1 *M*. The sample is introduced into the titration cell and enough ethanol added to make its concentration 20 per cent. Air is removed by passing nitrogen through the solution for about 15 minutes (the removal is complete when the residual current has become constant), and the titration is carried out at an applied potential of −0.7 to −0.8 volt.

NOTES. If a precipitate of barium phosphate is formed by the addition of ethanol, it is dissolved by adding 1 *M* acetic acid dropwise.

In the presence of large amounts of both calcium and sulfate the phosphate is first precipitated in ammoniacal medium and then treated as described above.

Iron if present is removed with cupferron as described above.

In the presence of organic substances like citrate, tartrate, oxalate, etc., which form complexes with uranyl and in the analysis of organic phosphorus, a destruction of the organic matter is necessary. In the latter instance the total content of phosphorus is determined as orthophosphate.

Summary

The polarographic waves of uranyl acetate in weakly acid media were studied.

A procedure has been given for the amperometric titration of phosphate with uranyl acetate at room temperature. The accuracy was 1 per cent or better with 0.01 to 0.0003 *M* con-

centrations of orthophosphate. In 0.0001 *M* phosphate the accuracy was of the order of 4 per cent.

Alkaline earth phosphates can be titrated by the standard procedure. Calcium in large amounts, iron, and organic anions interfere. Methods are described to eliminate the interference.

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Quantitative Determination of 2-Methyl-1,4-naphthoquinone

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THE increased use of 2-methyl-1,4-naphthoquinone as a therapeutic agent necessitated the development of an accurate and convenient method for its quantitative estimation in marketed preparations. The procedure described in this paper has been found reliable for the determination of 2-methyl-1,4-naphthoquinone in quantities as low as 0.05 mg., and has been employed successfully in the assay of oil solutions and of alcoholic extracts containing the drug. In a study of the reactions involved in this determination, the 2,4-dinitrophenylhydrazone of 2-methyl-1,4-naphthoquinone was isolated and characterized, and its absorption spectrum examined in both alkaline and neutral solvents.

Pinder and Singer (*3*) described a colorimetric procedure for the determination of 2-methyl-1,4-naphthoquinone which involves the interaction of that substance with ethyl cyanoacetate. The unstable, purple colored reaction product thus obtained is subsequently hydrolyzed with 6 *N* potassium hydroxide to produce a more stable yellow colored derivative in solution. The yellow solution is then compared with a suitable standard. Results of analysis obtained by the method of Pinder and Singer (*3*) were found, in this laboratory, to be sufficiently accurate, if the naphthoquinone could first be isolated by alcoholic extraction. The method appeared to be inadequate when applied to vegetable oil solutions from which the naphthoquinone could not be quantitatively extracted. Partial saponification of the vegetable oil, which occurred on the addition of potassium hydroxide, produced turbid solutions that required considerable treatment to effect clarification. It was observed also that the presence of oil complicated the formation and subsequent hydrolysis of the intermediate, unstable purple reaction product.

As an alternative, it was considered that the direct spectrophotometric determination of 2-methyl-1,4-naphthoquinone in oil solution, employing the absorption maximum at 3360 Å., might provide a convenient method of assay. However, direct spectrophotometric examination (*1*) was found to be limited in application to those solutions in which the oil used as a vehicle exhibited low absorption in the desired spectral region. The method was of little value when applied to certain commercial products which contained vegetable oil that showed complete absorption at 3360 Å.

Novelli (*2*) described a sensitive color test for 2-methyl-1,4-naphthoquinone and related substances which forms the basis for the method finally adopted in this laboratory for the quantitative estimation of 2-methyl-1,4-naphthoquinone. The procedure detailed below depends on (*a*) formation of the 2,4-dinitrophenylhydrazone of 2-methyl-1,4-naphthoquinone, (*b*) interaction of this dinitrophenylhydrazone with ethanolic ammonia to yield a green to blue-green colored solution, and (*c*) comparison of the intensity of this color with that produced in a control with known quantities of the naphthoquinone.

The 2,4-dinitrophenylhydrazone of 2-methyl-1,4-naphthoquinone in alcoholic ammonia solutions exhibits a bright blue color. The green color described by Novelli (*2*) was found to be a combination of this blue color with the yellow color of the 2,4-dinitrophenylhydrazine present in excess. For quantitative determinations it was found impracticable to eliminate entirely the yellow color of the excess reagent; conse-

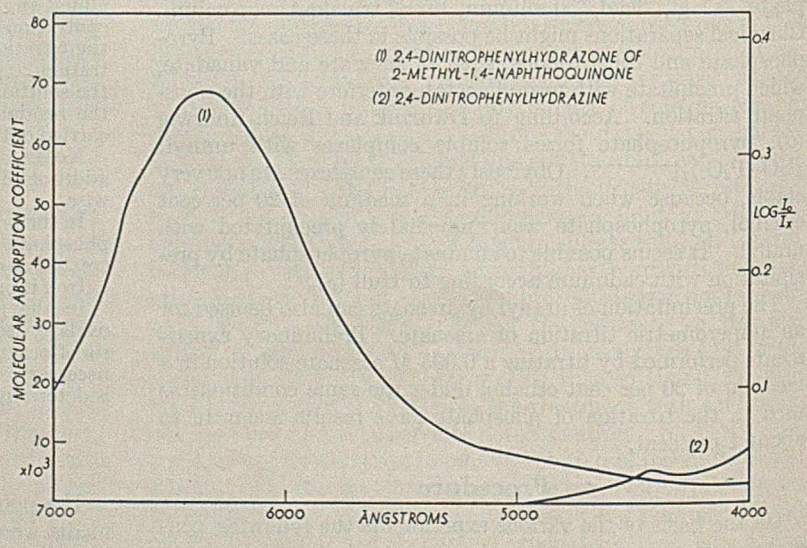

FIGURE 1. LIGHT ABSORPTION OF SOLUTIONS OF THE 2,4-DINITROPHENYLHYDRAZONE OF 2-METHYL-1,4-NAPHTHOQUINONE AND OF 2,4-DINITROPHENYLHYDRAZINE

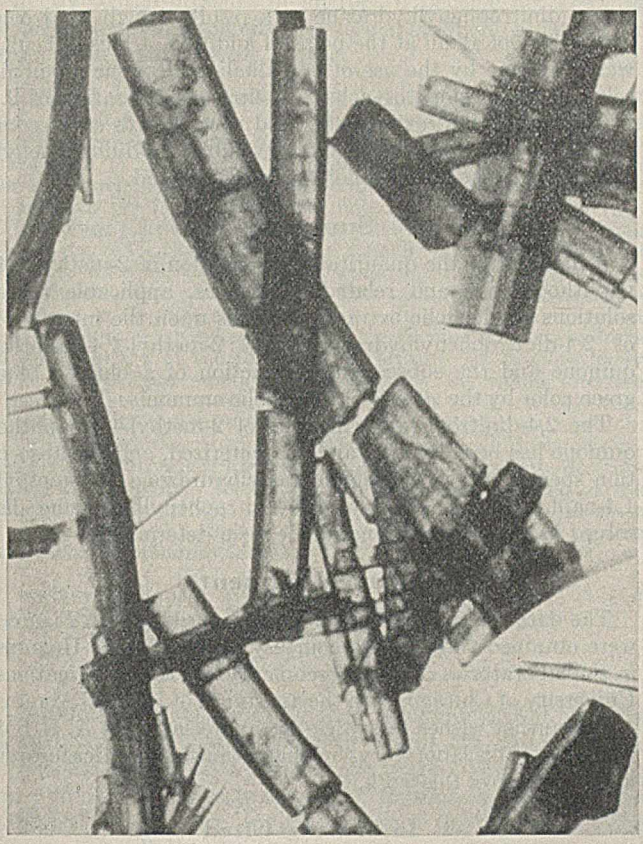

FIGURE 2. 2,4-DINITROPHENYLHYDRAZONE OF 2-METHYL-1,4 NAPHTHOQUINONE (× 100)

quently the solutions compared colorimetrically were always green to blue-green. Identical shades of green could be obtained in the known and unknown test solutions by controlling the amount of the unknown sample taken for a determination.

Curves representing the light absorption of the 2,4-dinitrophenylhydrazone of 2-methyl-1,4-naphthoquinone and of 2,4-dinitrophenylhydrazine are shown in Figure 1.

The data for these curves were obtained with a Cenco-Sheard spectrophotelometer. The 2,4-dinitrophenylhydrazone was dissolved in a mixture of one part ethanol and one part concentrated ammonium hydroxide (specific gravity 0.90). The solution of 2,4-dinitrophenylhydrazine was prepared by dissolving 2.5 mg. of this substance in 5.0 ml. of 2 *N* hydrochloric acid contained in a 100-ml. volumetric flask, adding 10 ml. of ethanol followed by 10 ml. of a mixture of one part ethanol to one part concentrated ammonium hydroxide, and finally filling to the mark with 95 per cent ethanol.

Molecular absorption coefficients were plotted for curve 1, Figure 1, representing the absorption of the dinitrophenylhydrazone, which has an absorption maximum at 635 mμ. The curve representing the absorption of 2,4-dinitrophenylhydrazine was then plotted to illustrate the relative absorptions of the two components in the solution as used for quantitative determinations. From the curves it may be noted that 2,4-dinitrophenylhydrazine, under the conditions of the determination, possesses no conflicting absorption in the region where the dinitrophenylhydrazone exhibits an absorption maximum. It is suggested that measurements with a spectrophotometer, adjusted for operation at 635 mμ, would increase the accuracy of the method.

The 2,4-dinitrophenylhydrazone of 2-methyl-1,4-naphthoquinone was readily obtained according to the following procedure:

A saturated solution of 2,4-dinitrophenylhydrazine in 2 *N* hydrochloric acid was added to an ethanolic solution of 2-methyl-1,4-naphthoquinone; the solution was warmed at 60° to 70° C. for a few minutes and then slowly cooled. Upon recrystallization from hot chloroform the crystalline product yielded bright orange needles and clusters (Figure 2), which sublimed when heated above 200° C. to form long orange needles, and melted with decomposition at 299° C. Analysis for nitrogen gave the following results:

Calculated for $C_{17}H_{12}O_8N_4$ (352.30)	N, 15.92
Found	N, 16.0

The crystalline dinitrophenylhydrazone was very slightly soluble in neutral solvents but dissolved readily on the addition of ammonia or other alkaline reagents with the production of a bright blue color (spectrum, Figure 1). The enhanced solubility and the change in color from orange to blue produced by the addition of bases was attributed to the formation of the aci-salt of the nitro groups in the dinitrophenylhydrazine nucleus, with the consequent production of an *o*- or *p*-quinone configuration coupled to the 2-methyl-1,4-naphthoquinone structure. By careful neutralization of the blue alkaline solutions, orange crystals of the dinitrophenylhydrazone could be recovered.

The *p*-nitrophenylhydrazone of 2-methyl-1,4-naphthoquinone was also prepared and found to exhibit properties similar to those of the 2,4-dinitrophenylhydrazone. However, the instability of *p*-nitrophenylhydrazine as a reagent prevented its use in quantitative determinations. 2,4,6-Trinitrophenylhydrazine was found too insoluble in hydrochloric acid solutions to be used as a reagent.

In Table I a comparison is shown of the results obtained on assay of marketed specimens of 2-methyl-1,4-naphthoquinone dissolved in vegetable oil by direct spectrophotometric measurement (*1*) and by the method described below.

Further controlled experiments indicated that 1 mg. of 2-methyl-1,4-naphthoquinone dissolved in ethanol could be determined with an accuracy of ±3 per cent by this method, employing a visual colorimeter; when the naphthoquinone was dissolved in vegetable oil the accuracy was about ±5 per cent. Chlorobutanol was found to have no effect on the accuracy of the determination.

TABLE I. COMPARISON OF DIRECT SPECTROPHOTOMETRIC AND 2,4-DINITROPHENYLHYDRAZONE COLORIMETRIC METHOD FOR DETERMINATION OF 2-METHYL-1,4-NAPHTHOQUINONE

Specimen No.	2-Methyl-1,4-naphthoquinone Content Found: Dinitrophenylhydrazone method	Direct spectrophotometric method	Difference
	Mg./ml.	*Mg./ml.*	*Mg./ml.*
A	0.93	0.94	0.01
B	1.90	1.96	0.06
C	0.82	0.77	0.05
D	0.75	0.70	0.05
E	1.00	0.94	0.06
F	2.06	2.02	0.04

Method

REAGENTS. Standard 2-methyl-1,4-naphthoquinone solution. Dissolve 25 mg. of 2-methyl-1,4-naphthoquinone, melting point 105–107° C., in 50 ml. of 95 per cent ethanol. This solution is stable for about one week if stored in a cool, dark place when not in use.

Dinitrophenylhydrazine reagent. Dissolve 50 mg. of reagent quality 2,4-dinitrophenylhydrazine in 20 ml. of 2 *N* hydrochloric acid.

Ethanolic ammonia solution. Mix one part of 95 per cent ethanol with one part of concentrated ammonium hydroxide (specific gravity 0.90).

PROCEDURE. Transfer a sample (oil solution or alcoholic extract) calculated to contain about 0.5 mg. of 2-methyl-1, 4-naphthoquinone to a 50-ml. volumetric flask. Place 1.0 ml. of the standard alcoholic solution of 2-methyl-1,4-naphthoquinone in a

second 50-ml. volumetric flask and adjust the volume in both flasks to 5 ml. with ethanol. If the unknown contains oil, add an equivalent amount of the same kind of oil, free from naphthoquinone, to the flask containing the standard solution and shake both flasks thoroughly. Add 1.0 ml. of the 2,4-dinitrophenylhydrazine reagent to each flask and place both flasks in a water bath maintained at 70° to 75° C. for 15 minutes. If oil solutions are being assayed, the flasks must be shaken vigorously once every 3 minutes to ensure complete reaction; otherwise, only occasional shaking is necessary. At the end of the heating period, cool the flasks to room temperature by immersion in a water bath and add to each flask 5.0 ml. of the ethanolic ammonia solution. Shake the flask thoroughly, fill to the mark with 95 per cent ethanol, and compare the solutions in a colorimeter. When oil is present in the flasks, allow the solutions to stand for 15 minutes in order that the oil may separate, and use the supernatant liquid for the determination.

Note. 2-Methyl-1,4-naphthoquinone is decomposed by prolonged exposure to light; consequently, it is best to carry out the determination in subdued light.

Discussion

Because the excess reagent contributes to the final color, the same volume of dinitrophenylhydrazine reagent solution must be added to the unknown and to the standard when the color comparison is performed visually. In practice, if the developed color of the unknown solution is found to vary more than 10 per cent from the standard, a second determination is made with an adjusted volume of the unknown sample. Consistently accurate results may be obtained in this manner.

Ammonia was found to be the most satisfactory alkaline reagent for the development of the blue color. Strong alkalies, such as sodium or potassium hydroxides in small amounts, led to extensive decomposition of the excess dinitrophenylhydrazine and to the production of colored derivatives, which interfere with color comparison.

It was observed that the addition of concentrated ammonium hydroxide solution produced some decomposition of the excess dinitrophenylhydrazine as a result of high local concentrations of alkali at the point of addition. This difficulty was overcome by the use of less alkaline alcoholic ammonia solution to develop the color. A final concentration of 0.6 *N* ammonium hydroxide was found necessary to effect complete conversion of the alcohol-insoluble 2,4-dinitrophenylhydrazone to the blue alcohol-soluble compound.

Summary

A method for the quantitative estimation of 2-methyl-1,4-naphthoquinone and related substances, applicable to oil solutions or alcoholic extracts, depends upon the interaction of 2,4-dinitrophenylhydrazine with 2-methyl-1,4-naphthoquinone and the subsequent production of a blue to blue-green color by the addition of alcoholic ammonia.

The 2,4-dinitrophenylhydrazone of 2-methyl-1,4-naphthoquinone has been isolated and characterized, and the absorption spectra of the 2,4-dinitrophenylhydrazone of 2-methyl-1,4-naphthoquinone and of 2,4-dinitrophenylhydrazine dissolved in alcoholic ammonia have been determined.

Acknowledgment

The data for the absorption curves reproduced in Figure 1 were obtained through the courtesy of Thorfen R. Hogness, in the laboratories of Spectroscopic Biological Investigations, University of Chicago, with the assistance of Richard Abrams.

The author wishes to express his appreciation to A. E. Sidwell, Jr., of this laboratory, for suggestions and criticisms during the course of this work.

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Colorimetric Determination of Low Concentrations of Sodium Nitrate in Sodium Nitrite

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A METHOD was needed for the determination of sodium nitrate in sodium nitrite samples in concentrations of about 1 per cent or less. In order to obtain fairly precise values, those methods were excluded from consideration which depend upon the determination of nitrates by the difference in the total nitrogen value and the nitrite value, because the errors would all be thrown upon the minor constituent. Of the direct methods which have been reported for nitrate, the colorimetric ones seemed most promising.

These fall into two classes. The first class comprises tests such as those with diphenylamine and with ferrous sulfate which are not specific for nitrates but are also given by nitrites. The use of such tests would require the preliminary removal of nitrites. In order to avoid the necessity for doing this, attention was turned to the second class—namely, tests which have been reported as being specific for nitrates and not subject to interference by nitrites. This claim has been made for two methods in particular; one by Wolf and Heymann (*11*) involves the formation of a color by means of 2,4-diamino-6-hydroxypyrimidine in the presence of sulfuric acid; the other by Pesez (*7, 8*) depends upon nitrating nitrobenzene to dinitrobenzene by means of the nitrate in sulfuric acid and reacting the product according to Janowski (*2*) with acetone and alkali to produce a color which is a measure of the nitrate present. The authors of both methods claimed that nitrites do not interfere. The present authors have been unable to confirm their claims in this respect; on the contrary, they find that nitrites do interfere. Even recrystallized sodium nitrite gives a variable intensity of color.

The reason for this is of fundamental importance in considering claims for the specificity of any method for nitrates in the presence of nitrites. Whenever it is necessary to carry out such an analysis by treatment of the sample, which still contains nitrite, in the presence of a considerable concentration of strong acid, then the analysis cannot be reliable, because some of the free nitrous acid which is formed will decompose to form nitric acid, presumably according to the well-known reaction:

$$3HNO_2 \longrightarrow HNO_3 + 2NO + H_2O$$

When analyzing samples consisting almost entirely of sodium nitrite, the amount of nitrate which is formed is sufficient to vitiate the analysis.

Since no method was available which could be applied

directly to the presence of nitrites, it became necessary to consider removing the nitrite before applying any method for the determination of the nitrate.

A number of reagents have been recommended for this purpose. Using hydrazine (*4*) sulfamic acid (*1*), urea (*5*), and hydroxylamine (*3*), the authors were unable to prevent the formation of nitric acid. Ammonium chloride was also tried in a manner similar to that described by Nelson, Levine, and Buchanan (*6*) in a slightly alkaline medium (pH about 7.5). The treated solution was then evaporated to dryness, after which it was reacted with silver sulfate in a small volume of added water to remove excess chloride, which would interfere with the nitrobenzene reaction. It was found that tests for nitrate could be obtained with pure sodium nitrite. Since the decomposition of the nitrite is carried out in a slightly alkaline medium, there is little likelihood of the formation of nitrate. It is more likely that some nitrite remains undestroyed. This would interfere with the final color test, if present as such, or if partially converted to nitrate by oxidation upon concentrating the solution. The procedure described by Nelson, Levine, and Buchanan requires a high excess of ammonium chloride for complete destruction of the nitrite. Because of the need for removing excess chloride in the authors' procedure, this was not practical, since they had about 0.1 gram of sodium nitrite and so would require too much ammonium chloride.

Sodium azide was a more satisfactory reagent. It is said to act in the following manner (*10*):

$$HN_3 + HNO_2 \longrightarrow N_2O + N_2 + H_2O$$

Sommer and Pincas (*10*), who recommended it for the destruction of nitrite in order to permit the determination of nitrate, were able to remove nitrite completely and found no conversion to nitrate when the latter was tested for by diphenylamine. The authors confirmed this report, but did not consider diphenylamine a suitable reagent for the quantitative determination of nitrate. One reason is its lack of specificity, since it would respond to contaminants such as ferric iron or other oxidants. Another reason is that the usual technique in applying the diphenylamine test, which involves the formation of a colored ring upon stratifying the sample solution on the diphenylamine reagent, did not seem promising for quantitative purposes. Pesez's method appeared more promising, but the authors found that the use of sodium azide as recommended by Sommer and Pincas did result in the formation of some nitrate from nitrite.

Presumably Pesez's method is more sensitive than the diphenylamine test. In order to use this method, the factors involved in the azide decomposition reaction were studied in order to avoid any formation of nitrate, while at the same time getting complete destruction of nitrite. It was found essential to control the relative concentrations of nitrite, sulfuric acid, and sodium azide. If too much azide is used, there seems to be some destruction of nitrate (possibly by the reducing action of the free hydrazoic acid). If too little is used, some nitrite will escape destruction and will be converted to nitrate during the application of Pesez's method. The concentration of sulfuric acid must be controlled so as to have only a small excess present over the amount which is just necessary to convert the nitrate to nitric acid and the sodium azide to hydrazoic acid. The effect of differences in the time and temperature of the reaction was studied.

After the nitrite has been destroyed, the solution is made alkaline and evaporated to dryness. Pesez's method is then applied to the solid residue. The influence of such variables as time and temperature, volumes of reagents, and moisture, both upon the nitration and upon the development of the color after nitration, was studied.

Precision and Accuracy

By using the method with the technique carefully controlled as described, it has been possible to distinguish without difficulty (in 0.1-gram samples) each of the members of the series—0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, and 1.0 per cent sodium nitrate—from the preceding and the following member of the series. This precision was sufficient for the authors' purposes, but it is possible that the method could be developed to a greater precision. Beyond a concentration of 0.6 per cent the higher intensity of color results in a loss in the sensitivity with which successively higher concentrations may be differentiated.

Method of Analysis

CAUTION. Because of the poisonous nature of hydrazoic acid it is well to carry out the analysis in a hood.

REAGENTS. Sodium Azide Solution (A). The sodium azide solution must be made up to be 0.0581 *N*. It is prepared by weighing out and dissolving in water enough solid sodium azide, which has previously been assayed according to the method of Reith and Bouwman (*9*), to make a solution of 3.7765 grams of 100 per cent sodium azide per liter. The azide solution is satisfactory if, using the procedure described, a colorless test solution is obtained with 100 mg. of recrystallized sodium nitrite and a pinkish-lilac color is obtained with 100 mg. of recrystallized sodium nitrite plus 0.1 mg. of sodium nitrate.

Sodium Nitrite Solution (B). C. P. sodium nitrite is recrystallized from water several times to ensure the absence of nitrates. A solution is made up to contain exactly 0.4000 gram per 100 ml.

Sodium Nitrate Solution (C). C. P. sodium nitrate is dissolved in water to give a solution which contains 0.1 mg. of sodium nitrate in 1 ml.

Nitrobenzene. A redistilled product with a freezing point not less than 5.2° C.

Concentrated sulfuric acid, *N* sodium hydroxide solution, 5 *N* sodium hydroxide solution, and *N* sulfuric acid solution.

PREPARATION OF STANDARD SOLUTIONS. Standard solutions are prepared by mixing 25.0-ml. portions of Solution B with Solution C. Each milliliter of Solution C corresponds to 0.1 per cent sodium nitrate. Standards are made up to correspond to 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, and 1.0 per cent sodium nitrate.

PREPARATION OF SAMPLE SOLUTION. The sodium nitrite sample solution is made up to contain exactly 0.1 gram of sodium nitrite in 25 ml. of water. For this purpose the sodium nitrite is assayed by titrating 0.1 *N* sodium sulfanilate in an acid medium with the nitrite, using a potassium iodide–starch paste as a streak indicator for the end point.

DESTRUCTION OF NITRITE AND DEVELOPMENT AND MATCHING OF COLORS. The standard solutions and 25-ml. portions of the sample solution (from a transfer pipet) are placed in 100-ml. beakers. From a transfer pipet 25 ml. of Solution A are added and then 3.5 ml. of *N* sulfuric acid. The solutions are stirred immediately and then occasionally during 15 minutes, at which time all gas evolution should have ceased. The solutions are then made just faintly alkaline with *N* sodium hydroxide solution, spotting on phenolphthalein indicator paper for the end point and using no more than one drop in excess. The amount of sodium hydroxide necessary varies from 0.5 to 0.7 ml. The solutions are then evaporated to a small volume on a hot plate, transferred to a steam bath, and evaporated to dryness two or three times with the aid of some added absolute alcohol to ensure complete absence of moisture from the solid residue.

The residue is powdered with a small stirring rod, the beaker is cooled in ice, and exactly 3 drops of nitrobenzene are added from a capillary dropper. (The same dropper is used for the standards as for the sample in order to be certain of uniformly sized drops.) The nitrobenzene is rubbed up with the residue, and then exactly 0.35 ml. of concentrated sulfuric acid (from a 1-ml. Mohr pipet) is mixed in. The mixture is allowed to stand for 3 minutes in the ice bath with occasional stirring. During this time 5 ml. of 5 *N* sodium hydroxide solution are transferred to a 20-ml. glass-stoppered test tube by means of a transfer pipet or a buret. The reaction mixture in the beaker is then rubbed twice with 5-ml. portions of C. P. acetone. The acetone is transferred to the tube containing the 5 *N* sodium hydroxide solution. (It is not necessary to remove the solid matter from the beaker.) The tube is shaken vigorously with an up and down motion 50 times, then allowed to stand for 3 minutes at room temperature, and finally immersed in an ice bath. After cooling, the colored acetone layer of the sample is compared with those of the standards within 30 minutes. The acetone may, if desired, be removed from the alkali layer. The color seems to fade less rapidly if that is done.

EFFECTS OF VARIATIONS IN CONDITIONS. The solution containing the sample (or standard) and sodium azide was allowed to stand up to 20 minutes before adding the sulfuric acid, with no effect upon the results. The sulfuric acid could be added rapidly rather than dropwise without influencing the values for nitrate. Instead of 3.5 ml. of *N* sulfuric acid as little as 3.0 ml. and as much as 4.0 ml. were used without adverse effects.

The effect of changing the temperature of the solution from room temperature during the addition of the azide and the sulfuric acid and during the subsequent stirring period was studied. At a temperature of 50° to 60° C. the colors were intensified and it was impossible to obtain colorless blanks. This may have been the result of some loss of hydrazoic acid (boiling point 38° C.) which would cause an incomplete destruction of nitrite, thereby allowing some of the nitrite to become converted to nitrate, which would produce a colored blank. No advantage could be observed in cooling; room temperature seemed to be most advantageous.

Summary

Methods for the determination of low concentrations of nitrates in the presence of nitrites have been examined for which claims have been made that nitrites do not interfere. It has been found impossible to substantiate these claims, since some conversion of nitrite to nitrate always occurs. The removal of nitrites by various reagents was then tested and sodium azide was found most suitable. A procedure was worked out which involves the removal of nitrites by means of sodium azide and the subsequent colorimetric determination of the nitrate by modifications of a reported procedure involving the nitration of nitrobenzene and the development of a color with acetone and alkali. Concentrations of sodium nitrate up to 1 per cent have been determined.

Acknowledgment

Grateful acknowledgment is made to Harold J. Rodenberger for calling attention to the use of sodium azide for the destruction of nitrites.

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Ultraviolet Absorption of Vitamin A in Various Solvents

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SOME disagreement exists regarding the numerical value of the absorption coefficient of vitamin A at the maximum of its absorption curve in the ultraviolet (*2*, *4*, *6*, *7*, *8*, *11*). Since very pure preparations of crystalline vitamin A are now available (*2*), a study of this value was made for solutions in many of the commonly used solvents which might be employed in the assay of vitamin A products. In this paper, vitamin A indicates vitamin A_1.

Experimental Procedure

The absorption values were determined with a photoelectric spectrophotometer recently employed in spectrophotometric studies on chlorophyll (*12*) and linseed oil (*9*). A large Müller-Hilger Universal double monochromator with crystal quartz optics was the dispersion instrument. A hydrogen arc of the Munch type (*10*), constructed of quartz, was the source of radiation for many of these measurements. An incandescent filament lamp with a clear Pyrex bulb was the source of radiation for most of the measurements above 3100 Å. All determinations were made on solutions at 25° C.

For these measurements, all slits were of uniform width, with the exit slit 6 mm. in length. Slit widths were as follows [numbers in brackets indicate the corresponding spectral region isolated, as defined by Hogness, Zscheile, and Sidwell (*5*)]: 0.30 to 0.80 mm. (6 to 16 Å.) at 2240 A.; 0.20 to 0.80 mm. (5.2 to 20.8 A.) at 2400 Å.; 0.08 to 0.15 mm. (4.2 to 7.8 Å.) at 3000 Å.; 0.12 to 0.20 mm. (8.2 to 13.6 Å.) at 3280 Å.; 0.20 mm. (16.4 Å.) at 3500 A.; and 0.08 mm. (8.5 Å.) at 3760 Å. The results were independent of slit width in the ranges employed. For instance, when the slit widths were varied from 0.06 to 0.50 mm., the absorption value at 3280 Å. remained constant. A decrease of only 1 per cent occurred when slits of 0.95 mm. were used.

The absorption spectra reported here are expressed in terms of $E^{1\%}_{1\text{ cm.}}$, defined as follows:

$$E^{1\%}_{1\text{ cm.}} = \frac{\log_{10} \frac{I_0}{I}}{cl}$$

where I_0 = intensity of radiant energy transmitted by solvent-filled cell
I = intensity of radiant energy transmitted by solution-filled cell
c = concentration in grams per 100 ml.
l = thickness of solution layer in centimeters

The cell length was 1 cm. and the concentration was adjusted to keep the $\log_{10} \frac{I_0}{I}$ values between 0.200 and 0.800 in order to obtain high precision (*5*).

"Iso-octane" was distilled over solid sodium hydroxide. Cyclohexane was purified by repeated recrystallization and distillation. Other solvents were purified in accordance with the practices of this laboratory (*13*). All alcohols were treated in the same manner as ethanol. Amber glassware (*3*) was not used in the experiments reported here.

The samples studied were purified in the laboratories of Distillation Products, Inc., by molecular distillation and subsequent crystallization (*2*). Samples B-152 and B-153 were received in vacuum, packed in dry ice. All observations were made 3 to 4 days after receipt of sample. Sample B-210 was received in vacuum at room temperature and stored in dry ice until studied, 6 to 15 days later. Samples were kept in vacuum at dry ice temperatures in the dark as much as possible between the necessary weighings for different solvents. Maximum absorption values of all samples were determined within 10 minutes to 1 hour after the weighings was made.

EFFECT OF INCANDESCENT FILAMENT RADIATION ON ABSORPTION VALUES. Certain tests were made to determine the effect of the radiation employed in the absorption measurements themselves.

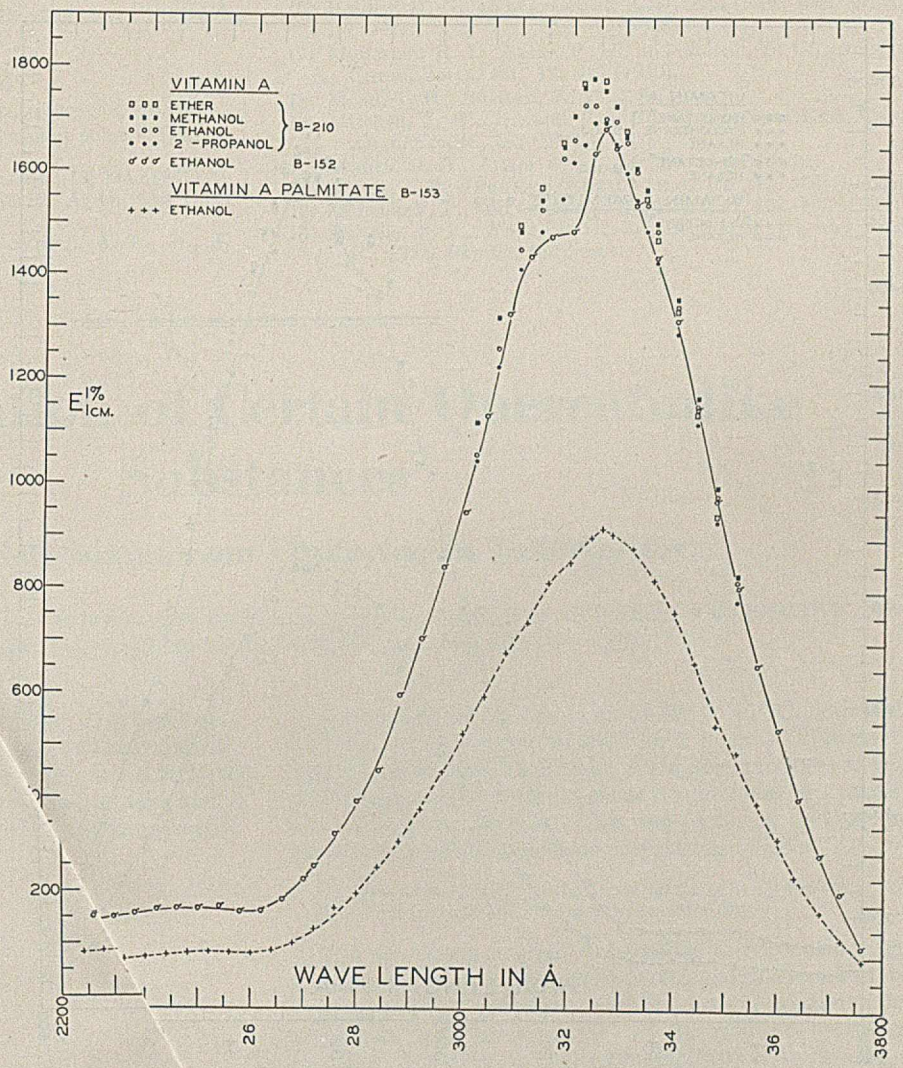

FIGURE 1. ABSORPTION SPECTRA IN ETHER AND ALCOHOL SOLUTIONS

The absorption of a fresh solution of vitamin A (B-210) in ethanol was measured at 3280 Å. An exposure of 6 seconds to a very low intensity of radiation from the exit slit of the monochromator is required for a single determination of $\log_{10} \frac{I_0}{I}$. Three successive determinations at 3280 Å. gave the same result. Then eight determinations were made from 3520 to 3300 Å., followed by three more at 3280 Å. Ten more determinations followed at intervals from 3260 to 3020 A., succeeded by three additional ones at 3280 Å. The values obtained at 3280 Å. remained constant to ±0.5 per cent throughout these measurements. Then the same solution in the absorption cell was held directly in front of the 72-watt incandescent filament radiation source for successive periods of 3, 9, and [illegible] minutes and the absorption at 3280 Å. was determined after each exposure. During this series, the absorption at 3280 Å. remained constant to ±0.2 per cent.

Results

Figure 1 presents the absorption data of vitamin A in diethyl ether and various alcohols and of vitamin A palmitate in ethanol from 2240 to 3760 Å. Figure 2 contains similar data for solutions in several hydrocarbon solvents. Lines are drawn through the points for ethanol and isooctane (2,2,4-trimethylpentane) only.

Tables I, II, and III present the numerical $E^{1\%}_{1\,cm.}$ values for all preparations in the solvents studied, at wave lengths 3260 and 3280 Å. Values at 3240 Å. are included in some cases. Data in columns numbered 1 are from the same solution; those in columns numbered 2 are from solutions made from different weighings of the original sample.

TABLE I. ABSORPTION COEFFICIENTS FOR VITAMIN A NO. B-210

(Determined 5 to 6 weeks after preparation)

Solvent	3240 Å. 1	3240 Å. 2	3260 Å. 1	3260 Å. 2	3280 Å. 1	3280 Å. 2
Ether	..	..	1778	1752	1723	1700
Methanol	1782	1734	1760	1734	1730	1696
Ethanol	1730	1712	1715	1712	1700	1682
2-Propanol	1698	1680	1698	1660	1655	1642
Isooctane	..	..	1660	..	1652	..
Cyclohexane	..	..	1605	1590	1605	1570
Hexane	..	..	1688	1662	1665	1660

TABLE II. ABSORPTION COEFFICIENTS FOR VITAMIN A NO. B-152

(Determined 19 weeks after preparation)

Solvent	3260 Å. 1	3260 Å. 2	3280 Å. 1	3280 Å. 2
Ether	[illegible]			
Isooctane	1685	1695	1660	1640
Hexane	1610	1630	1590	1600
	1600	..	1565	..

TABLE III. ABSORPTION COEFFICIENTS FOR VITAMIN A PALMITATE NO. B-153

(Determined 15 weeks after preparation)

Solvent	3260 Å. 1	3260 Å. 2	3280 Å. 1	3280 Å. 2
Ethanol				
Isooctane	920	930	910	926
Hexane	906	..	900	..
	[illegible]	878	862	870

Discussion

For most solvents, a d[illegible] curve in the region of 3150 Å. inflection appears in the sample B-152 than for B-2[illegible]his is more pronounced for maximum of absorption was fo[illegible]With one exception the [illegible]at wave lengths shorter

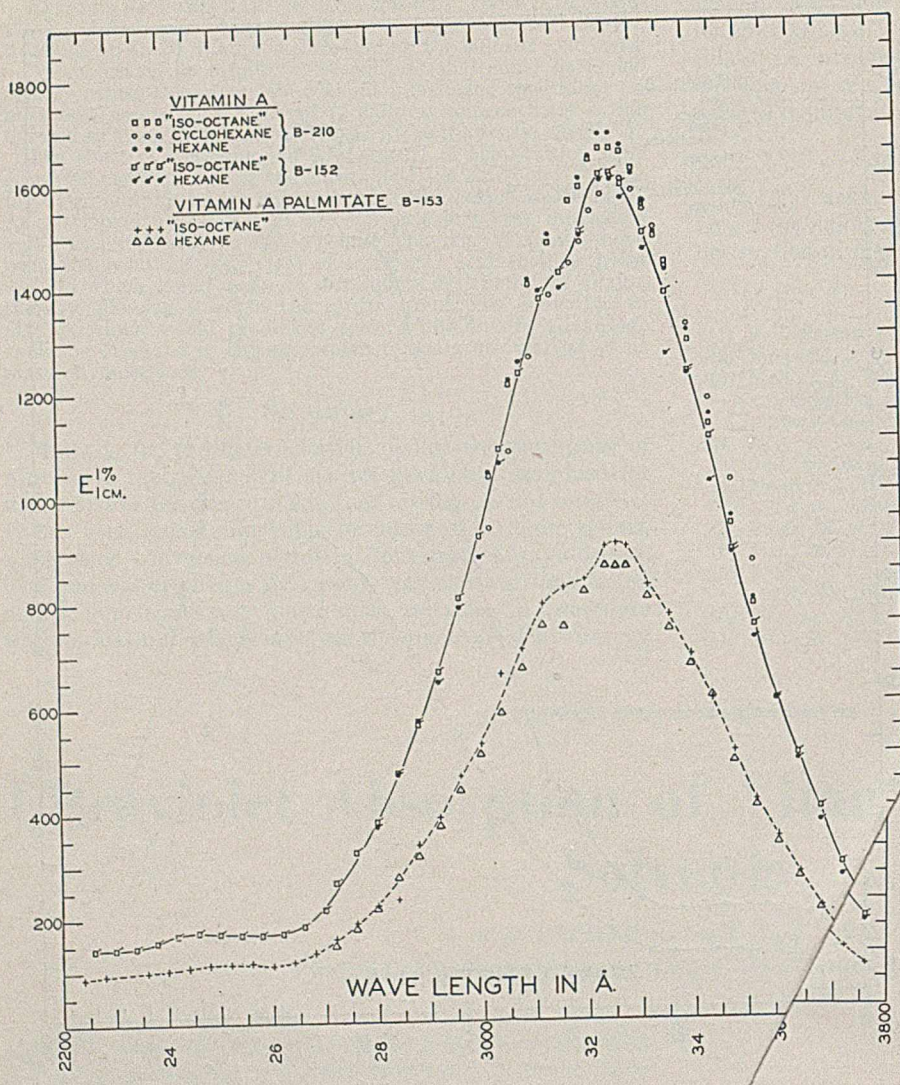

FIGURE 2. ABSORPTION SPECTRA IN HYDROCARBON SOLVENTS

than 3280 A., usually at 3260 Å. (at 3240 A. for vitamin A No. B-210 in alcohols). Baxter and Robeson (*1*) find maximum absorption at 3260 Å. for most preparations. Similar findings were reported by McFarlan, Bates, and Merrill (*2*). These findings are slightly different from the wave length 3280 Å. usually given for the maximum. The curves ths preparations examined in the region of lower wave n A have a second maximum (at 2450–2500 Å. for vi and 2500–2550 Å. for the palmitate).

It is noted that the maximum $E^1_{1\ cm.}$ % value sample B-210 is higher than that for B-152 in all sol s. Since tal period sample B-210 had been stored for a much shor ations were than B-152 before the spectroscopic deter for the former made, more emphasis should be placed on va evacuation of preparation. It is possible that incomp ial oxidation of air from the ampoule could have caused e slightly higher sample B-152. In general, the maxim solvents. in alcoholic solvents than in hydrocar following maximum

Baxter and Robeson (*1*) obtained ally at 3260 Å., soon $E^1_{1\ cm.}$ % values for ethanol solutions 30 for B-152, and 960 after preparation: 1740 for B-2 of $E^1_{1\ cm.}$ % reported here for B-153. The maximum va (average 1721 for sample for ethanol solutions of vitamme as the average maximum B-210 at 3240 Å.) are nearly t by Baxter and Robeson (*2*). value 1725 (at 328 mμ) rep

It is evident from their results and those reported here that small differences exist among the most carefully prepared samples. The solvent has a definite effect upon the maximum absorption value.

The average maximum $E^1_{1\ cm.}$ % values for vitamin A palmitate may be compared with those for the vitamin A sample (B-152) which is most comparable to it with respect to age when examined spectroscopically. The ratios of the corresponding values (0.547 for ethanol) agree well with the inverse ratio of the molecular weights

$$\left(\frac{\text{vitamin A } 286}{\text{palmitate } 524} = 0.547\right)$$

This agreement is better at 3260 Å. than at 3280 Å. For solutions in ethanol and hexane, the difference between ratios is only 0.37 per cent at 3260 Å.

Recently Embree (*3*) has discussed reasons for the instability of vitamin A in solution, especially when subjected to ultraviolet radiation, and has emphasized the importance of keeping such solutions in amber glassware. The above experiments on the effect of incandescent radiation indicate that the radiation conditions employed in these measurements did not cause any change in absorption values and that the slit widths employed were sufficiently small to ensure reproducible values. Other tests in the ultraviolet region indicate the absence of significant scattered radiation. Baxter and Robeson (*1*) have been unable to detect deterioration of vitamin A during spectrographic measurements.

Acknowledgment

The vitamin samples for this study were supplied through the generosity of K. C. D. Hickman and J. G. Baxter, Distillation Products, Inc., Rochester, N. Y. The authors are further indebted to Dr. Baxter for several suggestions on the

use of certain solvents and concerning the study on the effect of radiation during the measurements.

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Determination of Certain Quercetinlike Substances

Using a Klett-Summerson Photoelectric Colorimeter

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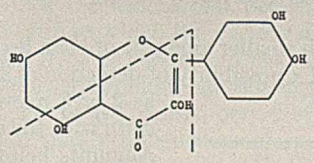
IT WAS observed by one of the authors that boric acid dried with lemon juice gave a brilliant yellow coloration (*3*). Further work led to the conclusion that this coloration was produced by a reaction between the boric acid and a flavone or group of flavones similar in structure to quercetin:



More specifically it has been postulated that the reaction is due to the grouping within the dotted line (*2*, *4*). Included in the materials thus reacting is citrin, believed by Szent-Györgyi to have vitamin activity and tentatively called by him vitamin P (*1*).

The necessity of a flavone-free diet in physiological work with citrin makes practical the test to be used, as its sensitivity will detect amounts as low as 2 or 3 micrograms. The Wilson boric acid test has been discussed and used considerably in a qualitative manner. However, it was the object of this work to develop some method of accurate quantitative measurement of flavones, using this test.

The color-forming substance, insoluble in toluene and chloroform, will dissolve in acetone saturated with boric acid to give a yellow coloration, the intensity of which is a quantitative measure of the amount of flavone present.

For color measurement it was convenient to use a Klett-Summerson photoelectric colorimeter with adaptor for the use of a test tube graduated in 5- and 10-ml. divisions. This eliminates the necessity of matching colors and is an accurate measure of the intensity of color produced. The instrument has a logarithmic scale, thus making possible the production of linear curves on ordinary graph paper.

The intensity of color is deepened considerably by an acid medium, but the strong acids react to give yellow colors with flavones. Acetic acid is too weak to develop the color fully. Anhydrous citric acid has been found suitable, considering strength, acetone solubility, and availability. (Citric acid hydrate may be conveniently rendered anhydrous by allowing it to effloresce completely in air at 30° to 40° C., followed by heating in a thin layer to 100° for 2 hours.) However, on long standing with boric acid, citric acid produces a yellow coloration, so that it was necessary to mix the two materials immediately before use. This was done by mixing equal parts of two filtered solutions prepared as follows:

A. Acetone 100 ml., anhydrous citric acid 10 grams
B. Acetone 100 ml., boric acid to saturate

This mixture is referred to hereafter as borocitric reagent. These separate solutions are apparently stable indefinitely.

It was found that metaboric acid gives a more intense coloration, but the sensitivity of the solution to traces of moisture, as shown by copious precipitates of boric acid, was too great, and the use of metaboric acid was discontinued. The authors recognized the possibility of advantageous use of metaboric acid if its concentration was restricted to the equivalent of boric acid saturation.

As the depth of color is affected greatly by traces of moisture, attempts were made to dehydrate the solvent acetone. As these methods of dehydration gave values which varied slightly, it was considered best to standardize with quercetin and to use one sample of acetone for all reagents. Merck's "Blue Label" acetone was found adequate as to dryness.

Method of Standardization

The standard used for the work with quercetin was prepared by C. E. Sando (Food Research Division, Bureau of Agricultural Chemistry and Engineering, Washington, D. C.) and repurified by Wilson. Weighed amounts of quercetin were dissolved in acetone and diluted to 100 ml. in volumetric flasks. The first sample of 56 mg. was found to give a color far too intense for the scale of the colorimeter. Therefore, further work was done with samples between 10 and 15 mg.

Using the industrial test-tube model of the Klett-Summerson colorimeter, the test tube was filled to the 10-ml. mark with a mixture of equal volumes of solutions A and B, and the zero point set for this reagent, using the blue filter No. 42 accompanying the instrument (value 400 to 465 millimicrons). The acetone-quercetin solution was then dropped into the test tube, using a serological pipet, graduated in 0.01-ml. divisions. After the addition of each 0.1-ml. the tube was removed from the instrument, shaken well to ensure proper mixing, and re-inserted and a reading was taken. The addition of 1 ml., in 0.1-ml. intervals, was found sufficient to determine response curves.

As the quercetin solution is itself yellow, it was necessary also to obtain a curve of colorimeter response to the acetone-quercetin

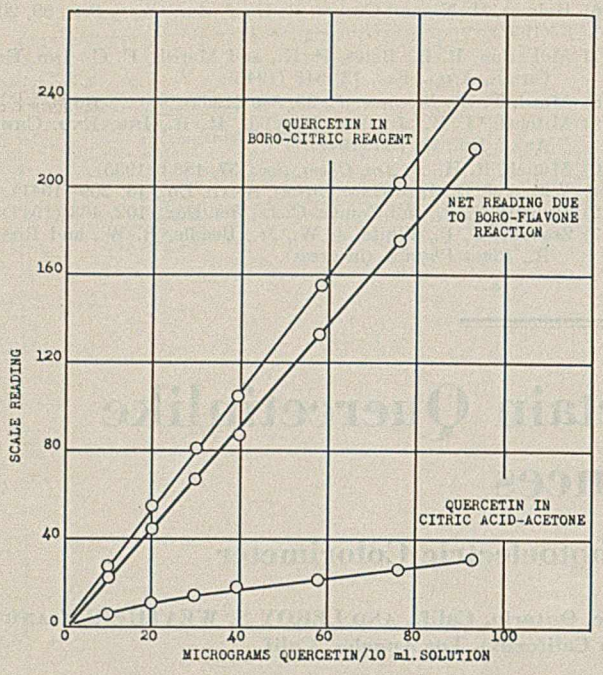


FIGURE 1

TABLE I. CALIBRATION OF COLORIMETER WITH QUERCETIN

(Quercetin strength: 10.2 mg. per 100 ml.)

Ml. of Solution Added to 10 Ml. of Reagent	Micrograms of Quercetin per 10 Ml. of Solution[a]	Scale Reading with: Borocitric reagent	Scale Reading with: Citric acid–acetone	Net Reading
0.1	10.1	28	6	22
0.2	20.0	55	10	45
0.3	29.7	81	14	67
0.4	39.2	105	18	87
0.6	57.7	155	22	133
0.8	75.6	202	26	176
1.0	92.7	248	31	217

[a] Quercetin solution was added to 10 ml. of reagent, but concentration of quercetin is calculated as micrograms per 10 ml. of total solution.

TABLE II. FLAVONE DETERMINATION IN BIOLOGICAL MATERIALS

Material	Weight Taken Fresh	Weight Taken Dry	Volume of Solution Taken, Ml.	Reading with: Borocitric reagent	Reading with: Citric acid–acetone	Net Reading	Micrograms of Quercetin Equivalent	Quercetin Equivalent, Mg. per Gram of Fresh Weight
Bean leaf	17.53	1.753	1.0	151	113	38	18.4	0.105
Lemon peel	25.75	6.799	0.1	113	13	100	44.0	1.71
Lemon peel	5.92	1.563	0.5	142	28	114	48.8	1.65
Lemon peel	6.89	1.820	0.5	170	30	140	60.0	1.74
Rabbit liver[a]	...	...	..	..	..	..	..	...

[a] Undetectable.

solution, free of the boroflavone reaction. This was done by mixing it with 5 ml. of solution A and 5 ml. of pure acetone and using the measuring technique described above. Thus a curve could be plotted for the boroflavone reaction and then for the acetone-quercetin solution alone. The net reading of these two curves gave the curve due to the boroflavone reaction with quercetin. From this curve were taken the data needed in determining quercetin equivalents of natural materials.

The reaction is so sensitive to moisture that all precautions must be taken to exclude even traces of moisture from the acetone and from the materials used. As little as 1 per cent of moisture decreases the color response by half. It was necessary to dry the quercetin in a vacuum desiccator for 2 days before use and to use care in mixing the borocitric with the quercetin solution. It was advisable in shaking the test tube to cover its lip with thin tin foil (previously washed in acetone) rather than to run the risk of picking up moisture from the fingers.

The net curves based on these data are shown in Figure 1. As these are straight lines, it is both simple and advisable to replot them for each lot of reagents.

Extraction Procedure

For the determination of the quercetin equivalents of tissues, the following procedure was used:

The tissue was dried in a 60° oven under vacuum, then ground, and a sample was taken, generally from 1 to 5 grams. This was extracted with methyl alcohol in a Soxhlet extraction apparatus, and the extracted liquid was evaporated to dryness on a water bath. Chlorophyll, fats, resins, etc., were next removed from the dry extract by digestion with chloroform. Sometimes, because of the quantity of substances extracted by the methyl alcohol, this chloroform extraction is incomplete, chlorophyll often remaining. In such cases the residue may be redissolved in a small amount of methyl alcohol, a few milliliters of toluene added, the methyl alcohol removed by evaporation, and the chloroform digestion repeated. The resulting residue is dissolved in acetone, filtered, and diluted to 100 ml. in a volumetric flask.

The readings with borocitric reagent shown in Table II were made by taking sufficient of the acetone solution of the extract to give a reading in the desired range (suitably 100 to 250) and adding sufficient reagent to fill the tube to the 10-ml. mark.

Blank determinations were then made, using the same quantities of the extracts and a mixture of equal parts of solution A and acetone.

The net reading is the difference between these two sets of readings, and the quercetin equivalent value was the result of calculating the quantity found on the net curve back to the original material.

Results on some typical plant and animal materials are shown in Table II.

In order to check the accuracy of the method of extraction, 0.82 gram of dried bean leaves (representing 8.2 grams of fresh material) was added to an acetone solution of quercetin containing 1.70 mg. of quercetin. The acetone was evaporated and the fortified leaves were extracted as outlined above. The final residue was taken up in acetone and diluted to 50 ml. Using 1 ml. of this solution, the net reading was 115, equivalent to 50 micrograms of quercetin or 2.50 mg. of quercetin for the 8.2 grams of fresh leaves. According to Table II this quantity of bean leaf contained 0.86 mg. The recovery of added quercetin was 2.50 — 0.86 or 1.64 mg.

Readings with smaller amounts of bean leaf and lemon peel extracts showed a possibility of detection of amounts as small as 2 or 3 micrograms of quercetin with the equipment used. Amounts below this would be difficult to detect.

Rabbit liver, an animal tissue, showed no detectable quercetin, whereas the plant tissues seem to be excellent sources. This may be explained in several ways: the unlikely possibility of a flavone-free diet; the possibility that the flavones are changed in the animal body into materials which do not give the boric acid color reaction; and the possibility that the flavones are conjugated to some form not solubilized by the procedure employed.

Summary

A method for quantitative determination of quercetinlike substances with the use of a Klett-Summerson photoelectric colorimeter has been described. Quercetin equivalent content of bean leaves was found to be 0.105 mg. per gram and of lemon peel, 1.70 mg. per gram. No flavone could be detected in a sample of rabbit liver. Pure quercetin added to dried bean leaves was quantitatively recovered.

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Analytical Determination of *p*-Toluidine in the Presence of Its Isomers

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THERE has long been a need for an analytical method for the determination of *p*-toluidine, particularly when present in small quantities in mixtures of the isomeric toluidines. Commercial *o*-toluidine contains small quantities of both the meta and para isomers, but accurate methods are available for the determination of only the meta isomer (*3*). The present investigation supplies a method for the determination of the para isomer.

Principles of the Method

In earlier work on the stability of diazo compounds (*2*) it was shown that at a temperature of 45° C. the stabilities of *o*- and *m*-toluenediazonium chlorides are identical and very much lower than the stability of the para isomer. For example, *o*- or *m*-toluenediazonium chloride is 99.9 per cent decomposed at the end of 36 minutes, whereas the para isomer requires 21 hours to reach 99.90 per cent decomposition. Thus, if a mixture of the isomeric toluidines is diazotized and allowed to decompose, virtually all of the *o*- and *m*-toluenediazonium chlorides will be decomposed in less than an hour, and all nitrogen which is evolved from the reaction mixture subsequently may be attributed to the decomposition of *p*-toluenediazonium chloride. Since careful determinations have been made of the rates at which the various diazonium compounds decompose (*2*), it is possible to calculate what percentage of the *p*-toluenediazonium chloride originally present will remain at the end of any chosen period of time. It was found, for example, that *p*-toluenediazonium chloride is 62 per cent decomposed after 3 hours at 45° C. Thus, if the nitrogen subsequently evolved from an unknown mixture of isomeric toluenediazonium chlorides after 3 hours of decomposition at 45° C. is gathered and measured, it will represent 38 per cent of the total para isomer which was present in the unknown mixture. By choosing 3 hours for the decomposition time, inaccuracies due to the presence of aniline are avoided, since 2.5 hours are required for benzenediazonium chloride to reach 99.90 per cent decomposition at 45° C. The analytical method described in detail below is an application of these principles.

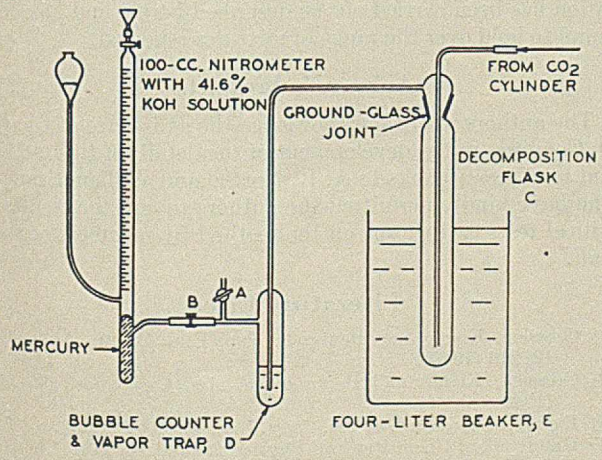

FIGURE 1. DECOMPOSITION AND NITROMETER ASSEMBLY

Apparatus

The earlier work carried out in these laboratories on the stability of diazo compounds involved the use of an automatic recording nitrometer (*1*), which was invaluable in working out the basis for the present analytical method. In the present work, however, a simpler apparatus has been found equally satisfactory. The setup used is shown in Figure 1 and consists of a cylindrical flask fitted with a ground-glass joint, a bubble counter or vapor trap, and a conventional nitrometer. A constant-temperature bath set at 45° ± 0.1° C. is also required but is not shown in Figure 1.

Details of Procedure

A 0.05-mole sample of the mixed toluidines (5.354 grams) is dissolved in 90 ml. of 5 *N* hydrochloric acid. The solution is cooled to 5° C. and *N* sodium nitrite added from a buret, the tip of which extends below the surface of the solution. The solution is stirred mechanically and the sodium nitrite solution added at a rate not greater than 3.5 ml. per minute at the beginning of the reaction and becoming even slower after the addition of 40 to 45 ml. of nitrite (theoretical requirement 50 ml.). It is very important to avoid an appreciable excess of nitrite at any time, as this seriously affects the final results. The temperature must not exceed 8° C.

The progress of the diazotization is observed by dipping a glass stirring rod into the solution and touching the rod to white starch-iodide paper. An immediate development of blue color indicates the presence of excess nitrite and the end point is reached when the immediate blue color which appears can be obtained repeatedly during a period of 5 minutes without further addition of nitrite. At the end of the 5 minutes the solution is transferred to flask *C* and diluted to 250 ml. with distilled water at 5° to 8° C. and the flask is immersed up to the neck and clamped in a constant-temperature bath held at 45° ± 0.1° C. (not shown in Figure 1). The flask is left open to the air and provided with a high-speed electric stirrer. The solution is protected from strong direct daylight and held thus at 45° C. for 180 (±1) minutes. Upon removal it is placed immediately in the ice and water bath (*E*, Figure 1) and cooled as quickly as possible to 0° C.

During the cooling period the assembly shown in Figure 1 is completed. Pinchclamp *B* is closed, stopcock *A* is opened to the air, and carbon dioxide from a pressure cylinder is run through the system in a very rapid stream and allowed to escape into the air at *A*. When the system has been thus flushed for 15 minutes, the rate of flow of the carbon dioxide is reduced to about one bubble per second, the pinchclamp at *B* is opened, and simultaneously the stopcock at *A* is closed. The carbon dioxide is dissolved in 41.6 per cent potassium hydroxide solution which fills the nitrometer. The air and nitrogen trapped in the system are collected over this potassium hydroxide solution. The value of the zero reading is not influenced measurably by the extremely slow evolution of nitrogen at 0° C. When a constant zero reading has been obtained (requiring about 3 minutes), the bulb is leveled, the reading is recorded, the flow of carbon dioxide is almost stopped (one bubble every 5 or 6 seconds), and the ice bath is replaced by a warm water bath. A flame is immediately applied to the water bath and the water is heated to the boil. After 10 or 15 minutes of boiling the flow of carbon dioxide is increased to 2 or 3 bubbles per second and the system flushed until there is no further increase in the volume of gas in the nitrometer. The bulb is then leveled and the volume of gas is read and recorded. The room temperature and barometric pressure are also recorded.

The barometric pressure is corrected for the vapor pressure of potassium hydroxide solution and, knowing the temperature and corrected pressure, the number of milligrams of nitrogen collected is calculated. This value is divided by the weight of the sample in grams and the value obtained is then divided by an empirical factor—namely, 96.06 ($\log_{10} = 1.98254$). This factor represents the milligrams of nitrogen per gram of sample if the sample had been pure *p*-toluidine, corrected by the extent to which *p*-toluidine is incompletely diazotized under the prescribed

conditions. The figure thus obtained is multiplied by 100 to give the per cent of *p*-toluidine in the sample.

The following typical example will serve to illustrate the method of calculation:

Weight of mixed toluidines diazotized = 5.354 grams
Zero nitrometer reading = 1.30 ml.
Nitrometer reading after complete decomposition = 26.25 ml.
Volume of evolved nitrogen = 26.25 − 1.30 = 24.95 ml.
Room temperature = 25.0° C.
Barometric pressure = 758.0 mm. of mercury
Vapor pressure of 41.6 per cent potassium hydroxide at 25° C. = 7 mm. of mercury (*5*)
Corrected vapor pressure = 751 mm. of mercury
Since the density of nitrogen at standard conditions is 1.25055 (*4*), therefore at 25° C. and 751 mm. pressure 1 ml. of nitrogen = 1.132 mg.

$$\% \text{ } p\text{-toluidine} = \frac{\text{corrected ml. of } N_2 \text{ evolved}}{\text{wt. of sample} \times \text{factor}} \times 100 = \frac{24.95\ (1.132)}{5.354\ (96.06)} \times 100 = 5.49\%$$

Experimental Results

In commercial *o*-toluidine the para content is seldom in excess of 10 per cent. Hence for purposes of testing this method it was considered sufficient to analyze synthetic mixtures of toluidines made up to contain from 0 to 15 per cent of *p*-toluidine. In Table I are given results of analyses of such mixtures of pure *o*- and *p*-toluidines.

The major part of the work in testing the method involved the use of mixtures of the ortho and para isomers. Two experiments were carried out, however, in which the para isomer was mixed with *m*-toluidine; as is indicated in Table II, the method operates as well in the presence of the meta isomer as in the presence of only the ortho isomer.

Table I. Analyses of Synthetic Mixtures of *o*- and *p*-Toluidines

Para Found %	Para Present %
1.31	1.34
1.68	1.44
4.92	4.86
5.13	5.17
13.81	13.80
14.19	14.59

As other impurities may be present in commercial *o*-toluidine, steps were taken to determine whether these impurities would interfere with the successful operation of the analytical method. A common impurity is moisture. Obviously, since the method is carried out in aqueous solution, moisture does not interfere with the reactions involved. However, for accurate work the total diazotizable material present should first be determined. This may be done by means of well-established procedures for determination of aromatic amines.

Table II. Analyses of Synthetic Mixtures of *o*-, *m*-, and *p*-Toluidines

Para Found	Present	
	Para	Meta
%	%	%
4.86	4.74	4.07
5.36	5.18	4.37

Certain high-boiling impurities are sometimes present in commercial material. In order to test the effect of these materials on the analysis, 75 ml. of a commercial *o*-toluidine were distilled until only 2 or 3 ml. of a highly-colored, nonvolatile liquid remained. This remaining impurity was mixed with half of the distillate and the resulting mixture analyzed by the present method. The remaining half of the distillate was likewise analyzed and since, as shown in Table III, there was no difference in the values obtained, it was inferred that the nonvolatile impurity in the commercial sample had no effect on the results of the determination.

Precautions

The most critical aspect of the analytical method is the diazotization. Apparently *o*- and *m*-toluidine diazotize much more readily than *p*-toluidine; however, the resulting diazonium chlorides vary in stability as stated above. Thus, if complete diazotization of the para isomer is to be attempted, considerable decomposition of the other two isomeric diazonium chlorides will result during diazotization, even at very low temperatures, and the lower the temperature the slower the diazotization of the para isomer. Theoretically, the decomposition of *o*- and/or *m*-toluenediazonium chloride during diazotization of the mixture should be of no consequence, since complete decomposition of the ortho and meta isomers is to be carried out before any measurements are made. Practically, however, if the ortho and meta isomers decompose excessively and an excess of nitrous acid is present, side reactions occur which appear to involve *p*-toluenediazonium chloride, decomposed *o*-toluenediazonium chloride, and probably nitrous acid, since very low values are obtained if appreciable excesses of nitrous acid are allowed to accumulate during diazotization.

Table III. Analysis of Commercial *o*-Toluidine in Presence and Absence of High-Boiling Impurities

	Para, %
Pure distillate	0.92, 0.90
Av.	0.91
Pure distillate + residue	0.90, 0.92
Av.	0.91

Consequently, the authors' method prescribes the addition of nitrite so slowly that the troublesome side reactions are virtually eliminated. The prescribed temperature is high enough so that the greater part of the para isomer diazotizes, and yet not enough of the ortho and meta isomers decompose to cause significant errors. The side reactions mentioned can be recognized by the development of a deep coloration during diazotization and/or decomposition. Thus, by use of the diazotization procedure outlined the *p*-toluidine is incompletely diazotized, but if the directions are carefully followed, the reproducibility of the degree of diazotization appears to be well within the limits of accuracy stated.

The accuracy of the analytical method depends not upon complete diazotization but upon diazotization to the same extent every time. It thus becomes necessary for the calculations to include a term which will embrace the extent to which diazotization of the *p*-toluidine is complete. The term 96.06 in the above calculations is the milligrams of nitrogen actually obtained per gram of pure *p*-toluidine when diazotization has been carried out as described herein and has been found to hold over the range of mixtures reported.

Acknowledgments

The authors wish to acknowledge the assistance of Charles D. Compton in the development of the details of this method and to express thanks to A. R. Norton and William Seaman, who have kindly permitted the authors to cite the results of critical tests carried out on the method by members of their staff.

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Electronic Timer

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This paper describes a resistance-capacitance circuit which, when used in conjunction with a thyratron-type tube, provides a time-delay relay instantly adjustable and capable of operating mechanical devices automatically at predetermined time intervals.

IN THE control of laboratory apparatus and in certain types of industrial control problems, it is frequently desirable to operate mechanical devices or electrical circuits in a cyclical manner with predetermined time intervals. Usually it is required that control devices be of such a nature that they will operate automatically and without attention over long periods of time.

An obvious means of accomplishing this end is to employ a motor-driven rotary switch which develops the required timing pulses. Such devices are not usually capable of instant variation over wide ranges of time intervals and frequently require changing gears or other speed-reduction elements. Rotating contacts are not always reliable for continuous operation, especially when relatively large currents are being interrupted. Thermally operated devices are also available but they usually present the same disadvantages as have been attributed to rotary apparatus.

A less commonly used method of timing is one which relies upon the time characteristics of a resistance-capacitance network for control of the time interval. Such a network, when used in conjunction with a tube of the gas-filled or thyratron type, will provide a time-delay relay which is capable of controlling an electromechanical relay having a current-carrying capacity of considerable magnitude.

The apparatus described here is essentially the same as that described by Goldberg (*3*) for a specific use in photography. Similar circuits with other adaptations have been described by Gilson (*2*), Smiley (*5*), and Mucher (*4*). Of course, the fundamental resistance-capacitance network is well known and is thoroughly treated in textbooks on the subject as well as in summaries in handbooks and similar publications (*1*). The principal advantage of the present apparatus is that it provides a repeated time pulse automatically, whereas that used by Goldberg requires resetting after each operation.

When a condenser is allowed to discharge through a resistance shunted across the condenser the time interval required for the condenser voltage to reach a given level is expressed by

$$t = RC \log_\epsilon \frac{E}{e} \qquad (1)$$

where E = voltage to which condenser is initially charged
e = voltage to which condenser falls in time t
R = value of shunt resistance in ohms
C = capacity in farads
ϵ = Napierian logarithmic base

Factor e given in Equation 1 as the potential to which the condenser falls in time t, is also the critical grid potential below which the thyratron will conduct a plate current. To determine its value in the absence of experimental data it is necessary to refer to the characteristics chart of the 885 (or 884) tube.

For any given values of E and e the time interval may be made almost any chosen value, since a large number of combinations of R and C may be obtained from parts which are commercially available. Furthermore, the time interval may be controlled by utilizing variable components for either R or C or both.

Figure 1 shows the diagram of a circuit which will deliver an electrical pulse at definite time intervals, with instant control of the interval by the aid of a calibrated dial.

The diagram indicates the use of type 885 gas triode with a 2.5-volt filament supply. The type 884 gas triode which requires a 6.3-volt filament supply may be substituted for type 885 if so desired. The parts used are all available through the usual radio trade channels. In certain instances manufacturers will supply equivalent parts which may be substituted. Care should be taken to secure apparatus of good design and construction. This is particularly true of the R-C network components where condensers of low leakage value and good stability to atmospheric changes are required if accurate and stable calibration is desired. Hence, care should be taken to see that the desired capacities are

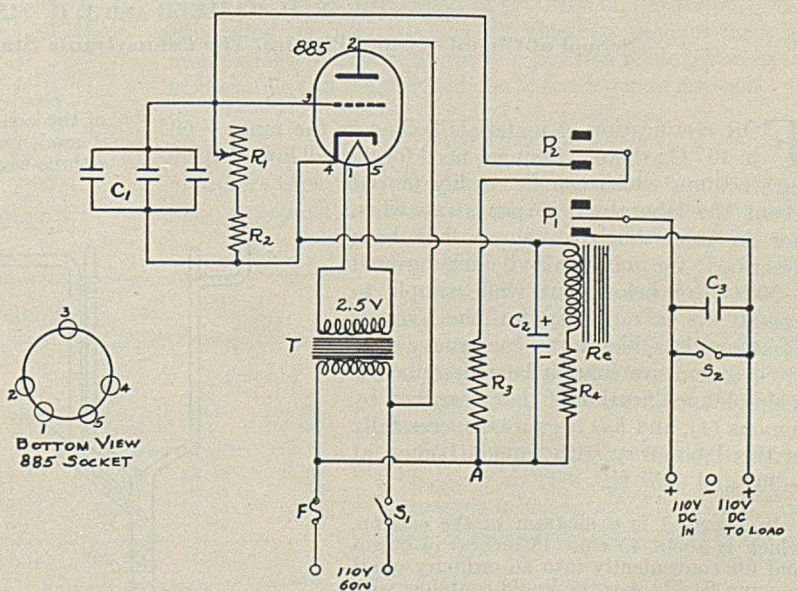

FIGURE 1. WIRING DIAGRAM

C_1. 3 Western Electric telephone type condensers (paper). Actual measured values = 2.23 + 2.18 + 1.10 = 5.51 total μfd.
C_2. 8-μfd. 250 WV electrolytic, Aerovox PBS-8
C_3. 1-μfd. 450 WV paper
R_1. 1-megohm potentiometer, Centralab No. 6 taper
R_2. 50,000-ohm 1-watt carbon
R_3, R_4. 10,000-ohm 1-watt carbons
T. 2.5-volt, 2.5-ampere filament transformer, Stancor P6141
Re. Double-pole double-throw relay, 2000-ohm coil, Guardian
885. RCA Type 885 gas-filled triode
F. 2.0-ampere Littelfuse
S_1. Single-pole single-throw switch
S_2. Single-pole single-throw switch, nonlocking, for manual control of load

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obtained. Frequently, "rated" or "replacement" values are given for paper condensers which are much greater than the actual effective capacities. Potentiometer R_1 should also be of good construction, so that it will repeat its setting and will not be subject to sudden and unpredictable resistance variations. The relay, *Re*, should be carefully selected, since many of the cheaper varieties are not reliable and may fail to make contact at times because of poor mechanical construction.

The operation of the circuit is simple and may be described by following through a complete cycle.

Let us start at that point in the cycle where a plate current has just started to flow and before the relay has had time to close. The thyratron acts as a half-wave rectifier. Because of the current in the plate circuit the relay coil is energized and there is also a potential drop across the relay coil and R_4 as indicated by the signs of C_2. As soon as the relay contacts have closed, this potential drop acts as an electromotive force in the circuit containing condenser C_1, the terminals of which are connected to the grid and cathode of the thyratron. As a consequence the condenser is charged and the potential of the grid becomes decidedly negative with respect to the cathode. This negative potential of the grid has no effect on the plate current during the time the thyratron is ionized. However, it prevents reionization following the deionization which occurs during that half of each current cycle in which the plate is negative. The rectified current, therefore, ceases and relay contacts P_1 and P_2 open, leaving condenser C_1 charged.

This charge then leaks off through R_1 and R_2 according to Equation 1, and as it does the potential of the grid becomes less negative. After the critical value is reached the tube reionizes as soon as the potential of the plate reaches a peak in the positive direction, thus completing the cycle.

The relay is de-energized most of the time. It receives short pulses at time intervals determined by grid circuit elements C_1, R_1, and R_2. If it is desired to have current flowing in the load circuit during the discharge interval instead of a momentary pulse, the "back contacts" of the relay may be used. The values given for R_1, R_2, R_4, and C_1 provide a time-interval range from 140 to 9 pulses per minute over the range of adjustment of potentiometer R_1. Resistance R_2 determines the minimum time interval available. Changing R_4 will change the time-interval range because of changes produced in the charging voltage, E. If a greater range is desired, a group of resistors may be used with a selector switch as shown by Goldberg (*3*), or other condensers may be switched into the circuit by similar means.

The relay indicated will carry currents up to 5 amperes at 110 volts through its contacts. If the controlled load requires a greater current, other relays may be selected or a second relay of high current-carrying capacity may be operated from points P_1. Either alternating or direct current may be used for load operations. In the particular application shown in Figure 1 the controlled load was actuated by current from a 110-volt direct current source. Condenser C_3 is used to prevent arcing at points P_1. This condenser should be selected to have the best value for the particular reactance and resistance characteristics of the controlled load.

The apparatus described here may be applied to a wide variety of timing operations. For time intervals not available with the components shown in Figure 1 it is only necessary to replace R_1, R_2, and C_1 with components having the desired values which may be calculated from Equation 1.

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Design for Rectifying Column

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FOR rectification of materials boiling in the range −60° to 10° C., there is frequent need for a small low-temperature column which can be readily moved about the laboratory or put away when not in use. Such a column has been described for materials boiling around −50° C. or below, but while simple to operate, it is not easy for the average amateur glass blower to construct. The low-temperature column here described is a simple modification of that described by Simons (*1*), and has been used successfully in this laboratory with liquids boiling at −30° and −5° C.

In Figure 1 is a diagram of the column, which is about 45 cm. (18 inches) in height and fits conveniently into an ordinary quart vacuum flask. *A* is the liquid container with a volume of about 50 cc. Sealed on the bottom is the nipple, *B*, which is wound with a heating coil of asbestos-covered Nichrome wire, B. & S. No. 26. The packed section, *C*, of the column is 9-mm. tubing and contains glass helices supported by a small glass cross bar. *D* is the take-off tube in which is sealed a small thermocouple well, containing a copper-constantan couple. The condenser, *E*, consists of two concentric tubes of 42- and 37-mm. outside diameter, sealed at the top. The 7-mm. tube, *F*, sealed at the top of the condenser, is to permit the escape of air as the material is condensed in *A*. The fractions may be obtained by the use of a three-way stopcock on *D*, or of a chain of traps in series.

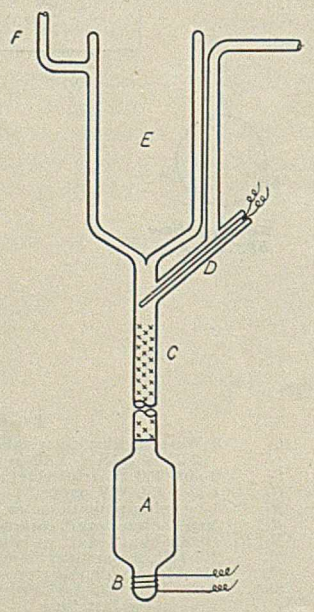

FIGURE 1

The column is very simple to operate. It is placed in a quart-size unsilvered Dewar flask, which is surrounded by a radiation shield of aluminum sheet containing slots cut into it for observation of the pot and reflux. Leaving tube *F* open, the material is condensed in *A* through the take-off tube with the aid of the cooling mixture in *E* and in the Dewar flask.

The Dewar flask is now removed, emptied, and replaced around the column. With *D* closed, *A* is heated if necessary to bring about a reflux. When equilibrium is established, *F* is closed and *D* is opened, and the condensate is received in a series of traps, the last one of which is open to the air. If the take-off is too slow, it may be increased by the use of a very slight vacuum obtained from an ordinary laboratory water-suction pump, applied to the last trap through a stopcock. The amount of take-off can be regulated by adjustments of both the heat supplied to the pot and the vacuum on the receiver. The temperature at the top of the column can be determined with a potentiometer.

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An Improved Soxhlet Extractor

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IN THE course of work on the analysis of commercial soy flours at the laboratory of the Agricultural Marketing Administration, Beltsville, Md., it was necessary to report the oil content values the same day that the samples of soy flour were received. The accepted method for determining the oil content of ground soybeans is to make the extractions in a Soxhlet or Butt extractor with petroleum ether. This method requires from 16 to 20 hours or a 4-hour extraction with regrind after 2 hours. It was much too slow for the needs of the laboratory workers, since results for reports were needed in from 4 to 5 hours.

Among the rapid methods for determining the oil content of oil-bearing seeds are the refractometric method (*5*), the method based on the change in density of a solvent (*1*, *2*), and various shaking procedures (*4*) that employ different types of solvents. All these are not accepted as standard or official for soy flour, but the extraction method is the one adopted by the Soybean Analysis Committee of the Oil Chemists' Society (*3*).

In the author's laboratory, where a battery of 36 Soxhlet extractors is in use, the color of the extract frequently indicates that some extractions proceed faster than others. These differences are probably due to variations in the porosity of the paper thimbles as well as to the manner in which the samples pack themselves within the thimbles. In some cases the retardation of the flow of the solvent is so great that the solvent will flow over the top of the cotton wad in the thimble, rather than through the sides and base of the thimble. Hence, in such cases, very little solvent really percolates through the sample, although if the extraction is allowed to proceed long enough, a complete extraction is obtained in 16 hours.

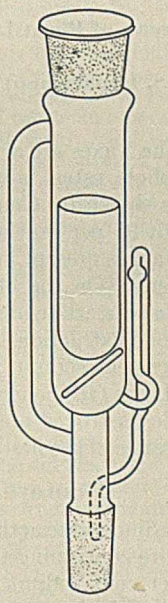

FIGURE 1

It occurred to the writer that having the base of the thimble next to the base of the extractor might retard the flow of solvent through the thimble. Therefore the thimble was elevated by inserting a piece of glass rod at an angle into the extractor and resting the thimble upon the rod (Figure 1). This rod was long enough to raise the thimble 3.75 cm. (1.5 inches) off the base, but the sample (10 grams) within this thimble was still below the overflow siphon of the extractor, so that at intervals the sample was completely immersed in the solvent and channeling was avoided. Another means of achieving the elevation was to make three depressions in the extractor, about 3.25 cm. (1.5 inches) from the base and 120 degrees apart, upon which to rest the paper thimble. The sample was then extracted with Skellysolve F at a rate equivalent to one siphoning about every 3 minutes, whereas with the common method about one minute was required.

The value of the improvement in the Soxhlet extractor can be seen by comparison of the results of extractions by the two methods (Table I). Extraction flasks were changed at the end of 1, 2, and 3 hours, so that each value represents the extraction for each successive hour except in the overnight extraction. All of the soy flours do not extract at the same rate of speed by any method. Extractions varied at the end of the first hour anywhere from 20 to 92 per cent of the total fat content. By use of the elevated thimble method, however, more than 98 per cent of the total fat was extracted during the first hour from all but one of the six samples. The differences between samples are probably due to the nature of each sample as regards its fineness and the distribution of the oil film through the sample. Differences that might be due to variations in porosity of the thimble were eliminated by interchanging the thimbles used in the common extraction in one sample to a position of elevation in the next sample and the elevated one to the common position.

TABLE I. EXTRACTIONS OF OIL FROM SOY FLOUR

(By common method of setting the thimble as compared with improved method of using an elevated thimble)

Fraction	Common		Elevated		Common		Elevated	
	Per cent fat	Per cent of total	Per cent fat	Per cent of total	Per cent fat	Per cent of total	Per cent fat	Per cent of total
	Sample 1				Sample 2			
1st hour	8.36	41.32	20.23	99.30	20.63	91.95	22.40	99.29
2nd hour	4.38	21.66	0.07	0.35	1.65	7.33	0.04	0.18
3rd hour	3.38	16.70	0.01	0.03	0.07	0.32	0.03	0.11
Overnight	4.11	20.32	0.07	0.32	0.09	0.40	0.09	0.42
Total	20.23	100.00	20.38	100.00	22.44	100.00	22.56	100.00
	Sample 3				Sample 4			
1st hour	21.28	95.71	22.02	99.34	10.81	48.66	22.53	99.67
2nd hour	0.79	3.57	0.05	0.20	8.89	40.01	0.00	0.00
3rd hour	0.07	0.31	0.00	0.00	2.52	11.33	0.02	0.09
Overnight	0.09	0.41	0.10	0.46	0.00	0.00	0.05	0.24
Total	22.23	100.00	22.17	100.00	22.22	100.00	22.60	100.00
	Sample 5				Sample 6			
1st hour	13.41	60.75	21.78	98.45	3.81	20.54	14.58	78.06
2nd hour	7.67	34.74	0.19	0.86	2.95	15.91	3.88	20.79
3rd hour	0.77	3.49	0.01	0.05	2.11	11.35	0.03	0.18
Overnight	0.23	1.02	0.14	0.64	9.68	52.20	0.18	0.97
Total	22.08	100.00	22.12	100.00	18.55	100.00	18.67	100.00

Summary

A more rapid method of determining the oil content of soy flour was sought, so that oil content values could be reported the same day samples of soy flour were received by the laboratory of the Agricultural Marketing Administration at Beltsville, Md. To this end an improvement was made in the Soxhlet extractor, the use of which, with petroleum ether, is an accepted method of extraction.

This improvement would no doubt help to shorten the time required to extract other types of materials, such as oil-bearing seeds and plant materials.

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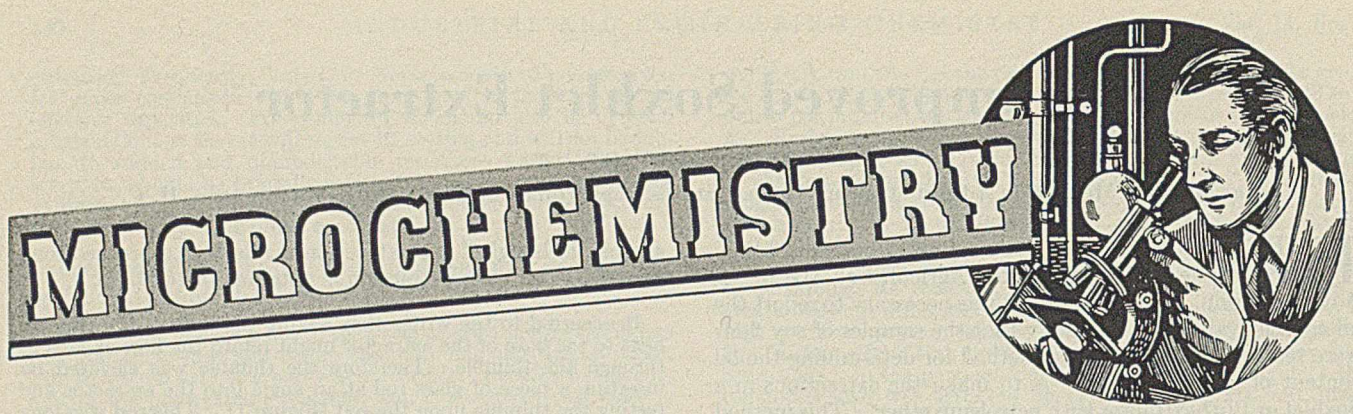

Quantitative Determination of Cellulose in Raw Cotton Fiber

A Simple and Rapid Semimicro Method

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ALTHOUGH cellulose is the major component of raw cotton fiber, its ratio to noncellulose constituents varies considerably with the growth conditions under which the fiber is produced. For example, analyses have shown that the cellulose of naturally opened bolls may constitute from less than 85 to over 97 per cent of the dry weight of the fiber. It is often desirable to know the cellulose content of cotton fiber. The use of the cellulose content as a criterion of cotton fiber maturity, as suggested by Sakostschikoff (*9*), Gontscharow and Burwasser (*4*), and more recently Conrad *et al.* (*2*), requires a rapid routine method for determination of the cellulose. Furthermore, the evaluation of the quality of cellulose in cotton fiber by means of such measures as copper number, alkali solubility, and fluidity of the cuprammonium solution, makes it necessary that these be expressed on the basis of the cellulose content, if the results are to be truly comparable.

For the routine determination of cellulose, it is desirable to select a method that is as simple and rapid as possible, while still possessing sufficient accuracy for the purpose in hand. Of the many techniques that have been proposed, a large proportion include steps designed to eliminate lignin which so frequently accompanies cellulose. In cotton fiber, lignin may be considered to be approximately, if not completely, absent. This greatly simplifies the procedure by making unnecessary any extensive chlorination treatment.

One of the most practical methods for determining cellulose in the absence of interfering substances is by oxidation and titration. A convenient description of such a procedure, employing potassium dichromate as the oxidant, is given by Launer (*6*); however, for the above-mentioned purpose, it is not necessary to separate the cellulose into its several fractions. In contrast to gravimetric procedures, the tenacious retention of ash constituents is of no consequence in volumetric procedures. However, it is necessary to remove noncellulose organic constituents before oxidation of the cellulose since their oxidation equivalents are similar to that of cellulose.

The chief noncellulose constituents of cotton fibers, aside from the ash, are waxes, pectic substances, nitrogenous substances, and small amounts of residual sugars and pigments.

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The waxes are a very heterogeneous mixture consisting of alcohols, esters, aliphatic acids, sterols, and hydrocarbons, but as shown by Conrad (*1*) they are rather easily extracted by hot 95 per cent alcohol. The sugars and certain other substances present in small amount are removed by the same solvent. On the other hand, the pectic and nitrogenous substances, while incompletely or not at all removed by the alcoholic extraction, are rather easily removed by boiling with 1 per cent sodium hydroxide solution. Whistler, Martin, and Harris (*10*) concluded that boiling 1 per cent sodium hydroxide destroyed and removed practically all of the pectic substance in the first half hour.

Removal of Nitrogenous Constituents

Since no exactly pertinent data could be found in the literature concerning the rapidity of nitrogen removal by boiling 1 per cent sodium hydroxide, some experiments were undertaken by the writers.

Duplicate samples of finely-cut alcohol-extracted cotton fibers were boiled with approximately 1000 volumes of the solution for 1, 2, and 4 hours. The solution was then filtered off and the residue washed with warm water. The amount of nitrogen was estimated in the residue, using the Kemmerer and Hallett (*5*) micro-Kjeldahl method, but collecting the ammonia in boric acid according to Winkler's (*12*) recommendation. The following results show that the small amounts of nitrogenous constituents remaining in alcohol-extracted cotton are rapidly decomposed by boiling 1 per cent sodium hydroxide and the amount remaining after 2 hours must be insignificant:

Time of Sodium Hydroxide Boil, Hours	Nitrogen, %
0	0.17
1	0.12
2	0.00
4	0.00

Effects of Treatments on the Cellulose

From the above discussion and results it would appear that the noncellulose constituents of cotton fiber may be removed by successive extractions with hot ethyl alcohol and boiling 1 per cent sodium hydroxide. However, attention must be given also to retention of the cellulose. Worner and Mease (*13*) found that continued extraction in a Soxhlet apparatus with hot alcohol and ether had no significant effect on either

the weight or the properties of cotton fibers after the first 6 hours. On the other hand, extended extraction with boiling 1 per cent sodium hydroxide resulted in progressive loss of the cellulose, at the rate, neglecting the first 6 hours, of 0.34 per cent per hour. The relatively large loss during the first few hours may be assumed to be due to removal of pectic materials. This loss of cellulose was accompanied by a progressive increase in the fluidity (average 0.46 rhe per hour) of the undissolved cellulose, indicating slow progressive hydrolysis of the chain molecules. The work of Davidson (*3*) indicates that the shorter chain fragments dissolve in the alkali. Thus, a compromise must be made in the case of the alkali extraction between complete removal of the noncellulose constituents and the dissolution of the cellulose itself.

Proposed Method

The proposed method allows for a 4-hour extraction of the fiber in a Soxhlet apparatus with hot 95 per cent ethyl alcohol, followed by drying and a 2-hour extraction with boiling 1 per cent sodium hydroxide. Experiments showed that the alcohol extraction, if continued at such a rate that the siphon operated once every 3 minutes, removed approximately 95 per cent of the waxy constituents and it may be assumed that the subsequent alkali extraction removes a considerable portion of the remainder. Since cotton fiber ordinarily contains less than 1 per cent originally, the error from the small residue of these substances must be inconsequential. The 2-hour alkali extraction is chosen as the best compromise for the destruction and removal of pectic and nitrogenous substances with a minimum attendant destruction and dissolution of the cellulose itself. While slight oxidation of the cellulose probably occurs under the conditions employed, its extent could have little effect on the analytical values obtained.

Extract the sample (10 grams or less) of cotton fiber in a large Soxhlet apparatus for 4 hours with hot 95 per cent ethyl alcohol. Remove the fiber from the apparatus, squeeze as dry as possible, and set aside overnight or longer to air-dry. When sufficiently dry, pass through a Wiley (*11*) or similar mill so that the fiber passes a 1-mm. sieve. Store. (When the results are to be used as a basis for reporting copper number, alkali solubility, etc., it is best to expose the fiber for 2 to 3 hours in the vicinity of the balance and weigh out samples for all determinations within a short interval of time.)

Weigh out duplicate 0.1000-gram samples of the finely divided fiber on a small watch glass and transfer with the aid of a camel's-hair brush to a 250-ml. Erlenmeyer flask. With a pipet, add 100 ml. of 1 per cent sodium hydroxide solution (free from carbonates) to the flask and mix with the fiber with a rotary motion. Connect flask to an upright reflux condenser supported over an asbestos wire gauze on a ring stand. Bring to boil and boil for 2 hours. Discontinue heating and filter the mixture with the aid of suction using a 30-ml. fritted Pyrex glass crucible of C porosity, supported in an adapter over a 1-liter filter flask. Wash the mat with 4 approximately 10-ml. portions of hot (60° C.) water. Discard the filtrate and washings.

With the aid of a pointed glass rod transfer as much as possible of the washed fiber mat from the crucible to the original Erlenmeyer flask. Insert into the latter a suitable 2-hole rubber stopper, containing a crucible adapter and glass tube connected to suction line. Insert the crucible in adapter and, with suction turned off, add 15 ml. of 12 molar sulfuric acid. Let stand for a short time (about 2 minutes) to dissolve any cellulose remaining in the crucible and then turn on the suction. Now wash the inside of the crucible twice with 5-ml. portions of 12 molar sulfuric acid, each time interrupting the suction during the addition of the acid, and then turning it on to draw the acid through. Finally wash the crucible with 5 to 10 ml. of distilled water and remove the stopper with adapter from the flask. Accurately pipet 25 ml. of 0.6 *N* potassium dichromate solution into the flask (the total volume should now be 55 to 60 ml.) and boil gently under a reflux condenser for one hour. Remove the flask, stopper, and allow to cool. Add approximately 40 ml. of water and 3 drops of *o*-phenanthroline indicator (1.485 per cent *o*-phenanthroline monohydrate dissolved in 0.025 molar ferrous sulfate solution), and titrate with freshly prepared 0.5 *N* ferrous ammonium sulfate (made up in approximately normal sulfuric acid) until the color changes abruptly from dark green to a deep pink or red.

The difficulty in detecting the color change from a very dark green to a still darker deep pink or red in the titration of the dichromate solution with ferrous ammonium sulfate has been entirely eliminated by titrating above a ground-glass platform beneath which an electric light is fixed.

The ferrous ammonium sulfate is standardized immediately before use by titrating 25-ml. portions of the 0.6 *N* potassium dichromate solution in a flask containing 12 ml. of 95 per cent sulfuric acid, 90 ml. of distilled water, and 3 drops of *o*-phenanthroline indicator.

The weight of pure cellulose in the original sample is computed on the assumptions that it is represented by the empirical formula $C_6H_{10}O_5$, that complete oxidation to carbon dioxide and water occurs, and that 1 ml. of normal potassium dichromate is equivalent to 0.00675 gram of cellulose. Actually Launer (*6*) found an average equivalent value of 0.00677 gram for cellulose from rags, pulp, and paper.

The percentage of cellulose may be expressed in terms of dry weight with the aid of a moisture determination, or the value as found may be used in lieu of a moisture determination as a basis for reporting copper number and other characteristics of cotton cellulose when a knowledge of the moisture content of the fiber is immaterial.

The method here described has many advantages to recommend its adoption. It requires a minimum of equipment, all of which is readily available in any chemical laboratory. The use of small samples results in material savings of reagents and conservation of sample, and permits use of the method where the quantity of available material is limited. Handling of the sample during analysis is reduced to a minimum and the whole sample is titrated rather than an aliquot portion.

Obviously, the method could be adapted readily to a gravimetric determination of the residual cellulose if other determinations on the cellulose sample are required, and it could be used in analyses of fabrics after removal of sizing. The method is not suitable, of course, for cellulosic materials containing more or less lignin.

Comparison with Other Methods

In order to appraise the proposed method, the results obtained with it on a series of ten cottons, selected for a wide range of cellulose content, were compared with those obtained on portions of the same samples by two other well-known methods. The methods chosen for comparison were the Norman and Jenkins method (*7*) which was applied after a 2-hour alkali extraction, and the monoethanolamine method described by Reid, Nelson, and Aronovsky (*8*). However, both of these latter methods were adapted to 0.1000-gram samples by reducing the quantities of reagents but maintaining the same concentrations, ratios of quantities to that of sample, and times for reaction as in the original procedures. In all other ways attempts were made to maintain as nearly as possible the conditions used in the macromethod. Also, in all methods the residual cellulose was determined volumetrically by dissolving and oxidizing the sample in acid dichromate and determining the amount of dichromate used. This same technique was employed for all three procedures, in order that the conditions at this stage of the analyses might be comparable.

In the case of the monoethanolamine method, a 19 × 125-mm. Pyrex test tube, fitted by means of a ground-glass joint to an 8-mm. air condenser 200 mm. high, was substituted for the refluxing apparatus described by Reid, Nelson, and Aronovsky (*8*). It was supported in a glycerol bath, provided with a false bottom, and maintained at 190° ± 3° C.

The samples were prepared for analysis by extraction of 10 grams of raw cotton with 95 per cent ethyl alcohol for 4 hours in a large (50 × 250 mm.) Soxhlet extractor. The cotton was then removed from the extractor, squeezed as dry as possible, and allowed to dry in the air overnight. The material was passed through a Wiley mill, equipped with a 1-mm. sieve, and placed in 120-ml. (4-ounce) bottles for storage. Subsamples, in duplicate, for each of the three cellulose methods and for moisture were weighed out consecutively in such a way as to avoid exposure to serious fluctuations in atmospheric moisture content. The results are shown in Table I.

By reference to Table I, it will be seen that the proposed method gave values intermediate, on the average, between those obtained by the Norman and Jenkins and the monoethanolamine methods. Cellulose content found by the monoethanolamine method is, on the average, higher by 1.33 per cent than that found by the proposed method, while by the Norman and Jenkins method it averages 0.63 per cent lower. Statistically, the mean deviations are in both cases highly significant, indicating that they are real and not accidental.

TABLE I. CELLULOSE FOUND IN ALCOHOL EXTRACTED COTTON FIBER

Sample No.	Cellulose[a] Found			Deviations of Proposed Method from:	
	Proposed	Norman and Jenkins	Monoethanolamine	Norman and Jenkins	Monoethanolamine
	%	%	%	%	%
1124	93.15	91.94	94.35	1.21	−1.20
1137	92.30	92.58	94.01	−0.28	−1.71
1138	93.22	92.88	94.08	0.34	−0.86
1144	90.18	89.76	92.24	0.42	−2.06
1410	84.06	83.37	84.53	0.69	−0.47
1411	90.38	89.20	91.80	1.18	−1.42
1412	83.98	83.40	85.90	0.58	−1.92
1414	87.50	86.13	88.50	1.37	−1.00
1415	93.21	92.14	93.96	1.07	−0.75
2494	83.19	83.48	85.07	−0.29	−1.88
Av.	89.12	88.49	90.44	0.629 ± 0.188	−1.327 ± 0.175

[a] Based on oven-dry weight.

The slightly larger average percentage of cellulose obtained by the proposed technique than by the Norman and Jenkins method is probably to be expected since the latter, designed for removal of lignin in lignin-bearing materials, involves treatment essentially oxidative and additive to that employed in the proposed technique. Whether the difference is due to a greater removal of the noncellulose impurities by the Norman and Jenkins method or to slightly greater loss of the cellulose itself cannot be stated with certainty.

Based on the findings of Worner and Mease (*13*), there should be a loss of 0.68 per cent of cellulose during the 2-hour period of boil with 1 per cent sodium hydroxide. The proposed method gives a yield of cellulose 1.33 per cent less than the ethanolamine method. Taking into account the 0.68 per cent of cellulose that would be lost during the 2-hour boil, there remains a difference of 0.65 per cent of cellulose still unaccounted for. A tendency for the ethanolamine method to give slightly higher yields of crude ash-free cellulose than either the Cross and Bevan or Norman and Jenkins method was noted by Reid, Nelson, and Aronovsky (*8*), using entirely gravimetric techniques. Thus, their results on five agricultural wastes and two spruce wood samples show an average of 54.11 per cent "crude cellulose corrected for ash" by the ethanolamine method, 52.61 per cent by the Cross and Bevan method, and 53.54 per cent by the Norman and Jenkins method. On the other hand, the average yields of "pure cellulose" on these same materials were 39.89, 40.40, and 39.69 per cent, respectively, indicating very little difference in the amount of actual cellulose retained by the three methods. The differences in yield between crude and pure celluloses were due, principally, to pentosans which are incompletely removed by any of the methods. The difference in the present case is therefore in the same direction as observed by Reid, Nelson, and Aronovsky.

Summary and Conclusions

A new, simpler, and more rapid semimicromethod for the quantitative determination of cellulose in raw cotton than heretofore available accomplishes the maximum removal of the accompanying organic noncellulose materials with a minimum of equipment, sample, and damage to the fiber itself.

The method consists of a 4-hour Soxhlet extraction with hot ethyl alcohol followed by drying and a 2-hour extraction with boiling 1 per cent sodium hydroxide. The cellulose is determined by dichromate oxidation of the whole sample and titration of the residual dichromate with ferrous ammonium sulfate.

The waxes, sugars, pectins, nitrogenous, and certain other substances present in small amounts in raw cotton are so thoroughly removed by the treatment that the error due to residues of these substances must be inconsequential.

In comparative analyses of ten different cottons, the average per cent of cellulose was slightly higher by the proposed technique than by a semimicroadaptation of the Norman and Jenkins procedure and lower than that obtained by a similar adaptation of the Reid, Nelson, and Aronovsky ethanolamine procedure.

The method requires no equipment which cannot be made readily, or assembled from apparatus and materials found in the average laboratory.

A special feature of the procedure is the titration of potassium dichromate solutions with ferrous ammonium sulfate using *o*-phenanthroline indicator over a ground-glass plate beneath which is an electric light. This is much more rapid and convenient than the usual titration with an outside indicator and spot plate.

The proposed method is recommended particularly for the determination of the cellulose content of raw cotton and may be used in analyses of desized fabrics. It is not suitable for cellulosic materials which contain lignin.

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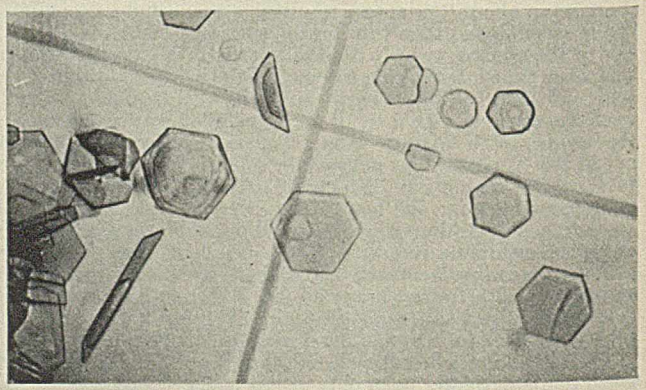

Courtesy, L. M. Willard

PHOTOMICROGRAPH OF POTASSIUM IODIDE

Reactivity of Substituted Thioureas with Inorganic Ions

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THE examination of the reactions of 60 substituted thioureas with 78 inorganic ions was undertaken to ascertain whether or not any of these reactions might be more sensitive than those given by thiourea, especially for bismuth.

The results presented in this paper show that the substituted thioureas—i. e., compounds containing the reactive group $=N-\overset{\overset{S}{\|}}{C}-N=$, give reactions similar to those of thiourea and that although a number of the reactions are as sensitive, or even slightly more sensitive for bismuth, they offer no advantage to justify their use in place of thiourea.

An attempt was made to correlate the reactivity or nonreactivity of certain ions with the structure of the various substituted thioureas. Because of the similarity of the reactivity no definite conclusions could be formulated. The differences in solubility of the various substituted thioureas undoubtedly play a more important part in their reactivity than does the presence of any particular substituted group. Moreover, no correlation of sensitivity and structure appears to be possible.

The reactions and the sensitivities of the more sensitive ones are given for the substituted thioureas, as well as those for thiourea.

Experimental

The experiments were performed on a spot plate by adding 1 drop of the solution of the reagent to 1 drop of the solution containing the inorganic ion. The reactions were carried out in aqueous, acid, or ammoniacal medium.

Solutions containing 1 mg. per ml. of the following 78 ions were used. (It is recognized that some of the ions are more complex than is indicated by the formulas in this list.): Ag^+, Al^{+++}, $AuCl_4^-$, As^{+++}, AsO_4^{---}, $B_4O_7^{--}$, Ba^{++}, Be^{++}, Bi^{+++}, Br^-, CO_3^{--}, Ca^{++}, CbO_4^{---}, Cd^{++}, Ce^{+++}, Ce^{++++}, Cl^-, Co^{++}, Cr^{+++}, Cs^+, Cu^{++}, Dy^{+++}, Er^{+++}, Eu^{+++}, F^-, Fe^{++}, Fe^{+++}, Ga^{+++}, Gd^{+++}, Ge^{++++} (0.5 mg. per ml.), Hf^{++++} (0.25 mg. per ml.), Hg_2^{++}, Hg^{++}, I^-, In^{+++}, $IrCl_6^{--}$, K^+, La^{+++}, Li^+, Mg^{++}, Mn^{++}, MoO_4^{--}, NO_2^-, NO_3^-, Na^+, Nd^{+++}, Ni^{++}, OsO_5^{--}, HPO_4^{--}, Pb^{++}, $PtCl_6^{--}$, Pr^{+++}, Rb^+, ReO_4^-, $RhCl_5^{--}$, $RuCl_4^-$, S^{--}, SO_4^{--}, Sb^{+++}, Sc^{+++}, SeO_3^{--}, SiO_3^{--}, Sm^{+++}, Sn^{++}, Sn^{++++}, Sr^{++}, TaO_4^{---}, TeO_4^{--}, Ti^{++++}, Th^{++++}, Tl^+, Tm^{+++}, UO_2^{++}, VO^+, WO_4^{--}, Y^{+++}, Yb^{+++}, Zn^{++}, Zr^{++++}.

An alcoholic solution of the reagent containing 10 mg. per ml. was used where the solubility permitted; otherwise a saturated solution was employed.

The reagents were obtained from E. I. du Pont de Nemours & Co., Inc., and the Eastman Kodak Co. and were used without further purification.

The reactions of the following 60 substituted thioureas were observed:

1. Allylthiourea
2. Monolaurylthiourea
3. Dilaurylthiourea
4. *s*-Diethylthiourea
5. Di-*n*-butylthiourea
6. Phenylthiourea
7. Benzylthiourea
8. *o*-Tolylthiourea
9. *m*-Tolylthiourea
10. *p*-Tolylthiourea
11. Xylidylthiourea
12. 2-Carboxyphenylthiourea
13. *o*-Chlorophenylthiourea
14. *p*-Hydroxyphenylthiourea
15. *m*-Nitrophenylthiourea
16. *p*-Fluorophenylthiourea
17. *p*-Ethoxyphenylthiourea
18. *o*-Methoxyphenylthiourea
19. *p*-Methoxyphenylthiourea
20. *p*-Isoamyloxyphenylthiourea
21. *o*-*n*-Butyloxyphenylthiourea
22. 2-Methoxy-5-methylphenylthiourea
23. 2,4-Dimethylphenylthiourea
24. α-Naphthylthiourea
25. Di-α-naphthylthiourea
26. β-Naphthylthiourea
27. *ac.*-Tetrahydro-β-naphthylthiourea
28. *N*-4-Ethoxyphenylpiperidylthiourea
29. Di-*o*-hydroxycyclohexylthiourea
30. Dimethylcyclohexylthiourea
31. Phenylethanolthiourea
32. *N*-(β-hydroxyethyl)-N^1-4-allyloxyphenylthiourea
33. *N*-(β-hydroxyethyl)-N^1-4-isoamyloxyphenylthiourea
34. *N*-Methyl-N^1-4-ethoxyphenylthiourea
35. N-Dimethyl-N^1-4-isopropyloxyphenylthiourea
36. *N*-Dimethyl-N^1-4-ethoxyphenylthiourea
37. *N*-(*n*-Butyl)-N^1-4-ethoxyphenylthiourea
38. *N*-(Di-*n*-butyl)-N^1-4-ethoxyphenylthiourea
39. *N*-Ethyl-N^1-4-isobutyloxyphenylthiourea
40. *N*-(β-Diethylaminoethyl)-*N'*-*n*-butyloxyphenylthiourea
41. *N*-(*p*-chlorophenyl)-N^1-acetylthiourea
42. *s*-Diphenylthiourea
43. *s*-Di-*o*-tolylthiourea
44. *s*-Di-*m*-tolylthiourea
45. *s*-Di-*p*-tolylthiourea
46. *s*-Di(2-methoxy-5-methylphenyl)thiourea
47. Phenyl-*o*-tolylthiourea
48. Diethoxydiphenylthiourea
49. N,N^1-di(*p*-*n*-propyloxyphenyl)-thiourea
50. N,N^1-di(*p*-bromophenyl)-thiourea
51. N,N^1-di(*p*-fluorophenyl)-thiourea
52. N,N^1-di(*p*-hydroxyphenyl)-thiourea
53. N,N^1-di(*p*-aminophenyl)-thiourea
54. N,N^1-di(*p*-acetylamino-phenyl)thiourea
55. N,N^1-di(*m*-trifluoromethylphenyl)thiourea
56. *m*-Phenylenedithiourea
57. *p*-Phenylenedithiourea
58. Benzidinedithiourea
59. Tolidinedithiourea
60. Poly-*m*-phenylenethiourea

Reactions with Thiourea

Ag^+. Tan precipitate (neutral); black precipitate (ammoniacal)
Bi^{+++}. Bright yellow color (acid)
Ce^{++++}. Decolorized (acid)
Cu^{++}. Brown precipitate (ammoniacal); white precipitate (acid)
Fe^{+++}. Light pink color (acid)
Hg_2^{++}. Gray precipitate (acid)
$IrCl_6^{--}$. Decolorized (acid)
OsO_5^{--}. Brown color (neutral); red color (acid); gray color (ammoniacal)
Pd^{++}. Yellow color (acid and ammoniacal)
$PtCl_6^{--}$. Brown precipitate (ammoniacal)
$RuCl_4^-$. Dark greenish-blue color (acid)
Sb^{+++}. Pale yellow color (acid)
SeO_3^{--}. Red precipitate (acid)
Tl^+. White precipitate (neutral); heavy white precipitate (acid)

Reactions with Substituted Thioureas

The following reactions are listed for each inorganic ion, the numbers referring to the organic compounds listed above. The descriptions of colors and precipitates are probably not complete or exact in all cases, because the reactions are grouped as much as possible for sake of brevity. They are, however, adequate for all practical purposes.

SILVER (Ag^+)

Neutral Medium
Tan to brown precipitates. 2, 3, 9, 11, 13 to 21, 24 to 27, 33, 34, 40 to 52, 55 to 59
White precipitate. 5, 12, 38, 39
Yellow precipitate. 60
Deep purple-brown precipitate. 53, 54

Acid Medium
White precipitate. 2, 3, 5 to 13, 16 to 24, 26, 27, 29, 30, 32 to 39, 41 to 49, 51, 55 to 59
Yellow precipitate. 60

Ammoniacal Medium
All brown to black precipitates except 12, 28, and 38
White precipitate. 38

GOLD ($AuCl_4^-$)
Acid Medium
Decolorized. 1 to 5, 7 to 10, 12, 13, 16, 20 to 24, 26 to 39, 41 to 45, 47, 49, 51, 55 to 57
Brown precipitate. 6, 11, 14, 15, 17, 18, 19, 25, 46, 53, 58, 60
Orange color. 40, 48, 50, 52, 54, 59

BISMUTH (Bi^{+++})
Acid Medium
Yellow color or precipitate in all cases except 12, 15, and 40

CERIUM (Ce^{++++})
Acid Medium
Decolorized. 1 to 11, 13, 14, 17, 18, 20 to 24, 26 to 39, 41 to 47, 51, 52, 53 to 57
Tan color. 12, 19, 60
Pink color. 25, 40
Pale gray color. 48
Blue color. 49
Purple flash, blue color. 53
Yellow color changes to gray precipitate. 58
Pink color changes to gray precipitate. 59

COPPER (Cu^{++})
Neutral Medium
White precipitate. 5 to 8, 10, 14, 16 to 24, 26, 27, 32 to 34, 37 to 39, 41, 42, 46, 54, 56, 57, 59
Tan to brown color or precipitate. 9, 44, 50, 51, 53, 60
Green color or precipitate. 12, 13, 25, 30, 40, 45
Unstable red color. 28, 29, 35, 36
Unstable tan color. 43, 47 to 49, 55
Unstable purple color. 52
Green color, changes immediately to blue precipitate. 58
Acid Medium
White precipitate. 5 to 8, 10, 16, 17, 18, 20 to 24, 26, 27, 29, 32, 33, 49, 54, 56 to 59
Pink color or precipitate 3, 9, 13, 25, 43, 44, 47
Brown precipitate. 30, 53
Unstable red color. 11, 28, 35 to 39, 41, 42, 46, 48, 50, 51, 55
Ammoniacal Medium
Gray black precipitate. 20, 21
Brown-black precipitate. 9, 14, 16, 22, 23, 33, 34, 44, 52, 57
Blue color discharged. 1, 7
Brown precipitate. 2, 5, 6, 8, 10, 11, 13, 15, 17, 18, 24, 26, 27, 28, 31, 35 to 43, 45 to 49, 51, 54, 56, 58, 59, 60
Gray-green precipitate. 12, 19, 25, 30, 32
Purple precipitate. 50, 53, 55

IRON (Fe^{+++})
Acid Medium
Pale yellow to tan color. 1, 4, 6, 8, 10, 11, 13, 16, 17, 27, 31, 56, 57, 58, 59
Yellow color, changes immediately to purple. 53

MERCURY (Hg_2^{++})
Acid Medium
Gray to black precipitate in all cases except 60
Yellow precipitate. 60

MERCURY (Hg^{++})
Neutral Medium
White precipitate. 5, 9, 11, 12, 13, 16, 18, 20 to 23, 25 to 27, 33, 34, 37, 39, 41 to 43, 45, 46, 47, 49, 51, 54, 57
Slightly gray precipitate. 53
Yellow precipitate. 60
Acid Solution
White precipitate. All except 1, 4, 6, 14, 15, 19, 31 35, 36, 52, 53, and 60
Yellow precipitate. 60

IRIDIUM ($IrCl_6^{--}$)
Acid Medium
Decolorized. All except 12, 15, 25, 53, 58, 59, 60
Slightly gray precipitate. 25, 53, 58, 59
Yellow-brown precipitate. 60
Ammoniacal Medium
Unstable blue color. 14

OSMIUM (OsO_5^{--})
Neutral Medium
Yellow to tan color or precipitate. 2, 5, 7, 8, 10, 17, 21, 23, 24, 28 to 30, 32, 33, 35, 36, 38, 43, 47, 48, 56, 58 to 60
Red to brown color or precipitate. 3, 4, 9, 11, 15, 16, 18, 42, 44 to 46, 54, 55
Pink color or precipitate. 6, 12 to 14, 19, 20, 25, 31, 34, 37, 39 to 41, 49 to 52, 57
Acid Medium
Pink color or precipitate. 5 to 8, 10, 13, 14, 17, 19, 21, 24 to 26, 29, 38, 41 to 43, 45, 47 to 49, 51, 53 to 59
Yellow to tan color or precipitate. 3, 30, 60
Blue color or precipitate. 28, 35, 36
Red to purple color or precipitate. 4, 9, 11, 15, 16, 18, 20, 22, 23, 31 to 34, 37, 39, 40, 44, 46, 52
Ammoniacal Medium
Yellow to tan color or precipitate. 2, 3, 12, 15, 20, 21, 23, 28, 31, 33, 37, 39 to 41, 43, 54, 59
Red to brown color or precipitate. 9, 11, 14, 16, 18, 22, 24 to 26, 32, 34, 42, 44 to 47, 49 to 51, 53, 56 to 58, 60
Purple color or precipitate. 6, 8, 10, 13, 17, 19, 52, 55

PALLADIUM (Pd^{++})
Acid Medium
Orange color or precipitate. In all cases
Ammoniacal Medium
Yellow color or precipitate. 1, 5 to 14, 16 to 21, 23 to 28, 32, 33, 37, 42 to 47, 51 to 53, 55 to 59

PLATINUM ($PtCl_6^{--}$)
Acid Medium
Yellow color or precipitate. In all cases except 15
Ammoniacal Medium
Slight tan color or precipitate. 1, 6 to 8, 10, 17
Red color. 14

RUTHENIUM ($RuCl_4^-$)
Acid Medium
Brown color. 2, 4 to 8, 10, 11, 14, 16 to 24, 26 to 29, 31 to 37, 39, 41 to 44, 47, 52, 56, 57
Green blue color. 1

ANTIMONY (Sb^{+++})
Acid Medium
Pale yellow color or precipitate. 2, 3, 5, 9, 11, 18, 20, 21, 23, 24, 27, 28, 30, 33, 34, 37, 39, 43, 44, 46, 49

SELENIUM (SeO_3^{--})
Acid Medium
Red color or precipitate. 1, 5 to 8, 10, 14, 16, 17, 19, 21, 24, 29, 33, 34, 39
Pink color or precipitate. 2, 4, 23, 27, 28, 31, 32, 36, 37, 43
Yellow color or precipitate. 9, 11, 20, 30, 35, 38, 41, 42, 44, 47, 52, 56, 57

THALLIUM (Tl^{++})
Ammoniacal Medium
White precipitate. 9, 11, 12, 18, 42, 44, 46, 47
Pink color or precipitate. 21, 33, 39, 55
Yellow to tan color or precipitate. 25, 43, 48, 51, 56, 58

Sensitivity

The sensitivities for the different inorganic ions as observed on the spot plate are reported for thiourea and for the substituted thioureas. Reactions not sensitive to 5 p. p. m. are not listed.

For thiourea: Bi^{+++}, 2 p. p. m.; Pd^{++}, 5 p. p. m.; SeO_3^{--}, 5 p. p. m.
For substituted thioureas:

BISMUTH (Bi^{+++})
0.5 p. p. m. 58
1 p. p. m. 3, 20, 27, 30, 37, 39
2 p. p. m. 2, 5, 9 (color fades), 11, 18, 38, 41, 44, 46, 56
3 p. p. m. 4, 10, 13 (color fades), 22 (color fades), 23, 24, 28, 47, 55
5 p. p. m. 1, 7, 8, 21, 60

COPPER (Cu^{++})
Acid Medium (color fades in all instances)
1 p. p. m. 11, 28, 30, 46, 47
2 p. p. m. 18, 25, 37, 41, 55
3 p. p. m. 35, 36, 38, 39
Ammoniacal Medium
1 p. p. m. 11, 14 (color fades)
2 p. p. m. 18, 25, 26 (color fades), 46, 52
3 p. p. m. 10, 13 (color fades), 22 (color fades), 23 (color fades, 24 (color fades), 27, 37, 38, 44 (color fades), 45, 47 (color fades), 50, 59
5 p. p. m. 9 (color fades), 16 (color fades), 17, 27 (color fades), 33 (color fades), 39 (color fades), 41, 42 (color fades), 48 (color fades), 51, 60

PALLADIUM (Pd^{++})

2 p. p. m. 9, 11, 18, 46
3 p. p. m. 3, 8, 13 (color fades), 16, 17, 21, 22, 24, 25, 27, 28, 30, 33, 36 to 39, 41, 43, 44, 48 to 50, 54, 55, 58
5 p. p. m. 1, 2, 4 to 7, 10, 12 (color fades), 20 (color fades), 23, 26, 29, 31, 32, 35, 40, 42, 45, 47, 51, 57, 59, 60

Summary

The reactions of 78 inorganic ions with 60 substituted thioureas and the sensitivities of the more sensitive reactions are reported. The reactions are very similar to those observed for thiourea and no definite relationship between reactivity and structure could be formulated. A number of the substituted thioureas give reactions with bismuth and copper that are more sensitive than those of thiourea; however, their low solubility is a disadvantage.

THIS investigation was supported partly by a grant-in-aid from the Carnegie Corporation of New York. It is the third of a projected series based upon studies of organic reagents in inorganic analysis. These studies are being conducted as a cooperative effort in which ten institutions are participating under the direction of John H. Yoe. Those cooperating with the University of Virginia are: Hampden-Sydney, Mary Baldwin, Randolph-Macon (Ashland), Virginia Military Institute, Virginia Polytechnic Institute, Washington and Lee, William and Mary, University of North Carolina, and Tulane University.

Micro-Kjeldahl Nitrogen Determination without Use of Titration Procedure

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THE work described in this paper has for its objective an extension of the Wagner (*1*, *2*) boric acid modification of the Kjeldahl nitrogen determination involving no standard titration solution. The ammonia is evolved in the usual manner, absorbed in dilute boric acid, and evaluated by the determination of the pH of the absorbing solution after dilution to a definite volume.

A recent critical survey of the Kjeldahl method has been made by Zakrzewski and Fuchs (*4*), whose survey includes a complete discussion with a bibliography of research on the method prior to 1937. No further reference to the literature of the subject is therefore necessary.

KJELDAHL METHOD AS MODIFIED BY WAGNER. In the Wagner procedure the digestion of the sample and the distillation of ammonia in the presence of excess alkali are carried out in the usual manner. The distilled ammonia is absorbed in 4 per cent aqueous boric acid solution and is titrated using standard acid with methyl red as indicator. The difficulty involved in the Wagner procedure, especially as applied to the microprocedure, is that of obtaining sufficiently sharp indicator change upon the completion of the neutralization of ammonia.

Present Modification of Wagner Procedure

The first attempt to improve upon the indicator titrational method of Wagner was to substitute a glass electrode pH meter for the methyl red indicator. This proved no more satisfactory than the use of the indicator because of the buffering effect of the boric acid present. This also explains the difficulty met in the use of methyl red as indicator for this titration.

The next attempt involved taking advantage of the buffer capacity of the boric acid by titrating the ammonium hydroxide with 0.01 *N* sulfuric acid to a definite pH, that of the pH represented by the boric acid solution alone. If the total volume of the solution is carefully controlled, and the total volume of boric acid accurately measured, very satisfactory results are obtained, as will be subsequently shown (Table I).

The last method of approach consisted in the determination of the amount of ammonia absorbed by the boric acid by determination of the change in pH of this solution due to the addition of ammonia. This procedure involves no standard titrating solution. Exact volumes of boric acid solution are treated with varying amounts of standard 0.01 *N* ammonium hydroxide and diluted to a definite volume. The pH's of a series of such solutions thus prepared are plotted as a function of the ammonia content. The unknown amounts of ammonia from Kjeldahl digestions and distillations are then determined by the same procedure, reading the amounts of ammonia present as a function of the pH of the solution by reference to the calibration plot previously obtained. By preparing a large volume of boric acid solution for absorption of the

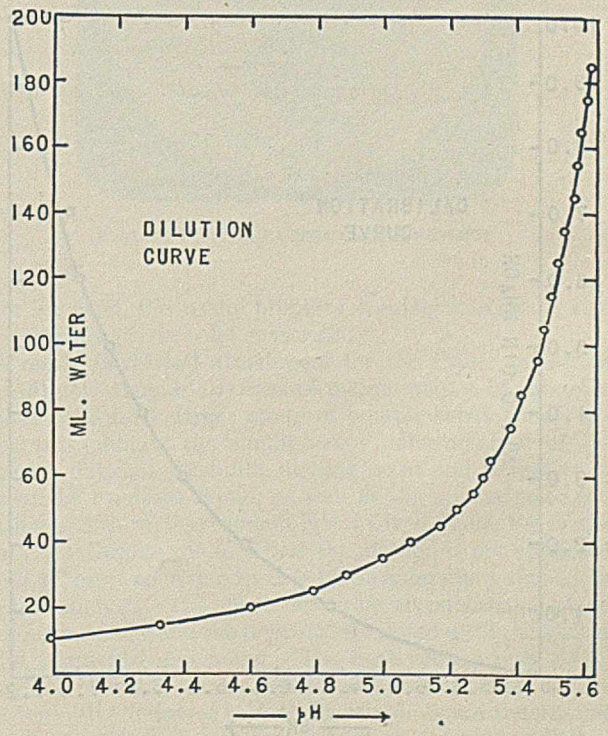

FIGURE 1. VARIATION IN pH WITH DILUTION
10 ml. of 4 per cent H_3BO_3

TABLE I. pH OF BORIC ACID SOLUTION AS A FUNCTION OF ADDED AMMONIUM HYDROXIDE

(10.00 ml. of 4 per cent boric acid diluted to 150 ml.)

0.01 *N* NH_4OH	Total Volume	pH	0.01 *N* NH_4OH Found		Error, 0.01 *N* NH_4OH	
		Found	By titration	By pH	By titration	By pH
Ml.	*Ml.*		*Ml.*	*Ml.*	*Ml.*	*Ml.*
2.15	152.2	6.53	2.16	2.20	+0.01	+0.05
2.62	152.6	6.60	2.65	2.57	+0.03	−0.05
3.26	153.3	6.72	3.20	3.35	−0.06	+0.09
3.74	153.7	6.77	3.73	3.72	−0.01	−0.01
4.31	154.3	6.83	4.35	4.25	+0.04	−0.06
4.87	154.9	6.90	4.80	5.00	−0.07	+0.13
5.44	155.4	6.94	5.44	5.42	±0.00	−0.02
5.80	155.8	6.97	5.83	5.85	+0.03	+0.05
			Av. algebraic error		−0.004	+0.023

evolved ammonia one calibration curve can be made to apply to a large number of determinations. A new calibration curve is required for each new lot of boric acid solution. The new procedure is therefore best adapted to use in a routine Kjeldahl process where large numbers of determinations are in demand.

Preparation of Calibration Curves

Since the measurement of pH had to be made at definite volume, it was necessary to determine the most favorable volume to employ—that is, to establish the minimum volume at which the change in pH of the boric acid solution would be negligible upon addition of a small increment of solvent. For this purpose a dilution curve was plotted by adding definite increments of water to 10 ml. of 4 per cent boric acid solution, followed by the determination of the pH. The data thus obtained are shown in Figure 1.

From a study of the data plotted in Figure 1, the dilution of 10 ml. of 4 per cent boric acid solution to 150-ml. volume was selected as suitable. At this volume a further dilution with 10 ml. of water altered the pH by only ±0.01 pH (an amount representing approximately the limiting precision of the usual industrial model direct-reading pH-meter).

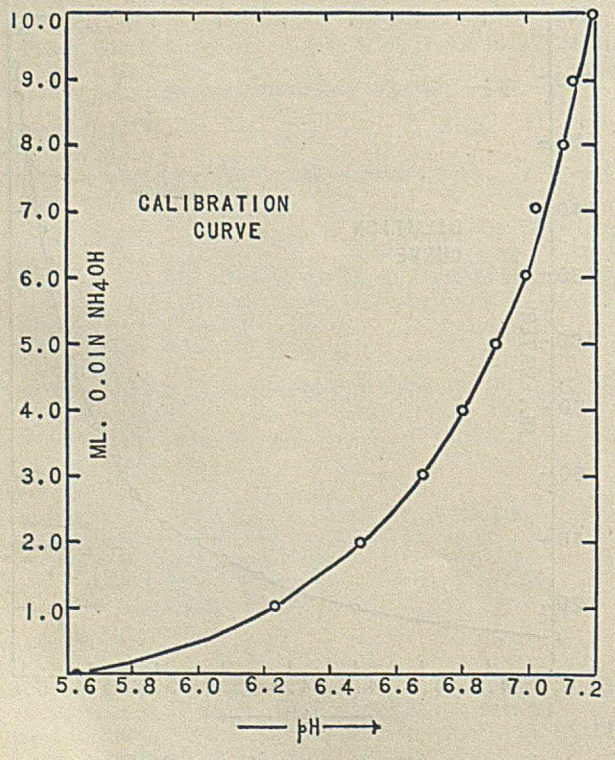

FIGURE 2. VARIATION IN pH AS A FUNCTION OF AMMONIUM HYDROXIDE

A calibration plot was then prepared by the addition of definite amounts of 0.01 *N* ammonium hydroxide solution to 10.00-ml. portions of 4 per cent boric acid solution diluted to 150 ml. with ammonia-free water. The pH of the solution was determined and in addition the ammonia was titrated using 0.01 *N* sulfuric acid to the pH represented by the original diluted boric acid solution. The results are shown in Table I.

The data of Table I, columns one and three, are plotted in Figure 2. The data of column five were taken from the plot thus found. The calibration curve of Figure 2 was used for the subsequent estimations reported in this work and checked by direct titration. The estimation of ammonia by determination of the pH is seen by the data of Table I to be somewhat less accurate than that obtained by direct titration to a definite pH.

To check further the accuracy of the new procedure, varying amounts of standard ammonium chloride solution were introduced into the Kjeldahl distillation apparatus and the ammonia evolved was distilled into 10.00 ml. of 4 per cent boric acid. The pH was measured at a volume of 150 ml. and the ammonium hydroxide was titrated, using 0.01 *N* sulfuric acid, to the original pH of the boric acid alone. The results are shown in Table II.

Entire Kjeldahl determinations were carried out using the modified procedure, with the results given in Table III.

TABLE II. DETERMINATION OF AMMONIUM HYDROXIDE FROM KNOWN AMOUNTS OF AMMONIUM CHLORIDE

(0.452 mg. of NH_4Cl per ml. = 0.845 ml. of 0.01 *N* NH_4Cl per ml. 10.00 ml. of 4 per cent boric acid solution used with final dilution to 150 ml. before determination of pH and titration of NH_4OH)

NH_4Cl Taken	pH Found	NH_4OH Found and Added			Error	
		Added	By pH	By titration	By pH	By titration
Ml.		*Ml.*	*Ml.*	*Ml.*	*Ml.*	*Ml.*
2.50	6.52	2.12	2.12	2.15	±0.00	+0.03
3.50	6.68	2.96	3.05	3.00	+0.09	+0.04
4.50	6.77	3.80	3.76	3.79	−0.04	−0.01
5.50	6.88	4.65	4.71	4.62	+0.05	−0.03
6.50	6.95	5.49	5.52	5.50	+0.03	+0.01
			Av. algebraic error		+0.026	+0.008

TABLE III. ANALYSIS OF PURE AMINOID-NITROGEN COMPOUNDS

Compound	Nitrogen Theoretical	Nitrogen Found
	%	%
$C_6H_5NHCOCH_3$ (acetanilide)	10.36	10.15
		10.55
		10.29
		Av. 10.33
$C_6H_5SO_2NH_2$ (benzene sulfonamide)	8.90	8.81
		8.82
		8.99
		Av. 8.87
$C_6H_4NH_2HSO_3H_2O$ (sulfanilic acid)	7.32	7.50
		7.40
		Av. 7.45

Detailed Procedure for Modified Wagner Micro-determination of Nitrogen

The apparatus used and the technique employed were those described by Niederl (*3*) and involve no modification of the original Pregl equipment. The sample, following digestion to decompose all organic matter in the usual way to convert all nitrogen to ammonium sulfate, is transferred to the standard Pregl distillation microapparatus. A slight excess of 50 per cent nitrogen-free sodium hydroxide is added and the liberated ammonia is distilled into a 125-ml. Erlenmeyer flask containing exactly 10.00 ml. of 4 per cent boric acid solution. When the total volume of distillate has reached 25 ml., the distillation is discontinued. (The amount of boric acid solution is accurately determined using a microburet.)

The solution is transferred to a 250-ml. beaker and diluted to 150 ml. with nitrogen-free boiled conductivity water. The pH of the solution is then measured by means of an industrial model direct-reading Beckman pH meter and the corresponding milli-

liters of 0.01 N ammonium hydroxide are obtained from the calibration curve obtained as previously described. From the data thus obtained the per cent of nitrogen in the original sample may be calculated.

The pH of the solution is dependent upon the amount of boric acid present as well as the amount of ammonia and thus it is necessary to measure the amount of boric acid very accurately. In addition, it is necessary to prepare a new calibration curve whenever a fresh stock of boric acid solution is prepared. Special conductivity water need not be prepared if the calibration curve is made using the same water for dilution or if a blank is run.

Conclusions

Two modifications of the Wagner micro-Kjeldahl procedure avoid the troublesome use of methyl red as indicator. The first method consists in a potentiometric titration to the pH represented by the boric acid absorption solution after dilution to a definite volume and estimation of the ammonia content by reference to a calibration curve. This method is more accurate, but involves the use of a standard titration solution.

The second method, while somewhat less accurate, is not dependent upon the preparation and storage of a standard acid and is admirably suited to routine analyses because of the saving of time otherwise required for a titration.

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Refractive Index Measurements at and above the Melting Point of Solids

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PROBABLY the most widely used method for determining the refractive index of crystalline or solid materials involves use of the "Becke line" phenomenon (*1*), for which a microscope and a series of standard liquids of known refractive index are required. For optimum conditions the liquid series used should include numerous duplicates, in order to obtain immersion media in which the solid material is insoluble. Even for the crystallographically simplest of compounds, isotropic in nature, the procedure is somewhat tedious and time-consuming.

Two courses are possible: (1) Crystals, or fragments, of the solid may be immersed in a progressively increasing or decreasing series of the immersion media of known index until a liquid of similar index is found, or (2) the particle may be immersed in a medium of lower refractive index and a second medium of higher index used for dilution until minimum visibility is attained. In this latter method the index of the medium finally attained must either be computed or be measured with a suitable refractometer. For anisotropic substances the procedure is still more complex. The refractive index varies with the direction of transmission and of vibration of the light in the specimen. Two constants must be obtained for uniaxial and three for biaxial crystals. This necessitates use of a polarizing microscope and proper crystallographic orientation of the material for each determination. The complexity of the measurements required may be understood by referring to the procedure recommended by Larsen (*10*). Inasmuch as most crystalline materials may be classified as anisotropic, it is easy to understand the recent statement that the values (refractive indices) for organic solids have not been so well collected as have those for liquids (*8*).

Many organic chemists working in qualitative organic analysis make routine refractive index measurements of liquids as an easily and conveniently determined physical constant to assist in their identification. Because of the complexity of the apparatus, the specialized technique required, and the labor involved, this useful property is rarely applied by such investigators (*2*) to solid materials.

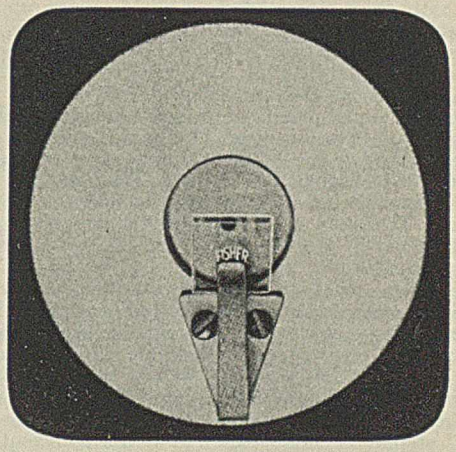

Figure 1. Refractometer Eyepiece

A newly developed attachment for the Fisher refractometer (*5*) now extends the ease of measurements of refractive indices to a much larger group of organic substances. The apparatus permits the simultaneous determination of two common physical constants, melting point and refractive index of the resultant liquid, as well as the approximate estimation of a third, less commonly employed value, the dispersion (*7*). Despite the fact that the method is not applicable to all types of compounds, it is of considerable value to the organic analyst. The substance to be investigated is placed on a heated stage and the temperature raised until the melting point is reached and noted. The heat control may be adjusted to maintain the temperature at the melting point and the refractive index of the resultant true liquid determined. An alternative procedure is to raise the temperature further to some reference point above the melting point and determine the refractive index and dispersion.

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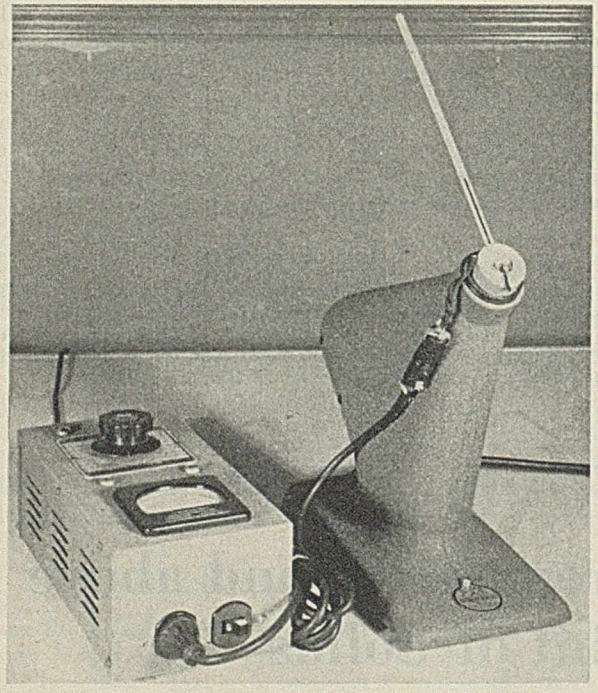

FIGURE 2. LABORATORY SETUP

Apparatus

The instrument employed is a Fisher refractometer, which embodies certain modifications of the principles suggested by Jelley (*6*) and also reported by Edwards and Otto (*3*). An accurately ground and polished glass prism is utilized (Figure 1) for the formation of a "liquid prism" of the substance to be investigated. Simultaneous observation of an illuminated slit and its virtual image, seen through the liquid prism, permits measurement of the vertical displacement of the image from the slit. This distance is dependent upon the refractive index of the liquid investigated. The scale used is calibrated directly in refractive index units from $n = 1.300$ to $n = 1.900$. Measurements are possible to ± 0.002 unit.

For measurements at controlled elevated temperatures a modified eyepiece, consisting of a nickel-plated brass block into which a Nichrome resistance wire heating unit has been sealed, is substituted for the one ordinarily supplied with the instrument. A thermometer, or thermocouple well, is also present. In the instrument used for obtaining the data reported in this paper, a 300° C., red column and magnifying front thermometer graduated in 1° steps was employed. The well is sufficiently deep to permit placing the bulb of the thermometer directly above the peep hole and slightly behind the circular optical glass backing plate.

The temperature was regulated and controlled by plugging the heater cord from the eyepiece into a Varitran (continuously variable auto-transformer) and adjusting the voltage applied between 0 and 60 volts. Fine, stepless, continuous adjustment is thus possible and the instrument may be brought to, and maintained at, any temperature between that of the room and 200° C. For mechanical reasons use of higher temperatures is not recommended.

The complete laboratory setup used for obtaining the data reported herein is shown in Figure 2.

Procedure

The glass plate and prism of the instrument are carefully wiped with a soft cloth, lens paper, or Kleenex, or if necessary, are cleaned by wiping with a cloth wetted with water, benzene, carbon tetrachloride, or any other readily volatile solvent. The prism is then placed on the eyepiece in the position governed by the spring clamp, with the beveled edge forming a well of V-shaped cross section, the lower edge bisecting the small hole behind the permanently mounted optical glass circular plate (see Figure 1). A crystal or fragment (or preferably a few milligrams of finely powdered material) is placed in this well, so that upon melting the liquid will be drawn to the apex and form a liquid prism. The size of sample required depends to a very large extent upon the volatility of the specimen. Two milligrams of sample will suffice for compounds exerting low vapor pressures at the melting point. The heater is plugged into the Varitran and the voltage turned up so that the temperature is rapidly brought to within 5° to 10° of the melting point. The Varitran then should be adjusted so that the temperature continues to rise 1° or 2° per minute.

At the melting point the fragments will liquify rapidly and the resultant liquid will flow into the prism. The exact temperature may now be noted and recorded. Upon depressing the refractometer light switch at the rear of the instrument, the refractive index of the liquid may be read directly from the scale, in the plane of the light slit, by peering through the small hole in the eyepiece. If desired, the heater may be adjusted to maintain the temperature at a predetermined point—i. e., 5°, 10°, 15°, etc., above the melting point—and the index measured under these conditions. Because the readings may be obtained practically instantaneously, it is not necessary to attempt to maintain constant temperatures for appreciable lengths of time. However, since the thermometer bulb is embedded in the block close to the heating element, whereas the sample is separated from the block by a glass plate 1 mm. thick, temperature changes should be effected slowly enough to compensate for thermal lag between the thermometer and sample.

It has been found best policy to clean the glass plate and prism immediately after recording the desired data. This may readily be accomplished by wiping while hot. The Varitran may then be turned to zero, and as soon as the thermometer drops below the melting point of the next specimen to be investigated, the fresh sample may be placed on the eyepiece and studied. A curve indicating the thermometer reading at various settings of the Varitran has been found extremely convenient. By its use approximate temperature settings may be readily made.

TABLE I. COMPOUNDS INVESTIGATED

Compound	Melting Point		Refractive Index at Melting Point		Dispersion Measurements		
	Detd.	Handbook (*9*)	Detd.	Handbook (*9*)	Red	Green	Scale No.
α-Naphthylamine	49	50	1.669	1.6703	1.662	1.698	4
Palmitic acid	62	63–4	1.435	1.430 at 90°	White image		0
Stearic acid	69	69–70	1.434	1.4335	White image		0
o-Nitroaniline	70	71–5	1.660	...	1.638	1.695	6
o-Nitrophenol	89	96	1.569	...	1.560	1.573	2
β-Naphthylamine	109	111–12	1.643	1.6493 at 98°	1.637	1.660	3
p-Nitrophenol	110	113	1.602	...	1.590	1.618	3
m-Nitroaniline	111	114	1.595	...	1.583	1.605	3
o-Tolidine	125	129	1.641	...	1.638	1.659	3
dl-Malic acid	129	128–9	1.450	...	White image		0
Pyrogallol	134	134	1.561	...	1.557	1.566	1
Anthranilic acid	144	145	1.578	...	1.570	1.593	3
p-Nitroaniline	147	146–7	1.670	...	1.655	1.715	7
Ammonium thiocyanate	150	150	1.590	...	1.586	1.598	2
Citric acid	152	153	1.460	...	White image		0
Tartaric acid	169	168–70	1.464	...	White image		0
Potassium thiocyanate	174	173	1.558	...	1.555	1.562	1
Succinic acid	190	189	1.405	...	White image		0

Discussion

With reasonable care in the adjustment of the heater, melting points may easily be determined within 1° or 2° of reported values. The principle is, of course, similar to that used in the Fisher-Johns melting point apparatus (*4*) which has been used by organic chemists for some four years.

Since a regular 110-volt, tungsten filament bulb is used as light source, the beam employed is not monochromatic but "white". For substances having low dispersion—i. e., where all wave lengths of light are similarly diffracted—the virtual image seen is a white image as narrow as the slit itself. For substances having appreciable dispersive powers the image obtained is not a line image but rather a multicolored band. The actual width of the band—i. e., distance between the far edges of the red and violet portions—depends upon

Table II. Difference between Readings Taken on Edges of Red and Green Portions of Image

	Dispersion Scale No.
0 (white image obtained)	0
0.00 to 0.01	1
0.01 to 0.02	2
0.02 to 0.03	3
0.03 to 0.04	4
0.04 to 0.05	5
0.05 to 0.06	6
0.06 to 0.07	7
0.07 to 0.08	8
0.08 to 0.09	9

the dispersive power of the sample. The scale of the instrument has been so constructed that reading of the yellow portion of the spectral band formed indicates the refractive index of the medium as generally determined by using an Abbe or Pulfrich refractometer with light of sodium D wave length. This yellow portion is generally sharply defined as the narrow region between the brilliant red and green portions of the image. The dispersive power of the sample investigated may readily be estimated from readings taken at the visible limits of the spectral band formed. With carefully performed observations the precision attainable, irrespective of the dispersion encountered, is ±0.002 unit. The temperature coefficient may also be ascertained by adjusting the heater so that the temperature slowly rises over the range desired and taking periodic readings on the refractometer as the thermometer reaches predetermined values.

Limitations Encountered. Because of the personal safety factor it is recommended that temperatures in excess of 175° C. be employed but rarely. In making a reading the eye must necessarily be brought close to the specimen; for this reason lachrymatory compounds will be difficult to study. Compounds that sublime cannot be studied. Data will be difficult to obtain for compounds exerting high vapor pressures at or slightly above the melting point. Salicylic acid is an excellent example demonstrating this type of difficulty. Nevertheless there are many organic compounds which do not fall in the above classes and which lend themselves admirably to study by the method and apparatus described.

Data. The data obtained in this preliminary investigation were chosen, not with any specific class of compounds in view, but with the intention of determining the constants on a representative group for the melting range recommended. A large enough group was chosen to include substances ranging from high to low refractive index and from zero to appreciable dispersion. In Table I are listed the compounds investigated, the determined and handbook values for melting point (and refractive index where available), and an indication as to the dispersion observed. The classification of the dispersive power is somewhat difficult to decide upon. Since monochromatic radiations were not used, accurate calculations were not possible (dispersion is usually defined as being proportional to the rate of change of the reciprocal of the velocity with wave length). It seemed likely, however, that some simple, definite, semiquantitative indication of the degree of dispersion would be useful. For that reason the compounds investigated have been classified according to an easily determinable arbitrary method. Readings were taken on the lowest edge of the red and the highest edge of the green portion of the band formed. Readings were not taken to the blue or violet because of the difficulty in locating the edge of these regions. The red and green edges are usually clearly discernible. These readings are also included in Table I and the dispersion scale numbers are based upon the relationships indicated in Table II.

Discussion of Data. For those few compounds for which data could be found in the literature the agreement between the author's values and those previously reported are satisfactory. The compounds used in this study were stock chemicals of the purest grade obtainable commercially but were not crystallized or further purified before use. The determined and handbook values for α-naphthylamine as well as for stearic acid agree remarkably. The handbook value for β-naphthylamine is reported as having been obtained at 98° C., about 14° below the melting point of the pure compound. The author's sample melted at 109°, indicating some impurity, and his value is somewhat less than that mentioned above. The difference between the two values (0.006 unit) may easily be laid to the differences in purity of the two samples and to the temperature difference at which the measurements were made. Small amounts of impurities are apt to have a much larger effect upon the melting point of a compound than upon its refractive index. For this reason the latter property may well be the better for identification studies.

Determination of the specific refraction for such compounds would be an interesting study, although density measurements at the reference temperature would be required. For many compounds the melting and solidification points may be accurately determined by noting the temperature at which the image appears and disappears upon slowly raising and lowering the temperature. Supercooling must be considered for certain samples.

The value of refractive index measurements at elevated temperatures for identification purposes will be enhanced greatly after sufficient data have been obtained and reported to permit the compilation of orderly data tables. In the meantime one may make use of the method by alternate determinations on the unknown material and on known compounds. Obviously, because of the scarcity of low-melting inorganic compounds, the method is not apt to find application in this field. It should prove of value in studies of natural and artificial waxes and similar materials, even though these substances are apt to have melting ranges rather than melting points. A compilation of data for this group of substances is now under way.

Summary

Refractive index measurements of organic compounds at and above the melting point have been proposed for identification studies. Such a procedure necessitates the determination of but a single value, rather than the two or three required on crystalline anisotropic material. This value may be determined more easily and rapidly than similar values for even isotropic materials in the crystalline state.

Apparatus for the simultaneous determination of melting points up to 200° C. and refractive indices between 1.300 and 1.900 has been described. The melting points may be obtained with an accuracy of 1° to 2° C., while the index measurements may be made to ±0.002 unit.

Estimations of dispersion may be made with the apparatus described and a dispersion scale is suggested for classification of compounds.

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Colorimetric Microdetermination of Arsenic

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METHODS for the determination of arsenic are legion for two principal reasons: first, because of the importance of the accurate determination of arsenic in food, biologicals, organic material, etc., and second, because the methods in use are not entirely satisfactory. The literature of arsenic determinations has been surveyed by Minot (*20*), Kleinmann and Pangritz (*16*), and How (*13*).

The methods for the microdetermination of arsenic may be placed in seven groups.

1. The Marsh-Berzelius method (*7, 18, 21*) depends upon the generation of arsine and its subsequent decomposition by heat with the formation of an arsenic mirror.
2. The Gutzeit method (*1, 13, 15*) depends on the generation of arsine, which in turn reacts with papers impregnated with mercuric chloride or bromide.
3. Variations of this method are the action of arsine on silver nitrate or on other silver and gold salts, as in Rose's method (*9*). Thus Truffert (*30*) used the action of arsine on photographic paper coated with silver citrate to ascertain the arsenic content of materials.
4. The bromate method (*1*) depends on the distillation of arsenious chloride and the subsequent estimation of this compound by a bromate titration.
5. The iodometric method of Cassil and Wichmann (*4*) depends on the evolution of arsine in a special generator; the liberated arsine is trapped by mercuric chloride solution which oxidizes it to arsenious acid, which in turn is estimated by titration with iodine.
6. An example of the nephelometric methods used for the determination of arsenic is that of Kleinmann and Pangritz (*16*). A reagent consisting of equal parts of 1 per cent potassium molybdate solution and 2 per cent cocaine solution, and 2 parts of *N* hydrochloric acid gives a turbidity with small quantities of arsenic pentoxide.
7. Molybdenum blue methods are discussed below.

Gutzeit Method

An official method for the determination of arsenic is the Gutzeit method (*1, 15*). In 1928, Clarke (*6*) said, "Although the results are very good in some instances, general experience has made it plain that not one of the various modifications of the Gutzeit method can be used by the average analyst with the assurance or probability that his results will be accurate unless he attains considerable experience in its use."

Studies of Gutzeit methods by Barnes and Murray (*2*), Neller (*24*), Gross (*12*), and Mühlsteph (*23*) show that relatively large errors often occur. Using disks, as required by the British Pharmacopeia, is no better than using strips, for arsine may pass unchanged through the disks.

The greatest difficulty in the use of the Gutzeit method is the inability to duplicate the brown arsenic stains on the mercuric bromide papers. There is sufficient evidence in the literature to show that arsine is quantitatively liberated by means of a Gutzeit generator (*13*). The greatest advantage of the Gutzeit method is the simplicity of the generator, the ease of generation of arsine, and therefore the ability to separate arsenic from interferences.

Molybdenum Blue Methods

The molybdenum blue method is probably the most sensitive and accurate for the determination of arsenic. This has been emphasized by Snell (*29*) and by Pierson (*25*).

Phosphorus reacts with ammonium molybdate to form a complex phosphomolybdate, which may subsequently be reduced with the formation of a complex molybdenum compound strongly colored blue. Arsenic undergoes an entirely analogous reaction with the formation of an intensely colored blue complex. This reaction of arsenic and its use in methods for the estimation of arsenic have been discussed by a number of investigators. The term "molybdenum blue complex" is applied to the series of complex oxides formed by arsenic and molybdenum, phosphorus and molybdenum, and molybdenum itself.

The principal variations of the molybdenum blue method for arsenic are those of Maechling and Flinn (*19*), Youngburg and Farber (*32*), Zinzadze (*33*), Morris and Calvery (*22*), and Chaney and Magnuson (*5*).

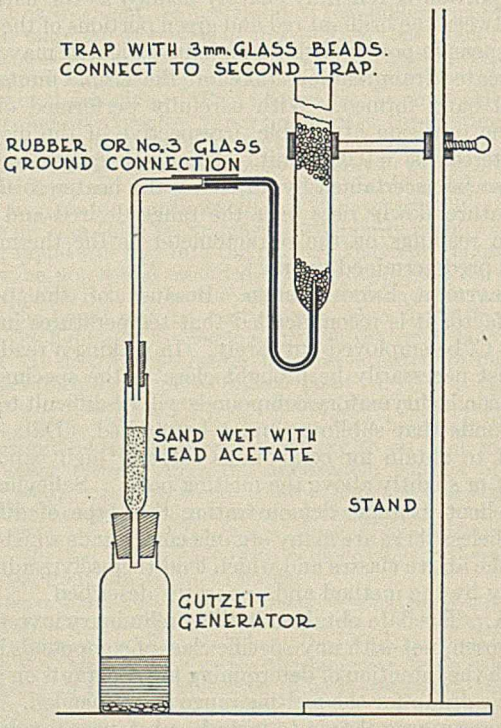

FIGURE 1. APPARATUS FOR ARSINE–MOLYBDENUM BLUE METHOD

The effects of concentration, ionic strength, and interferences on the molybdenum blue reaction were studied by Kuttner and Cohen (*17*) and Schricker and Dawson (*28*).

Arsine–Molybdenum Blue Method

The fact that the Gutzeit method is used so extensively despite its known faults indicates that the methods suggested to replace it do not have sufficient simplicity or adaptability for multiple determinations.

The following method, which is a combination of the official Gutzeit method for the generation of arsine and a simple modification of the molybdenum blue method, has the advantage of simplicity coupled with the ability to be adapted for multiple determinations.

If instead of permitting the arsine to impinge on paper impregnated with mercuric bromide, mercuric chloride, silver nitrate, silver citrate, or some analogous compound, the arsine is absorbed in some trapping solution which oxidizes it to

arsenate, the molybdenum blue method may be used directly for its determination.

The use of bromine water, sodium peroxide, hydrogen peroxide, potassium persulfate, potassium chlorate, potassium bromate, and sodium hypobromite was investigated by the authors. Robinson (*8, 27*) used a normal solution of sodium hypobromite in a Winkler spiral for the trapping of arsine. Deemer and Schricker (*8*) used concentrated nitric acid saturated with bromine but discarded arsine evolution for the trichloride distillation. Cassil and Wichmann (*4*) trapped arsine in mercuric chloride solution, but the authors found that the molybdenum blue method could not be used if mercuric chloride or silver nitrate were the trapping agents. Chaney and Magnuson (*5*) used potassium iodate to oxidize arsenious chloride to arsenate before applying the molybdenum blue test.

The authors found that complete absorption of arsine in one trapping device could be obtained by the use of 3 cc. of a mixture of 3 cc. of half-saturated bromine water and 1 cc. of 0.5 *N* sodium hydroxide solution. The use of bromine water alone necessitated the use of more than one trapping device. Solutions of hydrogen peroxide and sodium peroxide were also inefficient absorbing agents and required a series of bubblers. Potassium persulfate apparently did not oxidize arsine completely to arsenate, although it did oxidize arsenite quantitatively to arsenate.

Apparatus. The apparatus consists of the official Gutzeit generator (*1, 15*) and a trapping device similar to that used for the determination of benzene or toluene in air by the butanone method (*14, 31*). This arrangement is illustrated in Figure 1. If the benzene trap is not available, a simple trap can be made by bending a 17.6-cc. or 20-cc. pipet into the form of the trap shown in the illustration and then filling the bulb portion with beads. The tube containing the mercuric bromide test paper in the usual Gutzeit method is replaced by another tube leading the generated gases to the trapping device.

Preparation of Sample. Make an acid digestion or use the solvent procedure of Wichmann and Clifford as detailed by the Association of Official Agricultural Chemists (*1*) or Jacobs (*15*), or, if necessary, employ combustion methods (*3, 26*). Then prepare an aliquot for an official Gutzeit test as directed in the aforementioned texts. From this point proceed as directed below.

Reagents. Sodium hypobromite solution. Add 0.5 *N* sodium hydroxide solution to half-saturated bromine water in the proportion of 1 cc. of 0.5 *N* sodium hydroxide solution to 3 cc. of bromine water.

Saturated bromine water. Add 2 cc. of liquid bromine to 200 cc. of water in a 250-cc. glass-stoppered bottle, shake well, and allow to stand. Dilute with an equal volume of water before use in the preparation of the sodium hypobromite solution.

Ammonium molybdate solution. Dissolve 25 grams of ammonium molybdate in 300 cc. of water. Dilute 75 cc. of concentrated sulfuric acid to 200 cc. with water and add to the ammonium molybdate solution.

Standard arsenious oxide solution. Dissolve 0.300 gram of arsenic trioxide, As_2O_3, in 25 cc. of 10 per cent sodium hydroxide solution, make slightly acid with sulfuric acid (1 to 6), and dilute with water to 1 liter. Dilute this stock solution, which may be standardized against standard potassium bromate solution, if desired, to an appropriate dilution to make for ease in the removal of aliquots to be used for the standards in the development of the color. Thus, for instance, a 5-cc. aliquot of the stock standard solution diluted to 1 liter yields a solution, 1 cc. of which is equivalent to 1.5 micrograms of arsenic trioxide. This dilute standard should be prepared fresh from the stock standard solution.

Hydrazine sulfate solution. Prepare a saturated solution of hydrazine sulfate, $N_2H_4.H_2SO_4$. Dilute an aliquot of the supernatant liquid 1 to 1 with water.

Sulfuric acid. Prepare a 2 *N* solution of sulfuric acid and standardize against standard alkali in the usual manner.

Procedure. Allow the generation of arsine to proceed as directed in the Gutzeit method and trap the arsine in the bead device to which 3 cc. of sodium hypobromite solution have been added. After generation is completed—that is, after 1 to 1.5 hours—transfer the contents of the trap to a graduated colorimeter tube, Nessler tube, or volumetric flask. Wash the trap with six 2-cc. portions of distilled water, delivering the water to the trapping device with a 2-cc. pipet. Use a rubber bulb aspirator to blow the wash solutions out of the trap into the collection vessel. Press the aspirator bulb gently in this step. Add exactly 5 cc. of 2 *N* sulfuric acid and stir, add 1 cc. of ammonium molybdate reagent, and shake. Add 1 cc. of the half-saturated hydrazine sulfate solution and swirl, make to a volume of 25 cc., and allow to stand for 0.5 hour for full development of the blue color. Compare with standards or a standard treated in a similar way at the same time.

Preparation of Standards. Prepare the standards or standard from the diluted stock standard arsenious oxide solution. Add 3 cc. of sodium hypobromite solution to the aliquot or aliquots selected, dilute to 15 cc. with distilled water, add exactly 5 cc. of 2 *N* sulfuric acid, and stir. Add 1 cc. of the molybdate reagent, stir, add 1 cc. of half-saturated hydrazine sulfate solution, and stir. Make up to the same volume as the test solution. Run a blank on all the reagents as a check.

If a final volume of 25 cc. is to be used in making the comparisons, use exactly 5 cc. of 2 *N* sulfuric acid, in order to have the proper acidity for the development of the molybdenum blue color. If less than this quantity of acid is used, the blank may itself be reduced. If more than this quantity of acid is used, the development of the blue complex will be delayed.

Limitations of Arsine–Molybdenum Blue Method

In general, the procedure for checking this method was the following:

A standard solution of arsenious oxide was prepared as directed above and was standardized against standard potassium bromate solution. An aliquot of the standard arsenious oxide solution was then diluted to an appropriate volume, such as 5 cc. to 1 liter, yielding a solution which contained 1.5 micrograms of arsenic trioxide per cc. Gutzeit generators were prepared as directed by the A. O. A. C. (*1*). To each generator were added an aliquot of the diluted standard arsenious oxide solution and sufficient distilled water to make a total volume of 30 cc. From this point, the method as outlined was followed. Comparisons were made against standards in 25-cc. Nessler tubes. A few comparisons were made with a Duboscq colorimeter. Further comparisons with the Duboscq colorimeter were not made because the plungers often induced the release of gas bubbles.

Completeness of Trapping Arsine. In trapping gases by absorbing solutions in absorbers, incomplete recovery often results (*11, 14*). To overcome this difficulty, multiple absorbers are often used. However, if an absorbing medium is provided in which the gaseous substance undergoes a rapid chemical reaction to form a nonvolatile substance, complete recovery in one absorber is possible. The rate of flow of gas and the type of absorber are also important factors in the degree of recovery.

Using a Gutzeit generator, a scrubbing type of trap, and sodium hypobromite as the absorbing solution, it was possible to trap arsine completely in one absorber. All preliminary experiments were run using two traps in series. No arsenic was found in any of the second traps; hence, the second trap was discarded in making the other tests.

The completeness of trapping of arsine by sodium hypobromite solution was in marked contrast to the incompleteness shown by the use of bromine water, potassium persulfate solution, peroxide solutions, and other oxidizing agents. Indeed, in the case of bromine water, more arsine was found in the second trap, at times, than in the first absorber.

Recovery. Since the trapping of arsine in this method is complete, one would expect the recovery to be 100 per cent. This, however, depends also on the completeness of generation of arsine. The authors found that within the limits of experimental error of this method—that is, the ability to estimate 1.5 micrograms of arsenic trioxide—recovery is 100 per cent. In addition to the use of test solutions with no interferences, known amounts of arsenious oxide of the order of 15 micrograms were added to orange juice which was then subjected to an acid digestion. Orange juice was used because it gave a practically zero blank. Recovery was complete in these instances, also.

Sensitivity. Accurate estimation of arsenious oxide content by this method can be made down to amounts of the order of 1.5 micrograms. At this concentration—that is, 1.5 micrograms in 25 cc. of comparison solution—the color of the test solution is a greenish blue. Lower concentrations of arsenic can be detected, for faint greenish-blue tints are developed, but these cannot be accurately estimated. This in-

dicates that the arsine–molybdenum blue method is far more sensitive than the official Gutzeit method, for which 25 micrograms is considered the optimum concentration.

Amounts of the order of 50 micrograms are too deep to compare accurately by the arsine–molybdenum blue method using visual means. Schricker and Dawson (*28*) state that the limit of applicability of Beer's law for the molybdenum blue reaction is approximately 75 micrograms of arsenic and 30 micrograms of phosphorus. It is not desirable to work with such large amounts.

Interferences (*10*). Phosphorus and silicon form complex molybdates. The use of the Gutzeit generation in the arsine–molybdenum blue method is designed to eliminate these interferences. High concentrations of antimony interfere in the Gutzeit determination. A series of experiments was run to check on the interference of phosphorus, silicon, and antimony. Known amounts of antimony trichloride, disodium hydrogen phosphate, and sodium silicate were added to known amounts of arsenious oxide, of the order of 30 micrograms, in the Gutzeit generator before making a test. No appreciable interference was noted.

Table I. Comparative Determinations

Sample	Official Gutzeit Method			Arsine–Molybdenum Blue Method		
	Aliquot	As_2O_3		Aliquot	As_2O_3	
	Cc.	*Micrograms*	*P. p. m.*	*Cc.*	*Micrograms*[a]	*P. p. m.*
Canned clams	10	7.5	0.75	10	6.0	0.6
	20	12.0	0.60			
			Av. 0.68			
Canned clams	10	5.5	0.55	10	4.5	0.45
	20	8.5	0.43			
			Av. 0.49			
Apple butter	10	12.0	1.2	10	12.0	1.2
	20	22.0	1.1			
			Av. 1.15			

[a] Nearest standard, run in duplicate.

Effect of Concentration of Reagents. The effect of the reagents used in the Gutzeit test itself has been covered in the literature. The concentrations and volumes of the reagents used in the variation of the molybdenum blue method used by the authors must be rigidly observed, if correct results are to be obtained.

Too concentrated solutions of bromine water used for the formation of sodium hypobromite solution prevent the reaction from proceeding properly. The use of solutions of hydrazine sulfate stronger than that recommended—namely, half-saturation—may cause a reduction of the blank. Weaker solutions of hydrazine sulfate may cause no reduction at all or reduction only after long standing. The correct acid concentration is most important. If insufficient acid is present, the blank is reduced by the hydrazine sulfate with the formation of a blue color; on the other hand, too much acid will inhibit the formation of the blue complex in the test solutions.

By keeping the concentrations and volumes of the reagents as specified, the ionic strength of the test and standard comparison solutions is kept practically the same. This tends to make the color comparisons more accurate.

Time of Comparison. The colors are not fully developed until permitted to stand half an hour. At first, greenish tints may predominate over the blue and there may be an apparent nonuniformity of shade. The waiting period provides for color stabilization. The color remains relatively stable for at least one hour after the half-hour period.

Comparison with Official Gutzeit Test

To find the degree of correlation between the arsine–molybdenum blue method and the official Gutzeit test, determinations were made by both methods on products which contained small amounts of arsenic (Table I). No arsenic trioxide was added. One hundred grams each of two samples of canned clams and one of apple butter were prepared for arsenic determinations by means of acid digestions. The wet ash was made up to 100 cc. The official Gutzeit determinations were made by one chemist, while the arsine–molybdenum blue determinations were made by another, each working independently.

Summary

The combined arsine generation and molybdenum blue method for the microdetermination of arsenic is simple and convenient. It is more sensitive than the official Gutzeit test, for accurate determinations of as little as 1.5 micrograms of arsenious oxide can be made.

Its advantages are evident. It makes use of skills and reagents that are well known and combines them into a suitable framework. The apparatus is inexpensive and is available in practically every laboratory. The reagents are simple to prepare and last almost indefinitely. The relative hazard involved in an arsine generation as compared with a hydrochloric acid–arsenious chloride distillation is in the favor of the arsine generation, for while only a minute amount of arsine is ever generated, relatively large quantities of hydrochloric acid are distilled. While the time of generation is comparatively long in comparison with a hydrochloric acid distillation of arsenic trichloride, it is difficult to run multiple arsenious chloride distillations, whereas multiple arsine–molybdenum blue determinations can be run in banks similar to ordinary Gutzeit estimations.

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Orthonitrosophenol as a New Reagent in Colorimetric Analysis

Determination of Cobalt

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ORTHONITROSOPHENOL and some of its metal salts were prepared and described for the first time by Baudisch and co-workers (*3*, *4*). These metal salts are typical organic inner-complex compounds and therefore are usually highly colored. Baudisch mentioned as early as 1912 that the formation of the red-violet, water-soluble copper complex constituted one of the most sensitive qualitative reactions for cupric ions. However, this work was not followed up until recently, when Baudisch discovered new and very simple methods for preparing *o*-nitrosophenol, its homologs, and derivatives (*1*, *2*), based upon the formation of the radical NOH which reacts under suitable conditions with many aromatic compounds to form the corresponding *o*-nitrosophenols. The details of this work will be reported elsewhere.

A study of the metal complexes of about seventy *o*-nitrosophenol compounds revealed certain rules governing their formation and properties, which convinced the author that the *o*-nitrosophenols can be of great value in analytical chemistry.

o-Nitrosophenol forms, in weak acid solutions, strongly colored complex salts with several heavy metal ions. Those of cobalt (grayish brown), palladium (green), and trivalent iron (brown) are distinguished from all the others in that they are easily soluble in petroleum ether and can thus be separated from the original water solution.

The other group consists mainly of divalent copper, and mercury (reddish violet), nickel (red), zinc (red), and divalent iron (green). All these metal salts are soluble in water or certain organic solvents, such as ether, depending upon the conditions under which they are formed, but all are insoluble in petroleum ether.

The color of all the above complexes is stable for at least several hours, and is so intense that most of the ions can be easily detected in concentrations of one part in ten million. Therefore, it seemed worth while to investigate the usefulness of these new compounds for colorimetric analysis.

Preliminary experiments proved that the affinity of *o*-nitrosophenol for the above-mentioned metal ions is very strong. It is sufficient to shake a solution of *o*-nitrosophenol in a solvent, immiscible with water, for a few seconds with a solution of the metal ions in water to form the organic metal complex quantitatively. The color intensity of the solutions thus obtained is completely reproducible and, within the limits of solubility, proportional to the concentrations of the metal.

Unfortunately, because the author had no opportunity to measure the extinction coefficient for the colored solutions, it was impossible to determine exactly to what extent the colored system conforms strictly to Beer's law. However, as is shown in this paper, the system is entirely suitable for use in a photoelectric colorimeter.

Based on these observations, a colorimetric determination of cobalt has been worked out, in which the solution of the cobalt salt in water is shaken with a solution of *o*-nitrosophenol in petroleum ether. After the two liquids have separated, the color intensity of the petroleum ether is measured in a colorimeter.

There are very few limitations to the use of this method. The most important is that acids whose cobalt salts are insoluble at pH 4 must not be present (phosphoric acid, oxalic acid, and others). Of the complex cobalt compounds, only the potassium cobaltic chloride has been investigated and qualitatively it showed the same reactions as the simple cobaltous ions.

According to a private communication from G. H. Ellis, U. S. Plant, Soil and Nutrition Laboratory, Ithaca, N. Y., phosphates present in soil analysis do not interfere in the cobalt determination with *o*-nitrosophenol.

Palladium will not be considered further, since it is seldom present in a cobalt determination. However, since the color of the grayish-brown cobalt compound is quite different from that of the green palladium compound, the determination might be carried out with the help of suitable color filters. The theoretical considerations for the colorimetric analysis of a two-component color system are given by Knudson, Meloche, and Juday (*5*).

The interference of ferric salts can be eliminated in three ways. The surest of these is to precipitate the iron from an acid solution with cupferron, extract the excess cupferron with chloroform or ether, and remove the remaining organic solvent with warm air. A second way is to reduce the trivalent iron quantitatively to the divalent state with isoascorbic acid. However, since difficulties are frequently encountered in this reduction procedure, it has not been worked out in detail. The third possibility consists of the formation of complex ferric compounds, so that the concentration of free ferric ions is negligible. This method is the simplest and in most cases yields satisfactory results. However, in the presence of a large excess of iron, the cobalt value may be somewhat high, probably because the binding of the iron in the complex is not strong enough to exclude entirely its reactions with *o*-nitrosophenol.

In using *o*-nitrosophenol for colorimetric determination, the maintenance of the proper pH is important. The strongest color intensity for a given cobalt concentration is obtained at pH of 3.8 to 4.4. Therefore a pH of about 4.0 must be assured by use of a suitable buffer solution, which at the same time should form a complex compound with trivalent iron without affecting the state of the cobalt ions.

A mixture of sodium citrate and citric acid, made by dissolving 2.1 grams of citric acid in 88.5 ml. of water and adding 11.5 ml. of *N* sodium hydroxide, has been found to be the most suitable buffer solution. The pH of the mixture is 4.0.

A buffer made of tartaric acid and sodium hydroxide may also be used. Phosphate or oxalate buffers are not suitable because they form insoluble cobalt salts. An acetate buffer is not recommended, since its iron complex is not strong enough to prevent reaction of the iron with *o*-nitrosophenol.

The preparation of *o*-nitrosophenol is described by Baudisch (*1*, *2*). Since it can be prepared and is stable only in solution, the proper concentration cannot be given in per cent

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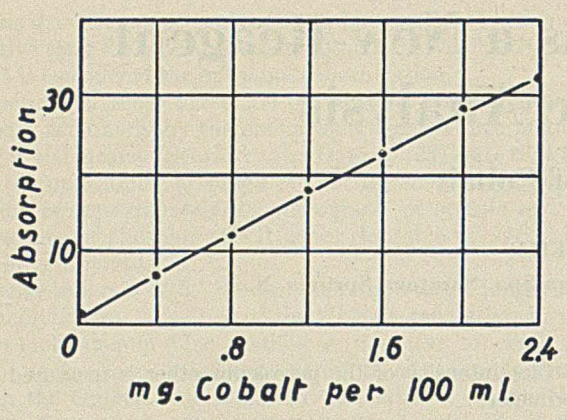

FIGURE 1. CALIBRATION CURVE FOR COBALT DETERMINATION
Absorption = deflection on "absorption" scale of colorimeter

values but must be controlled by the following tests, which should be made at regular intervals:

1. After shaking 1 ml. of the *o*-nitrosophenol solution with 5 ml. of copper sulfate solution (1 per cent) in a test tube, the petroleum ether layer should be absolutely colorless (test for purity).
2. After shaking 2 ml. of the *o*-nitrosophenol solution with 5 ml. of copper sulfate solution (10 mg. of copper sulfate pentahydrate in 1-liter of distilled water) the water solution should be violet while the petroleum ether layer should remain yellowish green (test for sufficient concentration).

The solution of *o*-nitrosophenol in petroleum ether is stable for 2 to 3 weeks if kept in a refrigerator.

Procedure

The solution of the cobalt salt is neutralized, if necessary (spot test with methyl orange or bromophenol blue), and diluted so that it contains not more than 1.5 mg. of cobalt in 100 ml. Ten milliliters of this solution are poured into a small separatory funnel, 5 ml. of the citrate buffer and 2 ml. of *o*-nitrosophenol in petroleum ether are added, and the mixture is shaken vigorously for 15 to 20 seconds. After standing for a short time, the brown petroleum ether layer is separated from the water solution and put into a glass-stoppered flask graduated at 12 ml. This procedure is repeated twice with 2 ml. of *o*-nitrosophenol solution each time. Then the water solution is washed twice with 2 ml. of pure petroleum ether, the washings are added to the brown cobalt extraction, and the whole mixture is diluted with petroleum ether to a volume of 12 ml. This solution is then placed in the 10-ml. cell of the colorimeter and the determination is made in the usual manner.

In some instances, as, for example, in spectrophotometric analysis, when it is necessary to remove the excess *o*-nitrosophenol, the solution of the cobalt complex in petroleum ether is shaken repeatedly with a very dilute solution (0.01 per cent) of copper sulfate until the latter no longer shows the formation of the red-violet copper complex.

Figure 1 shows the data obtained by using a photoelectric colorimeter (Pfaltz & Bauer, New York, N. Y.), following the above procedure. The curve is nearly a straight line. This means that the proposed method is entirely suitable for the colorimetric determination of cobalt, even with polychromatic light.

Furthermore, by means of this curve it is possible to determine the maximum error and the sensitivity of the proposed method. When a storage battery is used with the colorimeter, the fluctuations of the galvanometer are not greater than one-tenth scale division, and it is considered that the readings are made with an accuracy of one-quarter scale division. (This is far from being the limit; for an experienced worker, it is not difficult to estimate one-tenth scale division.) This corresponds to 2 micrograms of cobalt in 10 ml. Taking a reading from 25 to 30 with a corresponding cobalt content of about 200 micrograms in 10 ml. as most suitable, the maximum error in the determination is not greater than 1 per cent.

The sensitivity of the method can be calculated in a similar manner. The lowest limit for which a satisfactory reading can be made is two scale divisions, which corresponds to an absolute cobalt content of 12 micrograms in 10 ml. of petroleum ether. By using a larger cell it is possible to start with 50 ml. instead of 10 ml. of the original cobalt solution. Thus the cobalt in a solution containing as little as 12 micrograms in 50 ml. may be determined.

All the foregoing experiments and calculations were made with the so-called normal sensitivity of the colorimeter. By changing the resistance in the photoelectric circuit the sensitivity of the instrument may be increased ten times. Accordingly a deflection of the galvanometer of two scale divisions would be obtained by one tenth of the above cobalt concentration. However, in consideration of the increased possibility of error, it is safer to take a galvanometer deflection of four scale divisions as the minimum. This means the smallest amount of cobalt which can be determined by the described method is about 5 micrograms or about 8×10^{-8} mole of cobalt in 50 ml.

The values in Figure 1, upon which all these calculations are based, were obtained with a pure cobalt solution. In the presence of other heavy metals which form the water-soluble complex compounds, the method is essentially the same. One has only to consider that part of the *o*-nitrosophenol will be used by ions other than cobalt and therefore the amount of *o*-nitrosophenol has to be increased.

The only interfering ion of practical importance is trivalent iron, which may be eliminated by the use of a citrate buffer solution. The results of the determination of cobalt in the presence of ferric salts are given in Table I. It appears that the amount of ferric salts present, even if 25 times that of cobalt, is of little importance, provided a sufficient amount of buffer solution is present.

TABLE I. DETERMINATION OF COBALT

Cobalt Taken	Buffer Solution	Fe^{+++} Added	Cobalt Found
Mg.	*Ml.*	*Mg.*	*Mg.*
0.120	1	–	0.120
0.120	1	1	0.126
0.120	2	1	0.122
0.120	3	1	0.129
0.120	5	1	0.123
0.120	5	3	0.123

Daylight and strong artificial light should be excluded, because under their influence the ferric ions are reduced in the presence of *o*-nitrosophenol to ferrous ions. The latter form with *o*-nitrosophenol the green water-soluble ferrous complex salts, thus consuming a part of the *o*-nitrosophenol.

Summary

o-Nitrosophenol as a new reagent in colorimetric analysis offers many possibilities for the estimation of small amounts of cobalt, palladium, iron, copper, mercury, and nickel.

In the first application, *o*-nitrosophenol is used for the quantitative estimation of small amounts of cobalt. It is possible to estimate 5 micrograms of cobalt in 50 ml. with an error not exceeding 1 per cent.

The only interfering metals are trivalent iron and palladium. The interference of trivalent iron is eliminated by forming complex iron compounds which do not react with *o*-nitrosophenol. The interference of palladium can be removed by the use of suitable color filters.

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Determination of Divalent Iron

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IN A previous paper (*3*), *o*-nitrosophenol was introduced as a new reagent in quantitative colorimetric analysis. It forms strongly colored inner-complex compounds with a number of metal ions, and the different metal complexes are distinguished by their color and their solubility in different solvents. The most important metals of the water-soluble group are divalent iron, copper, mercury, and nickel. The complexes of these and other metals are red or reddish violet with the exception of divalent iron, which is grass-green colored. No other metal forms a green, water-soluble complex with *o*-nitrosophenol.

Based on this characteristic property, the authors have worked out a quantitative estimation of small amounts of divalent iron. Preliminary experiments (*3*) showed that the reaction between *o*-nitrosophenol and ferrous ions is, in every respect, suitable for a colorimetric determination of this metal. The new method makes use of a reagent dissolved in a water-immiscible organic solvent, a feature not yet common in colorimetric work. The affinity of the divalent iron for *o*-nitrosophenol is so strong that upon shaking the two solutions—i. e., the ferrous salt in water and the organic solvent—the complex green iron salt is formed immediately and quantitatively.

The use of a water-immiscible solvent for the reagent has a very important advantage. Since free *o*-nitrosophenol in an organic solvent is yellowish green, it can be seen directly whether or not the reagent has been used in excess, as should always be the case. If the solution of the free *o*-nitrosophenol becomes colorless after shaking with the ferrous salt solution, it is at once apparent that more reagent should be added.

In using this method the solution of the ferrous salt in water is shaken with a solution of *o*-nitrosophenol in petroleum ether, whereupon the water solution becomes deep green, and after the two liquids have been separated the green water solution is measured in a colorimeter in the usual manner. Petroleum ether is preferred as the solvent for the *o*-nitrosophenol because of the characteristic solubilities of the metal salts of *o*-nitrosophenol (*3*).

These characteristic solubilities account for the fact that by this method only the divalent iron is determined. Ferric ions too react with *o*-nitrosophenol, forming a brown-colored complex compound. However, the reaction between ferric ions and *o*-nitrosophenol is not quantitative and the ferric complex is easily soluble in petroleum ether and thus extracted from the water solution of the green ferrous complex.

The method can be used for the estimation of ferric ions after the latter have been reduced to the divalent state by means of isoascorbic acid. A description of this procedure will be published soon by Baudisch.

The green ferrous complex is stable for at least 24 hours in the absence of very strong oxidizing agents or of very strong light.

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The intensity of the green color formed is always reproducible and is in proportion to the concentration of ferrous ions originally present (curve 1, Figure 1). Unfortunately, the authors did not have the facilities to determine the range in which the colored system conforms strictly to Beer's law.

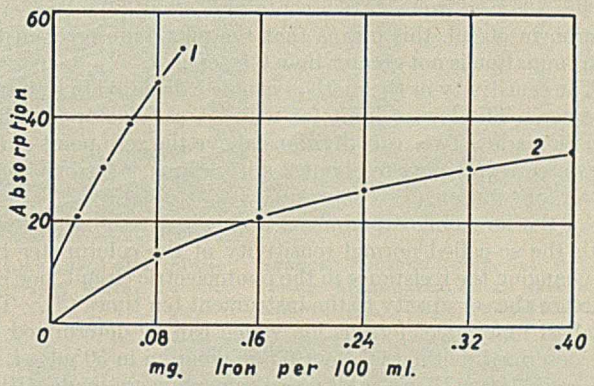

FIGURE 1. CALIBRATION CURVE FOR DETERMINATION OF FERROUS IONS
Absorption = deflection on the "absorption" scale of colorimeter
1, obtained with *o*-nitrosophenol; 2, with α,α'-bipyridine

The maintenance of the proper pH is of greatest importance in all colorimetric work with *o*-nitrosophenol. In the case of its water-soluble compounds, a low pH prevents the quantitative formation of the complex, owing to dissociation. If the pH is too high, a mixture of two compounds is formed whose general formula can be written as *N—Me—R* (I) and *N—Me—N* (II), where *N* represents the *o*-nitrosophenol group. These two compounds differ in their color and solubility. I is soluble only in water, while II is soluble in certain organic solvents like ether, but insoluble in petroleum ether.

For the determination of divalent iron the most suitable pH range is from 5.1 to 5.3.

The preparation of *o*-nitrosophenol is described by Baudisch (*1*, *2*). Since it can be prepared and is stable only in solution, the proper concentration of the reagent must be controlled by tests (*3*), which should be made at regular intervals. The solution of *o*-nitrosophenol in petroleum ether is stable for 2 to 3 weeks if kept in a refrigerator.

Procedure

The solution of the ferrous salt is nearly neutralized (spot test with methyl orange or bromophenol blue) and diluted so that it contains not more than 1 microgram of ferrous iron per ml. In a separatory funnel exactly 50 ml. of this solution are mixed with exactly 5 ml. of an acetate buffer solution of pH 5.2. After 5 ml. of *o*-nitrosophenol solution are added, the mixture is shaken vigorously from 15 to 20 seconds and then allowed to separate. The petroleum ether is removed as well as possible by means of a pipet with a rubber bulb and the green water solution is shaken with a second 5-ml. portion of *o*-nitrosophenol solution. After

the two liquids have separated, the petroleum ether should still be yellowish green because of an excess of free *o*-nitrosophenol. The green water solution is filtered through a paper filter directly from the separatory funnel into the 30-mm. cell of the colorimeter. The filtration helps to clear the water solution completely from the last droplets of petroleum ether and prevents the formation of bubbles which might cling to the cell walls and so influence the results.

Curve 1, Figure 1, shows the value obtained with the so-called absorption scale of a photoelectric colorimeter (a modified Lange photoelectric colorimeter manufactured by Pfaltz & Bauer, Inc., New York, N. Y.) using a pure ferrous chloride solution. The light source was a 6-volt incandescent bulb and no light filter was used. Therefore, the values in the curve do not represent the true absorption.

By means of this curve it is possible to determine the maximum error and the sensitivity of the proposed method. When using a storage battery the fluctuations of the galvanometer are not greater than one-tenth scale division, and the readings are made with an accuracy of one-quarter scale division (for an experienced worker it is not difficult to estimate one-tenth scale division), which corresponds to 0.3 microgram of iron in 50 ml. For an iron solution containing about 50 micrograms of iron in 50 ml. this means that the maximum error in the determination is not greater than 0.5 per cent.

The sensitivity of the method can be calculated in a similar manner. The lowest limit for which a satisfactory reading can be made is two scale divisions above the zero point of the curve, corresponding to about 2 micrograms of ferrous ion in 50 ml. of solution.

All the foregoing experiments and calculations were made with the so-called normal sensitivity of the colorimeter but by changing the resistance in the photoelectric circuit, one can increase the sensitivity of the instrument ten times (*3*). The smallest amount of ferrous ion which can be determined by the described method is about 0.5 microgram in 50 ml.—i. e., 1 part in 100 million. (All measurements were made with a regular incandescent bulb. The use of monochromic light of a suitable wave length will increase the sensitivity still more.)

The calculations indicate that the new method with *o*-nitrosophenol is one of the most sensitive for the quantitative determination of ferrous ions. An experimental proof is given in Figure 1 by curve 2 which was obtained with α,α'- bipyridine instead of *o*-nitrosophenol. A comparison of the two curves shows that under the present experimental conditions the new method is at least three times as sensitive as the bipyridine method. (Contrary to the literature, α,α'- bipyridine forms strongly colored red complex compounds with titanium salts. The authors made this observation when S. E. Ashley of the General Electric Company, Pittsfield, Mass., called their attention to the fact that titanium salts react in a similar manner with *o*-phenanthroline.)

The limitations for the new method are few. The most important is the necessity of having the divalent iron present in the ionic state. This means that complex-forming compounds such as phosphoric acid, oxalic acid, and others should not be present. Trivalent iron, cobalt, and palladium do not interfere because their *o*-nitrosophenol salts are soluble in petroleum ether and therefore will be shaken out during the reaction, but as these metals will bind a part of the free *o*-nitrosophenol the amount of the latter has to be increased accordingly. In the presence of trivalent iron strong light must be excluded, because under its influence the trivalent iron will be reduced to a certain extent by *o*-nitrosophenol and thus cause too high results.

Of the other heavy metals known to form water-soluble colored complex compounds with *o*-nitrosophenol, most important are copper, nickel, mercury, and zinc, because their color is so strong that they are detectable in concentrations as low as 10^{-7} to 10^{-8} molar. However, the nitrosophenol complexes of these and other less important metals are all red or reddish violet in color. In their presence, divalent iron can be determined by the use of suitable color filters. The theoretical considerations for the colorimetric analysis of a two-component color system are given by Knudson, Meloche, and Juday (*4*).

Alkali and alkaline earth salts do not interfere with the determination of divalent iron because their *o*-nitrosophenol salts are formed only at a pH above 7.

The described method has been applied to the determination of iron in natural Saratoga mineral waters, which are solutions of bicarbonates of alkalies and alkaline earths, together with an excess of free carbon dioxide. The iron is present only in the divalent state. Because of the bicarbonate content of these waters, the proper pH can be attained by adding only acetic acid. Owing to the high sensitivity of this method, the waters had to be diluted in some cases in the ratio of 1 to 25. Table I shows some of the results obtained.

To demonstrate the accuracy of the new method, the iron content of Lincoln water was determined gravimetrically, using cupferron as precipitating agent (Table I). The difference between the two methods does not exceed 1 per cent.

Table I. Determination of Iron in Natural Mineral Waters

Spring	Colorimetric	Gravimetric
	Mg./l.	*Mg./l.*
Lincoln[a]	12.15	12.20
Lincoln[a]	15.45	15.55
Geyser	2.22	...
Coesa	2.33	...
Hathorn No. 2	5.40	...

[a] Two samples taken at several days' interval.

Summary

A new method for the colorimetric estimation of divalent iron is based on the reaction of divalent iron with *o*-nitrosophenol, yielding a green inner-complex salt.

The method is one of the most sensitive colorimetric determinations for this metal, since 0.5 microgram in 50 ml. of solution can be estimated. Trivalent iron, cobalt, and palladium do not interfere. Copper, nickel, and mercury form red or reddish-violet compounds with the reagent. A comparison with a gravimetric determination of iron showed a very close agreement.

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Presented before the Division of Analytical and Micro Chemistry at the 101st Meeting of the American Chemical Society, St. Louis, Mo.

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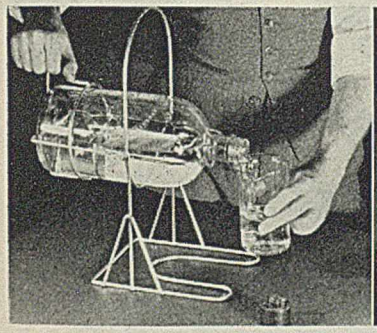

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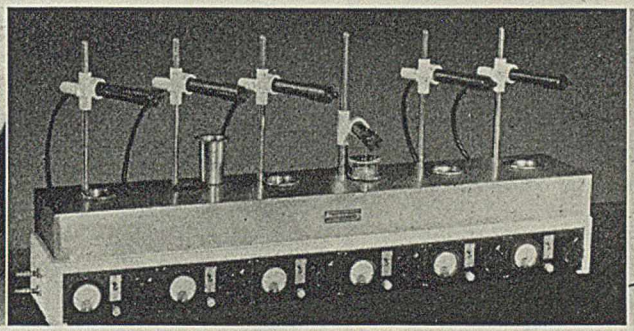

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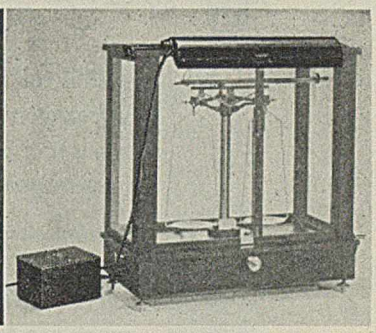

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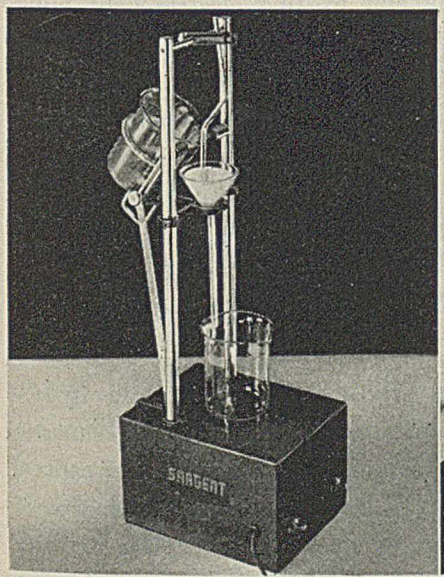

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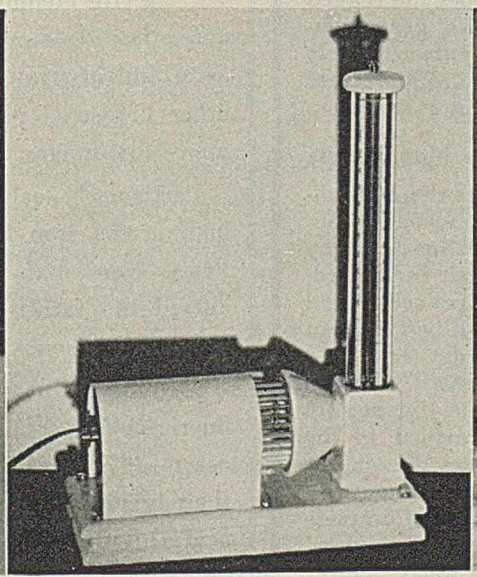

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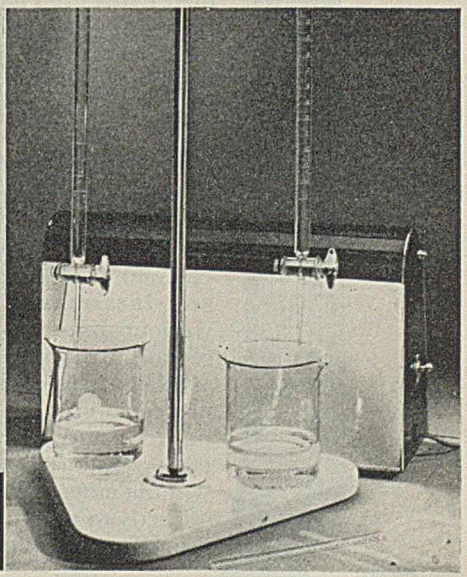

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