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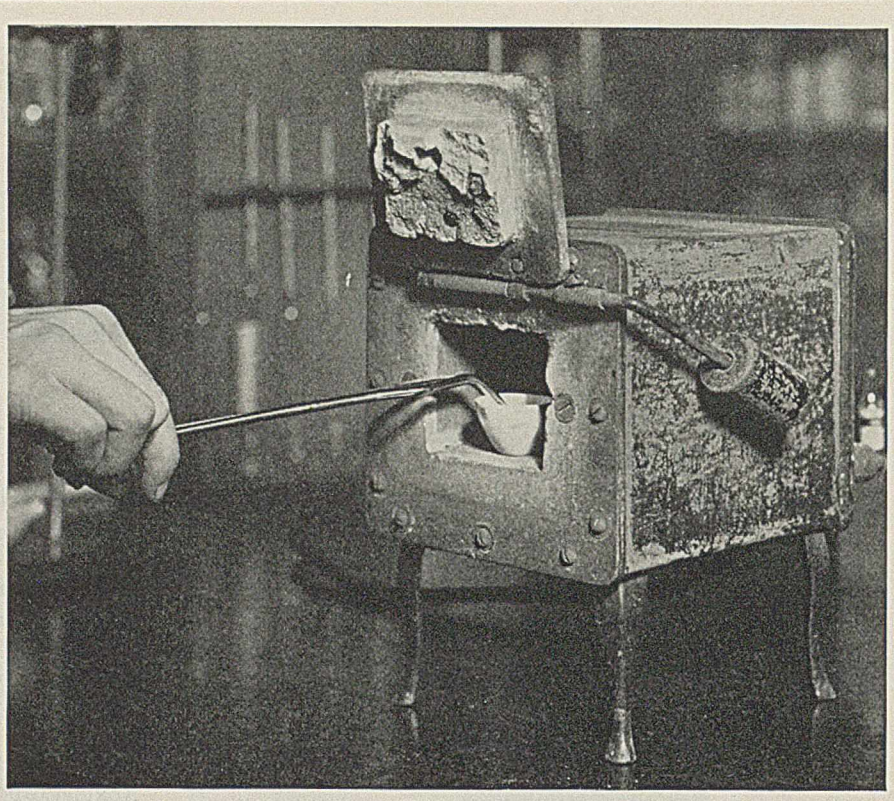

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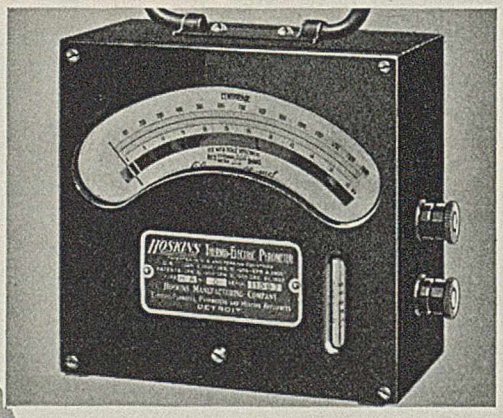

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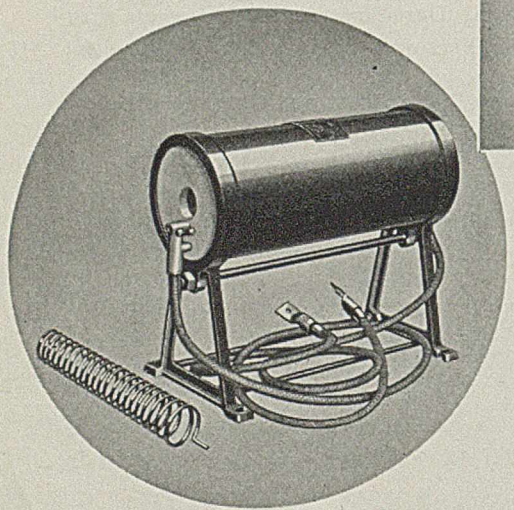

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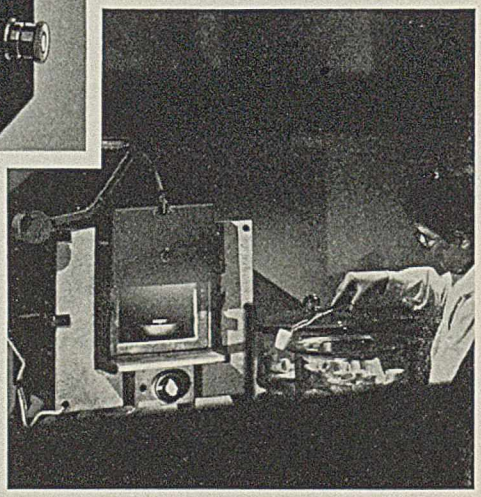

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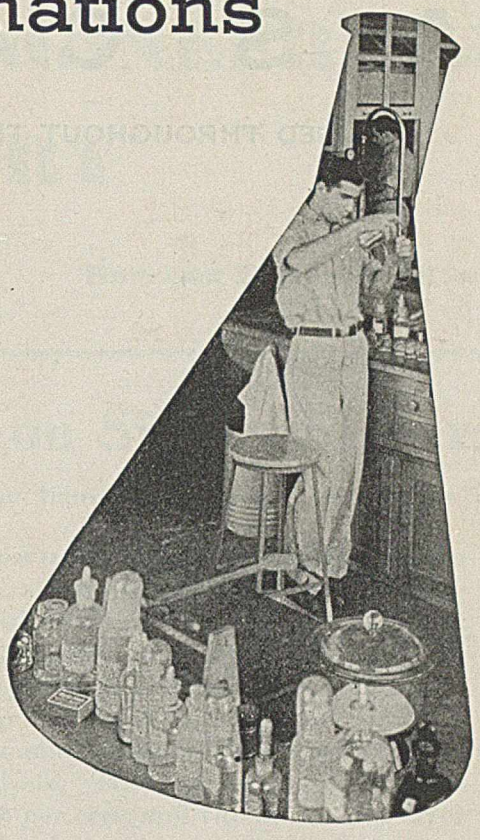

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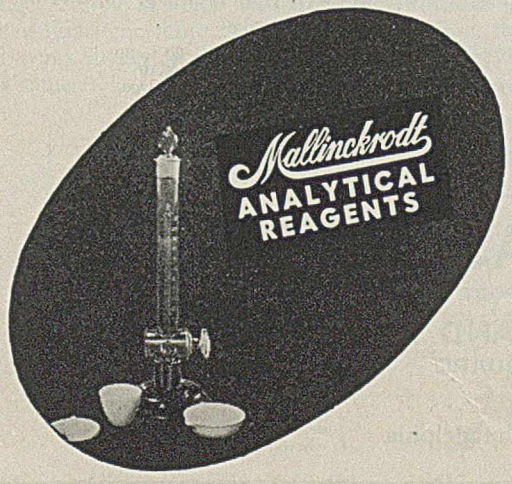

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INDUSTRIAL and ENGINEERING CHEMISTRY

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Quantitative Analysis Based on Spectral Energy

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An explanation is given for certain limitations of the commonly used internal standard method, and energy of spectral emission is suggested for measurement of concentration in place of intensity. Experimental work shows that the energy of spectral emission in a carbon arc is directly proportional to the weight of element causing the emission. For quantitative analysis, therefore, it is only necessary to determine energy per unit weight of element on known samples, and apply this value in the analysis of unknowns.

This procedure permits working over the entire range from the lowest limit of sensitivity up to 100 per cent. The presence of other elements appears to have no effect on the analysis. The average error was found to be 8.3 per cent and the maximum error was 18.5 per cent.

THE concept of intensity is as old as spectroscopy. We speak of lines as being intense or faint. Although we now use for observation the photographic plate, an integrating device, we still use the terminology appropriate to the spectroscope, an indicating device. It was natural, therefore, when the spectrograph came to be used for quantitative work, to apply this idea of intensity of an emission spectrum as a parameter of concentration.

The use of spectral intensity for analysis carries the implication that it is a property which varies only with the concentration. It is well known that a great many other factors affect intensity, so that it is necessary to impose the requirement that all conditions of the experimental procedure be held constant; the conditions here referred to are the current, optical setup, exposure time, photographic routine, and composition of sample. This last requirement is manifestly impossible to control, for this is the very thing we are trying to determine. Intensity methods have, therefore, purely from trial and error experience, been restricted in the main to so-called "simple samples" and low concentrations (such as the determination of impurities in metals of high purity) in which the composition of standards and unknowns is very nearly the same.

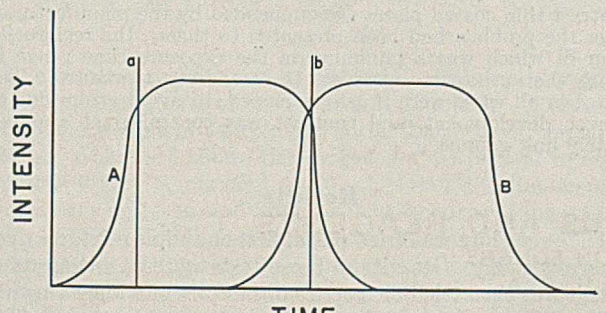

FIGURE 1

The literature of recent years contains several observations (*1–4*) to the effect that the various elements present in the arc (the spark has similar faults) do not emit their spectra simultaneously, or at a definite and constant intensity, but on the contrary show very wide variations, depending on the elements which make up the sample. The arc behaves like a small furnace, which in fact it is, volatilizing the substances in it in the order of their relative vapor pressures, the more volatile coming off first and the more refractory last.

How strikingly abrupt and clear-cut this differential volatilization may be is very well shown by Goldschmidt and Peters (*2*). They placed a partly cupeled lead bead containing silver, gold, and the six platinum metals in a carbon arc and completely vaporized the sample. The plateholder was shifted periodically during this process, allowing 20 seconds for each exposure and 5 seconds for the shift. The plate showed the lines of the nine metals in this order:

Seconds									
0 to 20	Pb	Ag							
25 to 45	Pb	Ag	Au						
50 to 70		Ag	Au	Pd					
75 to 95				Pd	Ru	Rh	Pt		
100 to 120				Pd	Ru	Rh	Pt	Ir	
125 to 145					Ru	Rh	Pt	Ir	Os
150 to 170						Rh	Pt	Ir	Os

The effect of this differential volatilization on an analytical procedure using intensity methods can best be explained graphically.

Let us examine the emission, during the course of volatilization, of some particular element of a sample introduced into such an arc. In other words, let us plot the variation of intensity of some particular line with respect to time (Figure 1). We should expect intensity to start from zero soon after the arc is struck, and gradually reach a maximum value, which would be maintained for an interval roughly equivalent to the absolute amount of the element present in the arc, and then drop back to zero again, when all had volatilized. This purely qualitative picture is represented by curve *A* of Figure 1.

Now if in a second sample, similar to the first and containing the same concentration of the element of curve A, there is present a more volatile element, say one of the alkalies, then curve A will be displaced to the right, as shown in curve B, for the alkali metal will volatilize before the more refractory one. But intensity methods assume, as they must, constancy of emission, and so their procedures consist in making an exposure for a fixed number of seconds, commencing either at the instant of striking the arc, or after a fixed interval, when it is hoped that constant conditions have been reached. This exposure is shown on the graph as the two vertical lines, a and b.

Area in Figure 1 represents energy (product of time and intensity). It is this energy that is recorded by the photographic plate as density of image, and thus the quantity measured by the intensity methods is the energy represented by the area enclosed by lines a and b and curve A. This procedure would be unobjectionable if the maximum of the intensity curve were reproducible under all conditions and the whole curve fixed on the time axis. But if, because of a change in composition, it shifts to, say, curve B, so that now the energy being recorded on the plate is represented by the area bounded by the two verticals and curve B, large errors result and the intensity method fails.

From these considerations it is evident that the principal cause of the trouble is the time factor entering into the exposure. Fundamentally, there is no physical relationship between intensity and mass or concentration of an element; they are two separate quantities. If we adopt a procedure involving a consistent relationship, the difficulty should disappear. With further reference to Figure 1, it seems reasonable to suppose that the total energy of the emission—i. e., the area under curve A, which does not contain the time factor—should prove to be a more robust parameter, not influenced by changes of composition. This is the basis of the method described in this paper.

There is considerable theoretical basis for this view. The carbon arc may be pictured as a furnace on which is set a gas-discharge tube, operating at atmospheric pressure and having walls of cool air—the surrounding atmosphere. The furnace discharges into this tube metallic vapors, whose atoms become excited and emit radiation and then pass out of the tube and out of the process. There is thus a unidirectional flow of atoms from the lower electrode into the zone of excitation and out into the cool air.

The intensity at any interval of time dt is a measure of the atoms present in the luminous zone during that interval (other conditions being the same). As the atoms are continuously lost out of the tube, while new ones take their place, the intensity integrated over the time of emission will be a measure of the number of atoms that have passed through during that time. If the time is taken from the instant of striking the arc until all the sample has burned off, the number of atoms that have passed through the zone of excitation will be the same as the number contained in the sample. Therefore, the integrated intensity, $\int I dt$, is a measure of the number of atoms in the sample, or of the mass of the element in the sample. The equation expressing this condition is

$$m = k \int I dt \qquad (1)$$

where m is the mass of element in the sample and k is a proportionality constant.

One other factor influencing energy emitted with respect to mass should be mentioned here. Excitation in an arc, whether due to thermal action or to impacts from cathode electrons, will be directly influenced by the current passing through the arc. A larger current, therefore, will cause more excitations among a given number of atoms than a smaller one. We are using the emitted energy as a means of counting the number of atoms passing through the arc, and if this count is to be reproducible from one exposure to another, the current must be standardized for any series of comparable tests.

Experimental

To test this hypothesis experimentally involves establishment of the validity of the above equation. It can be done in several ways; the author's procedure had, perforce, to be based on the equipment available, which consisted of a large Littrow-type quartz spectrograph equipped with a variable rotating sector, and a densitometer.

The portion of the equation at the left, pertaining to the mass of element consumed, obviously required the use of the carbon arc. Amounts of chemically analyzed samples were weighed on a microbalance and transferred with the aid of a small funnel into cored graphite electrodes. These formed the positive of the arc, and the discharge was maintained at constant current until there was no doubt that the entire sample had been consumed.

Evaluation of the portion of the equation at the right presented a much more complex problem. In the first place, the author realized that the usual method of illuminating the slit, by means of a spherical condenser, which throws a geometrical image of the source on the slit, was not suitable, because wandering of the arc would cause a corresponding shift at the slit; thus the plate would not be continuously illuminated during the whole of the exposure. A cylindrical condenser was therefore substituted (a suggestion of S. Jacobsohn, of the Gaertner Scientific Co., Chicago, Ill.), the axis of which was set perpendicular to the slit. This type of lens presented a horizontal segment of the arc column to the view of the spectrograph, so that while the light never fell outside the prism, the images of the incandescent poles were thrown above and below the slit opening, none of this light entering the spectrograph.

The integration required by the equation was to be done by the photographic plate. However, the response, which is in terms of density, had to be converted to relative energy values. The method decided upon consisted in giving each plate a series of regulated exposures, varying in intensity but constant in time, from a source that was reproducible from day to day. The source that most nearly met this requirement was a high-pressure direct-current quartz mercury-arc lamp with controlled input. The lamp and arc stand were mounted on a dovetail slide, so that either could be placed interchangeably in the optic axis of the spectrograph. The light path traversed by the standard radiation and the unknown was therefore the same. Controlled variation in intensity was obtained by means of the rotating sector, used as specified by Webb (*5*). This permitted the establishment of a characteristic curve (intensity scale) for each plate, from which densities could be converted into the corresponding energies, since both time and intensity of the causative radiation were known—that is, the curve expressed the relation between energy and plate response.

After development, the densities of a chosen mercury line and of a neighboring line of the unknown were measured by means of a densitometer. The mercury line densities were then used to construct the characteristic curve for each plate, and from this curve energies corresponding to the unknown's densities were read off. This is a null method, in which the unknown energy contained in a particular spectrum line is directly equated to the known energy of a mercury line, the plate being used merely as a null indicator.

A difficulty arose at this point through the inability of the photographic plate to integrate light correctly [the reciprocity error, a term applied specifically to photographic emulsions which exhibit failure of the Bunsen-Roscoe law. This failure manifests itself as unequal responses to dosages of energy which are equal, but at different intensities (or conversely, for different exposure times)]. The author was endeavoring to measure radiation of variable intensity in terms of constant radiation. Under these circumstances there is no way of avoiding reciprocity error. However, it was minimized by the use of Eastman contrast thin coated plates (recommended by the manufacturers after the problem had been presented to them), the reciprocity error of which was a minimum in the exposure time range in which the author was working, 15 seconds to 4 minutes. The plates in all cases were brush-developed, to avoid errors due to uneven development, and contrast was controlled at approximately unit gamma.

Results

The procedure was tried out at first on simple mixtures over a narrow range. Results of these tests were so encouraging that it was then tried on a series of samples of widely varying composition and over a large range of concentrations. The

metal whose radiation was to be measured was calcium (calculated in this paper as CaO) and the samples were various minerals and rock products, several of them Bureau of Standards standard samples.

The samples, with their CaO contents, are listed in Table I.

TABLE I. SAMPLES USED

Sample No.		Principal Constituents	CaO %
1	Bureau of Standards No. 98 (plastic clay)	SiO_2, Al_2O_3	0.21
2	Bureau of Standards No. 99 (feldspar)	$Na_2O \cdot Al_2O_3 \cdot 6SiO_2$	0.36
3	Feldspar	$(K_2O, Na_2O)Al_2O_3 \cdot 6SiO_2$	0.81
4	Bureau of Standards No. 102 (silica brick)	SiO_2	2.29
5	Bureau of Standards No. 104 (burnt magnesite)	MgO	3.35
6	Feldspar	$Na_2O \cdot Al_2O_3 \cdot 6SiO_2$	4.46
7	Tremolite-talc	CaO, MgO, SiO_2 (complex)	7.58
8	Tremolite-talc	CaO, MgO, SiO_2 (complex)	9.18
9	Dolomite	$CaO \cdot MgO \cdot 2CO_2$	34.02

Each sample was run in quadruplicate. In order that all exposures should fall within the latitude of the plate (it was not necessary that they fall only on the straight-line portion of the characteristic curve) the intensity was varied by means of the rotating sector, the transmission being successively reduced with increasing CaO content. The results were then calculated to a basis of 100 per cent transmission, to make all exposures comparable.

TABLE II. RESULTS OF QUADRUPLICATE EXPOSURES

Sample No.		Weight of Sample *Gamma*	Weight of CaO *Gamma*	Energy, Arbitrary Units	Sector Transmission %	Energy at 100 Per Cent Transmission
1	a	24,300	51.0	3.1	10	31
	b	23,400	49.2	2.2	10	22
	c	31,200	65.5	3.1	10	31
	d	28,000	58.8	3.1	10	31
2	a	25,700	92.5	2.6	5	52
	b	26,100	94.0	2.65	5	53
	c	25,500	91.8	2.65	5	53
	d	28,500	102	3.0	5	60
3	a	28,800	233	7.4	5	148
	b	26,300	213	6.6	5	132
	c	21,400	173	5.3	5	106
	d	26,200	212	6.8	5	135
4	a	30,100	690	10.0	2.5	400
	b	34,400	788	10.7	2.5	428
	c	34,800	797	11.0	2.5	440
	d	31,400	720	9.8	2.5	392
5	a	29,900	1,000	9.1	1.5	606
	b	24,500	820	8.0	1.5	533
	c	28,900	968	8.8	1.5	587
	d	28,800	965	8.7	1.5	580
6	a	28,600	1,273	18.8	2.5	753
	b	29,500	1,315	18.0	2.5	720
	c	25,500	1,135	15.5	2.5	620
	d	21,200	945	Lost	2.5	Lost
7	a	27,900	2,110	16.3	1.5	1,088
	b	21,100	1,600	13.0	1.5	866
	c	22,900	1,740	12.5	1.5	833
	d	27,800	2,110	15.0	1.5	1,000
8	a	24,800	2,280	11.6	1.0	1,160
	b	20,800	1,914	10.7	1.0	1,070
	c	22,700	2,080	10.9	1.0	1,090
	d	22,600	2,075	10.2	1.0	1,020
9	a	26,700	9,080	43	1.0	4,300
	b	21,600	7,350	38	1.0	3,800
	c	21,300	7,250	34	1.0	3,400
	d	20,800	7,070	33	1.0	3,300

The results are collected in Table II. Column 1 contains the sample numbers, corresponding to Table I. Column 2 shows the weight of sample placed in the cores of the electrodes. Column 3 shows the weight of CaO present in each sample (weight × per cent CaO). Column 4 is the energy, in arbitrary units, as read from the density curve of the standard radiation plotted for each plate. Column 5 shows the transmission setting used to obtain the values of column 4. Column 6 is the calculated energy at 100 per cent transmission (figures of column 4 divided by figures of column 5).

The calcium line on which the measurements were made was at 3179 Å., the mercury line was at 3125 Å.

The spectra for the lower concentrations of CaO showed background. The energy values for these tests were high, indicating that background correction was necessary. This was accomplished by converting the density readings of background, taken adjacent to calcium 3179 Å., into energy values by means of the mercury curve, and subtracting these from the energy values obtained for the calcium line (as in column 4 of Table II). When this was done the corrected points were consistent with those obtained from background-free spectra. The figures given in column 4 of Table II have been corrected for background in this way.

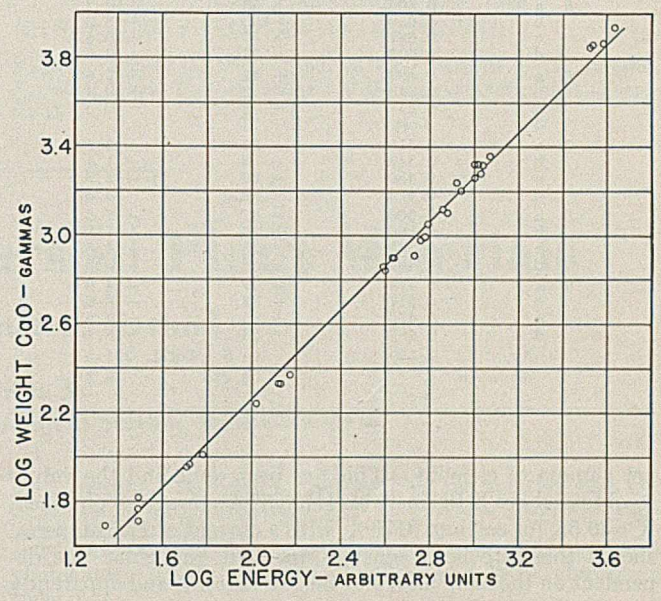

FIGURE 2

Figure 2 shows the relation between weight of CaO in gamma (a gamma is one microgram or 10^{-6} gram) volatilized in the arc and energy of the resultant emission (in arbitrary units) plotted on a logarithmic scale. A straight line drawn at 45° to the axes fits the points within the experimental errors. The equation of such a line is

$$\log m = \log E + C \quad (y = x + a)$$

or

$$\frac{E}{m} = k$$

where m is the weight of element, E the energy of emission, and k a constant. The results therefore agree with the equation given in the introduction.

Discussion

Simply stated, the experimental work described herein outlines a method of measuring elemental masses by measuring (relative) radiation from an electric arc. It should be applicable to various procedures and instruments, but is restricted in this discussion to those in common use at present, the spectrograph and densitometer.

In the experimental work radiation was measured and compared with known mass. The process obviously can be reversed and mass determined from radiation. If we rearrange Equation 1 thus

$$K = \frac{\int I dt}{m}$$

and measure the energy $\int I dt$ in any convenient units and m in gamma, then K has the meaning of so many energy units

TABLE III. VALUE OF K

Sample No.		K	Deviation ($\times 10^{-3}$)	Per Cent Error
1	a	0.608	+ 58	+10.5
	b	0.448	−102	−18.5
	c	0.473	− 77	−14.0
	d	0.527	− 23	− 4.2
2	a	0.563	+ 13	+ 2.4
	b	0.563	+ 13	+ 2.4
	c	0.577	+ 27	+ 4.9
	d	0.585	+ 35	+ 6.4
3	a	0.635	+ 85	+15.5
	b	0.620	+ 70	+12.7
	c	0.613	+ 63	+11.5
	d	0.636	+ 86	+15.6
4	a	0.580	+ 30	+ 5.4
	b	0.543	− 7	− 1.3
	c	0.552	+ 2	+ 0.3
	d	0.545	− 5	− 0.9
5	a	0.606	+ 56	+10.2
	b	0.650	+100	+18.2
	c	0.607	+ 57	+10.4
	d	0.602	+ 52	+ 9.5
6	a	0.592	+ 42	+ 7.6
	b	0.548	− 2	− 0.3
	c	0.547	− 3	− 0.5
	d	Lost	...	...
7	a	0.515	− 35	− 6.4
	b	0.542	− 8	− 1.4
	c	0.480	− 70	−12.7
	d	0.474	− 76	−13.8
8	a	0.508	− 42	− 7.6
	b	0.560	+ 10	+ 1.8
	c	0.525	+ 25	+ 4.5
	d	0.492	− 58	−10.5
9	a	0.473	− 77	−14.0
	b	0.517	− 33	− 6.0
	c	0.470	− 80	−14.5
	d	0.467	− 83	−15.1
Av.		0.550	45.8 Total	291.5
Av. error			8.3%	8.3%

per gamma of element. This has been done and the values so obtained are listed in Table III. The average so obtained, $K = 0.55$, for calcium 3179 Å. with a current of 12.5 amperes, should therefore be a constant for that particular line dependent on the lamp used and the arc current, and apparently on no other factors.

With the constant K thus established, the concentration of an element in a mixture can therefore be determined by means of the equation

$$C = \frac{E}{KM}$$

where M is the weight of sample taken in gamma.

It is apparent that this method based on total energy has several marked advantages over the customary intensity methods. It eliminates at one stroke all the difficulties inherent in the use of an internal standard; finding a suitable pair of lines for one concentration range and another pair for another range; knowing the ratio between unknown and base material; the necessity that both these metals volatilize at the same rate and during the same portion of the exposure; and the necessity of preparing an extensive series of graduated standards to fix the calibration curve.

So much for the negative virtues. On the positive side may be mentioned the ease with which the working constant may be evaluated, requiring but one or two standards; the consequent saving of time, permitting the method to be used for research or occasional samples; the ability to handle samples of any concentration without altering the procedure; and the possibility of correcting for background.

Data are here presented for a single line of a single metal. But the writer's experience, so far as it has gone, indicates that any line of any metal can be used for quantitative work, that any line will show this constant relation between mass and energy. Anomalous effects, such as are usually ascribed to "arc and spark lines," have not been detected. The choice of line is governed only by convenience.

To study the influence of one type of atom on the excitation of another type has been one of the objects of the experiment, but no such influence has been detected within the resolution of these measurements. Three of the samples used (the feldspars) contained considerable concentrations of sodium and potassium. The view is generally held that the presence of alkalies in the arc changes the intensity relationship in the spectrum. For instance, Harrison (*3*) says, "The alkali metals have unusually low ionization potentials, so their presence in quantity in a sample tends to suppress the excitation of other atoms." This was not the author's experience, for these three samples are consistent with the others. These samples were included in the series because of this general opinion that the alkalies are the worst offenders; if they did not affect the excitation of the calcium atoms under the conditions of the author's procedure, it was felt that no other elements would.

Two other samples may be mentioned in this connection. In No. 4 the calcium is present in an SiO_2 base and in No. 5 in an MgO base. These also showed no inconsistency. It is of course unsafe to make the categorical statement, based on such meager data, that the various atoms have no effect on each other; this point can be settled with assurance only by extensive work with various combinations. It is perhaps unnecessary to remark that lines terminating in the ground state should be used with caution for quantitative work, because of their tendency to show reversal.

As to accuracy, here also the comparison is favorable. The accuracy obtainable with the best of the intensity methods is of the order of 5 per cent error for a single determination. The errors for the data of this paper have been calculated and are shown in column 4 of Table III. These are higher than should be expected in an actual analysis, as the data have been taken over a much greater range than will usually be experienced in practice. Some reciprocity error has been unavoidable (as evidenced by the slight concavity of the series of points towards the log weight axis, Figure 2). Also, errors in chemical analysis, while small, had some effect on the results. Probably further experience will show that synthetically mixed samples are simplest and best. The error, then, should be found in practice to be of the same order as the most refined of the present methods.

A principal difficulty of the total energy method is with the standard light source. Of the two sources commonly available with sufficiently high reproducibility to be useful, the incandescent lamp and the mercury arc, neither is entirely satisfactory. The former can be used only from the infrared to about 4500 Å., and even in this range the change of intensity with wave length is sharp. Filters could conceivably equalize this fault, but only at the expense of reduced intensity, which is already too low compared to the monochromatic intensity of arc lines. (It must be remembered that exposure time cannot be increased without limit, but must be comparable to emission time of the element under investigation.) The principal fault of the mercury arc lamp is the paucity of lines in the mercury spectrum. A procedure that does away with the necessity for a standard lamp, but which is suitable only for occasional samples, has been tried in this laboratory. It consists in photographing the spectra, on the same plate, of a series of known samples and of the unknown, the weights of all samples being taken. The density of a particular line in each of the known spectra is measured and these values are plotted against log weight of element in the knowns. The densities of the unknown samples are then interpolated in this curve (which should be a straight line if the densities fall on the straight-line portion of the characteristic) and the unknown weight of element thus read off. This, divided by weight of sample taken, gives the concentration.

It appears possible, from the relation between mass and

emitted radiation found here, to devise a method which dispenses with both the photographic step and the standard lamp. For instance, the radiation from the arc, as an analyzed sample of known weight is volatilized, can be dispersed by a monochromator and a chosen line allowed to fall on a suitable radiation measuring device (photocell, thermopile, etc.). The response could then be integrated over time of emission by some such means as a photon counter and the constant K thus determined. Investigation in this direction could possibly lead to the development of a practical device for chemical analysis by purely mechanical means—an analytical machine. It is hoped that this phase of the problem will be attacked when more experience with the general procedure has been acquired.

The work described here is part of a broader research by the Nonmetals Division of the Bureau of Mines, having in mind the adaptation of quantitative methods of spectrochemical analysis to the nonmetallic minerals. Orthodox chemical methods, even for constituents occurring as commonly as titania and zirconia, are so long and difficult that there is room for much improvement.

Acknowledgment

The author takes pleasure in acknowledging the help received from his colleague, Alton Gabriel, whose suggestions and discussion during the whole course of this work were of very great help.

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Determination of Sugars in Plant Materials

A Photocolorimetric Method

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FOR several years the method used in this laboratory for the determination of reducing sugars in plant materials has been a combination of the Quisumbing-Thomas (*1*) and the Shaffer-Hartmann (*7*) methods. Cuprous oxide was precipitated according to the former method and determined directly without filtering according to the titration procedure described by the latter. The results have been fair, but the procedure possesses many disadvantages, such as the time and extreme care required for determinations, the number of reagents necessary, and the large amount of plant material low in sugar which must be extracted in order to obtain enough sugar for an analysis.

Several quantitative methods for reducing sugars have been based on the reduction of ferricyanide to ferrocyanide. In Strepkov's method (*8*) for the microdetermination of carbohydrates in plant materials, the excess ferricyanide was determined by an iodometric titration. Hassid (*4*) determined quantitatively the ferrocyanide formed by titration with a standard ceric sulfate solution. In a procedure for the determination of glucose in blood and urine, Hoffman (*5*) made use of the fact that ferricyanide solutions are yellow whereas ferrocyanide solutions are colorless. Glucose was thus estimated by measuring in a photoelectric colorimeter the diminution in yellow color of an excess of ferricyanide. The present author has adapted this method to the determination of reducing sugars in plant materials. The method is rapid and accurate, the procedure is simple, and only one standard solution is necessary. Precise results can be obtained with samples containing from 0.05 to 0.4 mg. of reducing sugars.

Solutions and Apparatus

ALKALINE FERRICYANIDE REAGENT. Potassium ferricyanide (1.8000 grams), purified according to Peters and Van Slyke (*6*), and 40 grams of anhydrous sodium carbonate were made up to 1 liter with distilled water. When kept in an amber-colored bottle and stored in a dark place, this solution remained stable for 3 months. In order to be sure that the solution had not deteriorated, a blank reading on the reagent was made with each series of determinations.

The photoelectric colorimeter used was a Cenco-Sheard-Sanford photelometer equipped with a blue filter and 12-cc. absorption cells.

Description of Method

CALIBRATION OF PHOTOELECTRIC COLORIMETER. Two cubic centimeters of solutions containing from 0 to 0.4 mg. of pure glucose were placed in test tubes or centrifuge tubes marked for 15 cc. Exactly 3 cc. of the alkaline ferricyanide reagent were added to each tube. The tubes were then immersed in boiling water for 5 minutes, cooled under the tap, and diluted to the mark. After mixing the contents of the tubes, the color intensities were determined in the colorimeter set at 100 with distilled water using a blue filter. The microammeter readings were plotted against milligrams of glucose on semilogarithmic paper. This standard curve has been found to be unchanged after 5 months.

PREPARATION OF SAMPLES. An accurately weighed sample of green or quick-dried plant material was extracted with hot 80 per cent alcohol in the usual manner and the alcohol removed by evaporation on a steam bath. Accurately measured volumes of plant juices were heated in a boiling water bath in order to destroy enzymatic activity. The extract or plant juice must be clarified so as to be free of all coloring matter and must be water-clear. The method as outlined by Hassid (*3*) has been found entirely satisfactory by the present author.

PROCEDURE. A sample of plant extract or juice containing 5 to 35 mg. of reducing sugar was evaporated to about 10 cc. on a water bath, cooled, and treated with 5 cc. of a saturated solution of neutral lead acetate. The excess lead was removed by adding

TABLE I. EFFECT OF REAGENTS AND CLARIFICATION

	10-Mg. Sample			20-Mg. Sample			30-Mg. Sample		
Replication	1	2	3	1	2	3	1	2	3
Colorimeter reading	48.1 48.0	48.0 48.0	48.0 48.0	53.5 53.5	53.7 53.5	53.5 53.7	60.7 60.8	60.8 60.8	60.8 60.5
Glucose recovered, mg.	10.1	10.0	10.0	19.7	19.9	19.9	29.8	29.8	29.6
Glucose recovered, %	101	100	100	98.5	99.5	99.5	99.3	99.3	98.9

10 cc. of a saturated disodium phosphate solution. After the addition of about 0.3 gram of Norite decolorizing charcoal, the mixture was allowed to stand with frequent shaking for 30 minutes, and was then poured onto a Büchner funnel provided with a thin layer of talc as described by Hassid (*3*). The original container and funnel were washed several times with a small volume of distilled water and the filtrate was transferred to a 100- or 200-cc. volumetric flask. An aliquot of not more than 2 cc. containing 0.1 to 0.35 mg. of glucose was transferred to a 15-cc. centrifuge tube, diluted to 2 cc., and treated as described above for the standard glucose solutions. After the photoelectric colorimeter reading was obtained, the weight in milligrams of glucose in the aliquot was read directly from the calibration curve.

In order to determine total sugars, aliquots of 50 cc. of clarified extract were placed in 100-cc. volumetric flasks. The solutions were brought to the acid color of methyl red with dilute acetic acid. The quantity of acid necessary was determined on a separate 5- or 10-cc. aliquot. Two to four drops of a 1 per cent solution of Wallerstein invertase scales were added and the solutions allowed to stand overnight at room temperature. A blank on the invertase solution was run simultaneously. The flasks were then diluted to volume and aliquots taken for the determination of reducing sugars as described above.

Experimental Results

In order to determine whether there were any loss during clarification and any interference by the reagents, solutions containing 10, 20, and 30 mg. of glucose were placed in three Erlenmeyer flasks and diluted to 10 cc. These were carried through the clarification process in triplicate and diluted to 200 cc., using 2-cc. aliquots for determinations. The results in Table I indicate no loss by clarification and no interference by the reagents used, and show a close agreement between replicate determinations over the range of the procedure.

Table II shows the effect of variations from the 2-cc. dilution and 5-minute heating time as called for in the procedure, using 0.15 mg. of glucose. These figures indicate a very slight increase in the amount of ferrocyanide formed when the dilution is reduced to 1 cc. No significant increase resulted in a longer heating period. This table also indicates that the colors are stable for at least 30 minutes but have increased after standing 2 hours.

Six plant materials were analyzed for reducing sugar and total sugar by the photocolorimetric and Quisumbing-Thomas methods. The results expressed as glucose are recorded in Table III. The photocolorimetric method gave values from 0 to 6.20 per cent higher than the volumetric method.

Table II. Effect of Variations in Procedure

Variation from Procedure	Colorimeter Reading			Av.
No variation	50.5	50.5	50.7	50.6
Same as above after standing 30 minutes	50.5	50.5	50.5	50.5
Same as above after standing 2 hours	49.7	49.5	49.7	49.6
1-cc. dilution, heated 5 minutes	50.8	51.0	50.8	50.9
5-cc. dilution, heated 5 minutes	50.7	50.7	50.5	50.6
2-cc. dilution, heated 10 minutes	50.7	50.8	50.5	50.7

Table III. Comparison of Photocolorimetric with Quisumbing-Thomas Method

Material	Before Inversion: Glucose in Dry Material			After Inversion: Glucose in Dry Material		
	Colorimetric	Volumetric	Difference	Colorimetric	Volumetric	Difference
	%	%	%	%	%	%
Dallis grass	2.74	2.58	6.20	7.54	7.35	2.58
Cabbage	39.3	37.9	3.69	39.6	37.4	5.88
Peas	0.89	0.89	0.00	22.8	22.5	1.33
Corn leaves	2.34	2.31	1.30	2.81	2.70	4.07
Celery	2.31	2.22	4.05	2.35	2.30	2.17
Orange juice[a]	3.60	3.58	0.56	7.60	7.44	2.15

[a] Recorded as per cent of glucose in original juice.

Table IV. Recovery of Pure Glucose Added to 1 Gram of Dallis Grass

Initial Glucose Content	Glucose Added	Glucose Found	Glucose Recovered	
Mg.	*Mg.*	*Mg.*	*Mg.*	%
18.1	10	28.2	10.1	101
18.1	10	28.0	9.9	99
24.0	10	33.8	9.8	98
24.0	10	34.0	10.0	100
24.0	10	33.8	9.8	98

Table V. Recovery of Pure Sucrose Added to 1 Gram of Dallis Grass

Initial Sucrose Content	Sucrose Added	Sucrose Found	Sucrose Recovered	
Mg.	*Mg.*	*Mg.*	*Mg.*	%
19.0	90	108.7	89.7	99.7
19.0	90	107.1	88.1	97.9
38.8	20	58.4	19.6	98.0
38.8	20	59.0	20.2	101.0
38.8	20	58.6	19.8	99.0

Several determinations by the photocolorimetric method were carried out in order to ascertain the recovery of added glucose and sucrose from 1-gram samples of Dallis grass. These results are recorded in Tables IV and V. All reducing values are recorded as glucose. The sucrose was hydrolyzed and determined along with the glucose as total sugar. The sucrose recovery was calculated from the total sugar and reducing sugar by using the factor 0.97 (*2*). There was good recovery of both glucose and sucrose.

This method has also been used successfully for the determination of starch and hemi-cellulose in plant materials, the starch or hemi-cellulose being hydrolyzed to reducing sugars and treated as described in the procedure for sugars.

Summary

A rapid and accurate photocolorimetric method for the determination of sugars in plant materials is described. The procedure is simple and only one standard solution is required. The clarification process and reagents do not influence the color as read in the colorimeter. Slight variations in the procedure cause no appreciable differences in the results.

Results obtained on plant extracts and fruit juice by the photocolorimetric method compare favorably with those obtained by the Quisumbing-Thomas method, the results being from 0 to 6.20 per cent higher by the former. The method gives good recovery of glucose and sucrose added to plant material.

Acknowledgment

The author wishes to thank J. R. Neller for his helpful suggestions and advice on this work.

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A Rapid Potentiometric Method for Determination of Sulfate

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WITH the recent applications of vacuum-tube technic to potentiometric work, a number of new devices have been developed for analytical work. These setups are particularly suited for routine control, in that they are rapid, compact, and easy to operate. Such instruments as the electron beam spectrometer can be operated under conditions in which the change in potential may be as small as 50 millivolts. The use of this technic, being limited to those reactions which can be determined electrometrically, has stimulated considerable research for new indicator electrodes and potentiometric methods. It is apparent that any means by which such common ions as sulfate, nitrate, sulfite, etc., could be estimated electrometrically would greatly enhance the usefulness of these instruments.

Because of the great need (especially in control work) for a rapid method of determining sulfate ion, the electrometric possibilities of solving this problem were studied. As far as the authors were able to ascertain from the literature, there were no satisfactory methods for such a determination (*1*). Muller and Wertheim (*3*) describe an indirect method using the ferri-ferrocyanide system, but this, according to Kolthoff, is not convenient for practical use (*2*).

Preliminary experiments on the precipitation of the sulfate ion in the presence of such ions as persulfate had indicated that there was a slight change in the electrode potential at the equivalence point. This striking behavior seemed worthy of further investigation. Since barium persulfate is fairly soluble, attempts were made to adapt the persulfate ion to an electrometric determination of the sulfate ion.

Experimental

The usual apparatus used in the classical method of potentiometric titrations was employed in this work. A shiny platinum electrode was used in conjunction with a calomel half cell.

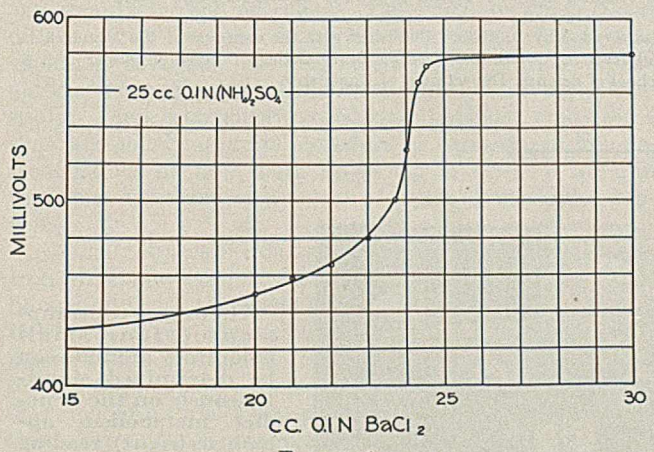

FIGURE 1

Initial experiments were carried out in an aqueous medium, using 0.1 *N* solutions. To 25 ml. of the sulfate solution a trace of persulfate was added and this in turn was titrated with 0.1 *N* barium chloride. The procedure was analogous to that employed when using a ferri-ferrocyanide electrode. These preliminary experiments indicated that the break at the end point of the reaction, though definite, was neither large nor very sharp. In Figure 1 is shown a typical titration curve.

One of the factors which appeared to affect the magnitude of the break at the end point was the quantity of persulfate present. The best results were obtained when the amount of persulfate used was small (1 mg. or less). When larger charges of persulfate were employed, the break did not appear to be quite so sharp.

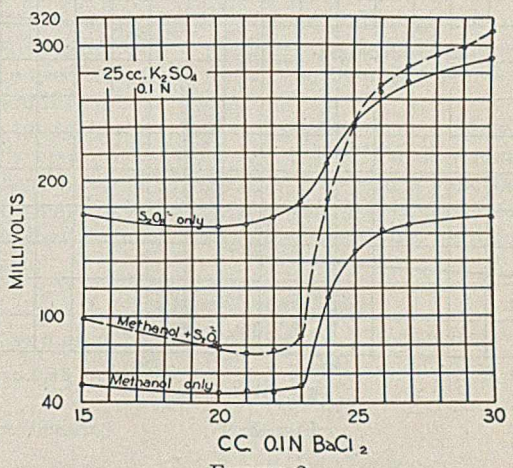

FIGURE 2

Once having established the fact that a change of electrode potential did occur in the vicinity of the stoichiometrical equivalence point, experiments to determine the effect of other factors were instituted. It was evident that a sharper break at the end point than shown in the preliminary tests would be desirable if this behavior was to be adapted to analytical purposes.

Effect of Solvents

It is well known that the addition of alcohol has a stabilizing effect on many electrode potentials. In the attempt to improve the character of the sulfate titration curves, experiments were conducted using aqueous solutions of acetone, ethanol, and methanol. Although all three solvents gave better results, the behavior of methanol solutions was particularly striking in that a relatively large break was observed, while the potential (in the vicinity of the end point) came to equilibrium in a few minutes. Further experimentation established the fact that a break at the end point could be obtained when methanol was used in the absence of persulfate. It appeared that the effect of alcohol was additive to that of persulfate, resulting in a much larger break when both were employed simultaneously (Figure 2).

The best results with alcohol were obtained when the initial concentration of the methanol was in the range of 25 to 60 per cent. The sulfate concentrations which gave the best results were within the limits of 0.05 to 0.25 *N*. In the more dilute solutions the break was too small for analytical pur-

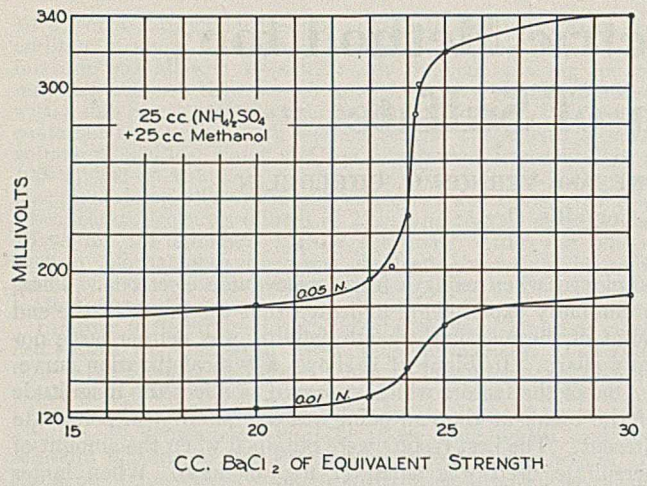

FIGURE 3

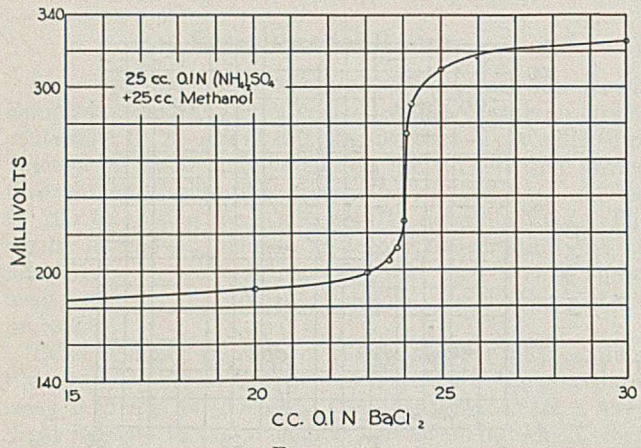

FIGURE 4

poses. These data are given graphically in Figure 3. When the concentration exceeded 0.25 *N* the precipitation was too heavy to give best results.

Although the solutions were carefully prepared and the data were always reproducible, the end point as shown by the titration curves did not occur at the stoichiometrical equivalence point but at that point where approximately 95 per cent of the sulfate had been precipitated (Figure 4). The authors were unable to account for this behavior.

Since the potential even in alcoholic solutions had a tendency to drift, the procedure for carrying out the titration was

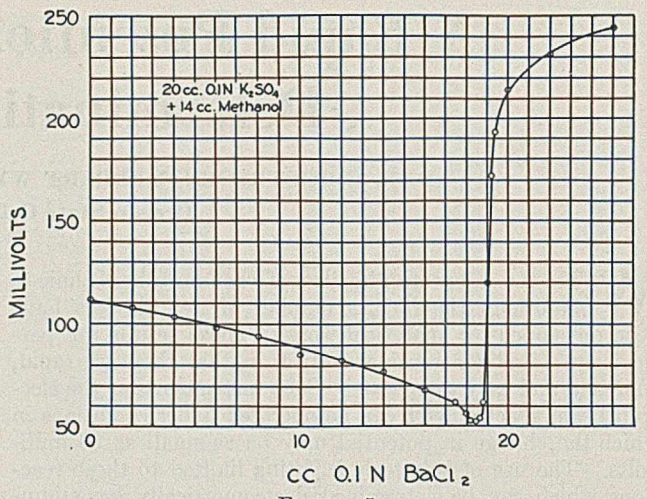

FIGURE 5

standardized as follows: The titration was carried out in the usual manner except in the matter of time. Instead of waiting for equilibrium, the e. m. f. was immediately determined and a minute later it was redetermined. In no case were more than 2 minutes allowed to elapse between each addition of barium chloride. Although the curves (obtained by plotting the potential after equal time intervals) were not as regular, considerable time was saved and the same results were attained in the end (Figure 5).

Interfering Ions

In order to determine other limitations of this method, the effect of foreign ions on the potential was studied. The presence of sulfite, sulfide, and thiosulfate ions completely eliminates the break in potential at the equivalence point. However, this should not detract from the value of the procedure, since these ions can readily be eliminated from solution. The chloride and nitrate ions have no apparent effect on the titration curve other than to alter slightly the position of the maximum $\Delta E/\Delta c$. Very poor results are also obtained when the titration is performed in distinctly acid or basic solutions.

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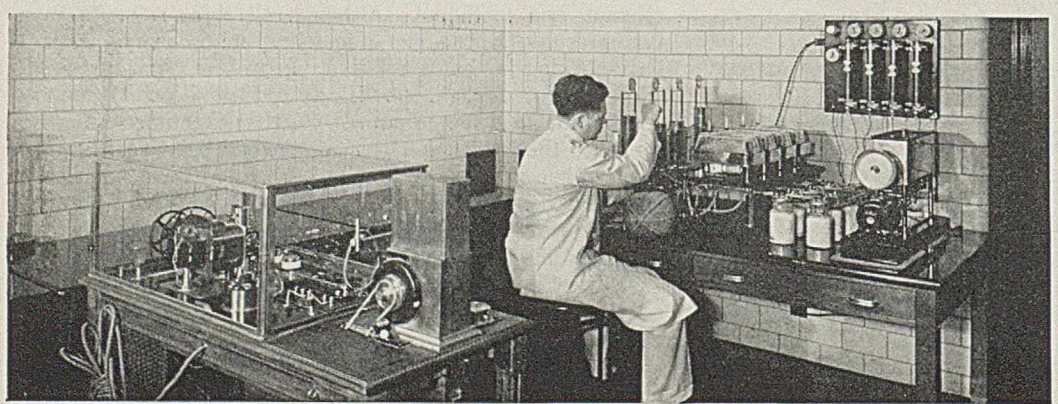

The oxygen consumption of four small laboratory animals can be determined simultaneously on the Benedict metabolism apparatus (*right*), reading directly on the four small gasometers in front of the operator. Electrocardiograph in the left foreground.

Courtesy, Lilly Research Laboratories

An Aspiration Method in Determining Ammonia and Other Volatile Gases

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ASPIRATION methods have been described for the microdetermination of ammonia and other volatile gases, but they appear not to have been adopted widely by the profession as a whole. This is the more surprising in view of the considerable advantages of aspiration over straight distillation, and the well-known disagreeable tendency of the latter to give trouble by bumping.

A step in the right direction has been made by Green (*1*) who has proposed distillation and agitation by steam. In this method the steam has a triple function: it heats, it prevents bumping by stirring, and it sweeps out the escaping gases. While steam is available at some points in most laboratories, yet there are times and places where it is not convenient to use it. For instance, the author wished to include the determination of nitrogen as part of the regular laboratory work in large classes, and it was out of the question to set up a sufficient number of stills, since steam was not piped to the desks in the analytical laboratory. However, suction was available, and it was found possible to determine nitrogen very quickly, simply, and accurately by aspiration.

The sample may be contained in either a Kjeldahl or an Erlenmeyer flask, fitted with a thistle tube which reaches the bottom and an outlet tube leading to a petticoat bubbler in an absorption bottle, preferably of tall narrow form; an outlet tube from the latter leads directly to the suction line. It was determined by experiment that no entrainment of the reagents occurred from either of the flasks, even with vigorous aspiration; at the same time the absorption of the ammonia, or other gas, is complete when an efficient bubbler is used.

The gas may be liberated by addition of the reagent through the thistle tube while aspiration is in progress, with no danger of loss. Some heat must be applied, since quantitative removal of the gas is very slow at room temperature; it may be supplied by direct heating or by a water bath. The process is complete in from 10 to 30 minutes, depending upon the volume and the rapidity of heating. In the case of ammonia, the gas may be absorbed in a small excess of standard acid, and titrated with standard base, using methyl red as indicator. The aspiration method has a further advantage over straight distillation in that little dilution of the absorbent occurs. No water-cooled condenser is necessary, although one may be used if desired. Only a small excess of the liberating agent need be used and, since there is no bumping, practically no attention is required.

The time necessary for the complete removal of ammonia depends upon the rapidity of heating. Small volumes and rapid heating are of advantage where speed is important; on the other hand, a water or steam bath is to be preferred where it is desired to run a series of analyses with a minimum of attention.

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RECEIVED April 27, 1938.

Direct Determination of Iron in Malt Beverages

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IT IS KNOWN that relatively small amounts of iron impair the colloidal stability of beer and cause development of abnormal tastes and off colors. The extent to which traces of iron, as well as other metallic elements, may function as oxidation catalysts has been under consideration in the authors' laboratories for some time and is receiving special study. There is obviously a need for a rapid and, at the same time, adequately accurate procedure for carrying out iron determinations on beer, both as an aid in research work in these directions and as a means of regularly scheduled control.

Normally, traces of iron are present in practically all beers. Iron which may occur in water and brewing materials, if not filtered out with the spent grains in the mash tub, appears to be very largely eliminated with the coagulum which forms during kettle operations. Nevertheless, traces remain and may be augmented by subsequent contact with iron surfaces. The amounts of iron found are usually 0.5 part per million or less. Yet amounts even slightly in excess of this concentration have been known to result in the development of a bitter taste, gradual acquisition of a dark color, loss of brilliance, and chill-haze development. It is also believed that, as a result of its catalytic influence on oxidation, the shelf life of packaged beer is definitely shortened.

Usual procedures for determining iron in beer, based on classical methods, call for a rather lengthy, involved treatment to reduce the sample to a form suitable for analysis. Methods generally applicable may be found in reference books (*4*, *6*, *7*).

Siebenberg and Hubbard (*5*) adapted the ferrocyanide method for use in beers. This method calls for evaporating and ashing, dissolving the ash in hydrochloric acid, precipitating copper, lead, and tin with hydrogen sulfide, removing hydrogen sulfide, oxidizing the iron, a phosphate separation to separate the iron from any nickel, and finally dissolving the iron-containing precipitate and determining the iron colorimetrically with ferrocyanide solution. Aside from the length of time involved, it is evident that opportunity for loss or contamination, representing unknown sources of error, may be presented by the number of manipulations involved in such a procedure.

In the method as developed by the authors, it has been found possible to determine the iron in beer quickly and directly without having to ash the beer or to subject it to any other preliminary treatment. In contrast with the considerable time required for existing methods, results are obtainable within 45 minutes.

The reagent utilized in this procedure is 2,2′-bipyridine (α,α'-dipyridyl). Hill (*2*) investigated and described its use for the determination of iron and reported on its applicability

for determining iron in various biological materials. While Bode (*1*) investigated the use of this reagent for determining iron in beer, his method requires a lengthy digestion with sulfuric acid and hydrogen peroxide for the purpose of destroying organic matter and hence offers no advantage over the other methods described. Hill's paper may be referred to for a bibliography of methods for preparing the reagent. However, 2,2′-bipyridine is now available through regular chemical supply houses, so that the need for the tedious preparation and purification of this compound is eliminated.

Table I. Effect of Sodium Hydrosulfite

Iron Added	Iron Found: On reduction with sodium hydrosulfite	Iron Found: Without sodium hydrosulfire
P. p. m.	*P. p. m.*	*P. p. m.*
None	0.1	0.1
1.0	1.1	1.1
2.0	2.1	2.1
3.0	3.1	3.0
4.0	...	4.2

This reagent reacts with ferrous iron to give an intense red coloration. Ferric iron does not give this color and it is usually necessary, in employing this reagent, to reduce the iron to the ferrous state before applying the test. Hill originally used iron-free sodium hydrosulfite for this purpose. Recently, Kohler, Elvehjem, and Hart (*3*) suggested the use of hydroquinone, as easier to purify and superior to hydrosulfite as a reducing agent for the purpose. However, the authors' work shows that iron in beer exists in the ferrous condition, and that no preliminary reduction is necessary in carrying out routine tests by this procedure. Within the limits of error of visual matching, the intensity of color is found to be the same whether reduction with hydrosulfite has or has not been employed (Table I).

For the amounts of iron usually encountered in beer, the color intensity of the test solutions ranges between a faint orange and a deep reddish orange. The orange shade is due to a mixture of the yellow color of the beer itself with the red color of the iron complex.

Table II. Analysis of Beers

Sample	Iron Added	Iron Found: 2,2′-Bipyridine method	Iron Found: Siebenberg-Hubbard ferrocyanide method	Iron Found: Thiocyanate[a] method
	P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*
A	None	0.5	0.5	...
A	1.0	1.5	1.4	...
A	3.0	3.5	4.0	...
A	5.0	5.0	5.6	...
B	None	2.5	2.2	...
C	None	1.8	1.5	...
D	None	1.9	1.9	...
E	None	1.7	2.0	...
F	None	1.4	1.5	...
G	None	0.5	...	0.4
H	None	2.5	...	2.5
I	None	3.5	...	3.4
J	None	1.5	...	1.3

[a] This method is widely used and consists in evaporating and ashing the beer, taking up with hydrochloric acid, heating to hydrolyze any pyrophosphates, oxidizing with $KMnO_4$, and then adding thiocyanate.

The method has been worked out for practical use and requires no special photometers or other complicated color-matching equipment. Results are generally satisfactory for ordinary control work. However, for work requiring a higher degree of precision than that offered by simple visual matching of color, the method may be easily adapted to the more precise visual or photoelectric photometers. In matching the colors of the sample with standards, use is made of the Walpole effect whereby the tube containing the standard is viewed through a tube containing untreated sample in order to compensate for the beer color. A simple block comparator is used, similar to those used for colorimetric pH determinations.

Method

Apparatus Required. Uniform test tubes graduated at 10 ml. and a block comparator with six holes. The tubes and comparator are similar to those commonly used in pH work.

Reagent. To 100 mg. of 2,2′-bipyridine add 2 ml. of acetic acid (1 + 2). Stir and dilute to 50 ml. with distilled water.

Standard Iron Solution. Weigh out 3.512 grams of ferrous ammonium sulfate hexahydrate (Mohr's salt), dissolve in distilled water, add 2 drops of hydrochloric acid, and dilute to 500 ml. with water. Dilute 10 ml. of this solution to 1 liter. This final diluted solution contains 0.01 mg. of iron per ml. One milliliter of this solution in 10 ml. is equivalent to one part per million of iron. This solution is used to prepare the permanent standards and should be made fresh before use.

Sodium Hydrosulfite Solution. Prepare immediately before use a small quantity of an approximately 2 per cent solution from iron-free sodium hydrosulfite.

Preparation of Permanent Standards. Pipet the desired quantity of standard iron solution into the test tube, add 0.5 ml. of the sodium hydrosulfite solution, and make volume to 10 ml. with distilled water. Add 0.5 ml. of the reagent and mix. Cork the test tube and seal with paraffin. A convenient series of standards for beer may be prepared to contain 0.00 to 0.05 mg. of iron in steps of 0.005 mg. (0 to 5 parts per million in steps of 0.5 part per million). These standards will keep for months in the dark.

Method. Place 10 ml. of degassed beer in each of three test tubes. Add 0.5 ml. of the 2,2′-bipyridine reagent to one of the tubes and mix. Heat in a water bath at 70° C. for 30 minutes to develop the color. At the end of this time, compare the color with the permanent iron standards in the block comparator, arranging the tubes as shown in Figure 1.

Accuracy and Recovery

A beer of low iron content was selected and small amounts of iron, as indicated in Table II, were added. The resulting beers were then analyzed by the above method and also by the procedure of Siebenberg and Hubbard with the excellent recovery noted. In Table II also appear results by the thiocyanate method, showing the agreement to be expected with the existing procedures.

Possible Interferences

In order to check up on the extent to which small amounts of other metallic elements would be capable of interfering with the accuracy of the procedure, three beers were prepared containing, respectively, 1, 3, and 5 parts per million of iron. Each was divided into separate portions and to each portion were then added 5 parts per million of aluminum, chromium, cobalt, copper, lead, manganese, nickel, tin, and zinc. One hundred per cent recovery was observed in all cases within experimental error.

Since the amounts of the different metals used in this test far exceed amounts which are normally to be encountered, the procedure is demonstrated to be entirely satisfactory from the standpoint of possible interferences under usual conditions.

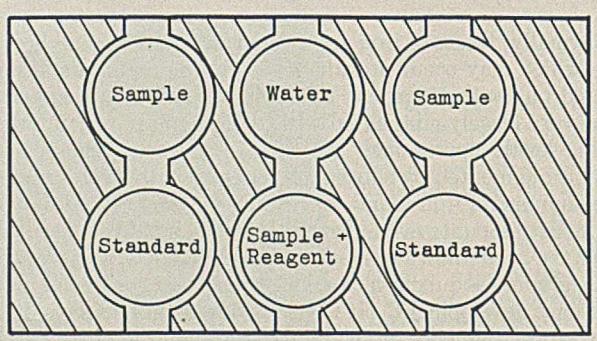

Figure 1. Cross Section of Block Comparator Showing Arrangement of Tubes

In connection with studies which resulted in the development of this method, tests were also carried out on the applicability of other iron reagents and included 1,10-phenanthroline. This is an oxidation-reduction indicator, forming a colored iron complex, and is structurally similar to 2,2′-bipyridine. The results obtainable were similar to those found for 2,2′-bipyridine, though 2,2′-bipyridine is to be preferred. The color with 1,10-phenanthroline tends toward the orange rather than the red, making visual comparisons much less sensitive than with the 2,2′-bipyridine. Moreover, this reagent was more susceptible to interferences by traces of certain metals, particularly cobalt, nickel, and copper.

Over a period of more than 3 years, many hundreds of samples of beer, ales, and the like have been subjected to the above iron test in the authors' laboratories, with entirely satisfactory results. It has the special advantage, by reason of the rapidity with which results may be secured, that the iron content of beer in process, during storage, and in the finished form, may be regularly and carefully controlled. Such regular control is impracticable with the more time-consuming methods. By means of such regularity of control, it has been possible to discern danger signals far ahead of actual trouble and to take necessary remedial steps.

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A Precise Method for the Determination of Carotene in Forage

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A REPORT of recent methods for the determination of carotene in forage is given by Munsey (*2*) and his referees. These methods give consistent results only when special precaution is taken. The Guilbert method (*1*) has been used in this laboratory for some time, but the use of ethyl ether in the extraction of the carotene from the saponified mixture is objectionable. Peterson and Hughes' (*3*) modification of Guilbert's method eliminates the use of ethyl ether and extracts the carotene directly from the saponified mixture with petroleum ether, forming emulsions which result in a loss of carotene.

During the past year a modification of this method (*1, 3*) has been used in the authors' laboratory; it permits the use of definite quantities of reagents, avoids the formation of emulsions, and gives more precise results.

Procedure

Reflux a 5- to 10-gram sample 40 minutes with 200 cc. of ethyl alcohol (approximately 95 to 97 per cent). Filter the hot alcoholic solution through a No. 31 Whatman paper placed in a Büchner funnel, and wash the residue with hot ethyl alcohol until the alcoholic filtrate comes through clear (150 cc. of hot alcohol are usually sufficient). Make the alcoholic filtrate up to 400-cc. volume, transfer one-half to a 250-cc. volumetric flask, and add 25 cc. of 10 per cent alcoholic potash. Shake and let the alkaline alcohol solution stand for 2 hours at room temperature to ensure complete saponification, or place the flask containing the alkaline alcohol solution in hot water for 0.5 hour at 80° C. to hasten saponification; cool and make up to volume. Transfer 25 cc. of the saponified alcoholic carotene solution to a 100-cc. separatory funnel. Add 15 cc. of petroleum ether (b. p. 40–60°) and shake the alcohol and petroleum ether vigorously. Add 7 cc. of water to the contents in the separatory funnel and again shake vigorously. Drain off the alcoholic solution into a similar separatory funnel and extract twice more with 10 cc. of petroleum ether. The last extraction of the petroleum ether will be colored.

Tests have shown that the carotene is almost completely removed with the first extraction of petroleum ether. Combine the petroleum ether extracts and wash gently with 25-cc. portions of distilled water until the wash water no longer gives a color with phenolphthalein. Extract the xanthophyll from the petroleum ether solution with 25-cc. portions of 85 per cent methyl alcohol until the alcohol is colorless. For the first extraction with 85 per cent methyl alcohol, pour the alcohol gently down the sides of the separatory funnel so as not to disturb the small amount of water left in the bottom of the separatory funnel. Drain off approximately 5 cc. of the alcohol and water and then shake the remaining alcohol and petroleum ether gently. Subsequent extractions with 25 cc. of 85 per cent methyl alcohol can be shaken more vigorously without danger of forming emulsions. Finally, extract once or twice more with 25 cc. of 90 per cent methyl alcohol. Filter the petroleum ether–carotene solution through anhydrous sodium sulfate, which is placed over a cotton plug in the stem of a funnel, into a 50-cc. volumetric flask, and make up to volume with petroleum ether. Compare the carotene solution against the standard dye solution as described by Guilbert (*1*) or determine the carotene spectrophotometrically.

Smaller quantities of alcohol can be used for the extraction of carotene from the forage if the size of the sample is decreased. Reflux a 1- to 2-gram sample with 50 cc. of alcohol, filter, and wash the residue with small portions of hot alcohol through a No. 3 fritted-glass crucible directly into a 100-cc. volumetric flask containing 5 cc. of 20 per cent potassium hydroxide–alcohol solution under a bell jar. Proceed as described above.

Results and Discussion

Determinations of carotene on commercial dehydrated alfalfa meal by Guilbert's method and the modified method are shown in Table I. More carotene is recovered by the modified method. The difference of the two methods may be due to the loss of carotene during the evaporation of the ethyl ether from the carotene. Results of duplicate analyses of

Table I. Determination of Carotene in Dehydrated Alfalfa by the Guilbert and Modified Methods

Sample No.	Guilbert Method	Modified Method
	Mg./100 g.	*Mg./100 g.*
1	7.6	8.3
2	7.8	8.6
3	16.0	16.5
4	6.7	8.1
5	7.1	8.5

carotene in dehydrated alfalfa with a carotene content of 10 to 20 mg. per 100 grams are reproducible within 0.1 mg. per 100 grams when the carotene content is determined photoelectric-spectrophotometrically, using Peterson and Hughes' (3) extinction coefficient for β-carotene.

To determine whether the extraction of carotene was complete with 95 per cent alcohol, the residue from the alcoholic extract was analyzed for carotene by the Guilbert and Peterson-Hughes methods. No measurable amount of carotene was found in the residue.

The use of 95 per cent alcohol permits rapid filtering, washing of the alcohol-carotene solution from the forage residue, and the use of definite quantities of reagents in the extraction of carotene. To prevent the formation of emulsions and to obtain a quantitative extraction of the carotene from the alkaline alcohol solution with petroleum ether, it is important that the concentration of the alcohol be between 70 and 75 per cent. At this concentration the separation of the petroleum ether from the water and alcohol phase is clear cut.

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Determination of Organic Sulfur in Gas

Titration of Sulfate in the Sulfur Lamp with Barium Chloride Using Tetrahydroxyquinone as an Indicator

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AN ADAPTATION of the A. S. T. M. (1) method for the determination of sulfur in motor fuels, with which it is possible to determine the concentration of organic sulfur in gas, has been described (3). The speed and convenience of this method, as well as its accuracy, have proved of great value, and it is now extensively used.

In this procedure, the gas is burned, and the sulfur dioxide formed by the combustion of sulfur compounds in the gas is absorbed in standard sodium carbonate solution. The excess sodium carbonate is titrated with standard hydrochloric acid, and the sulfur concentration is calculated from the amount of carbonate used and the volume of gas burned. Thus, the sulfur is determined through the acidic character of the sulfur dioxide formed.

In some cases this has been found objectionable, since any other constituent of an acid character absorbed by the carbonate will be determined as sulfur, and the result will be too high. The sulfur can be determined gravitrically as barium sulfate, but at a sacrifice in time and convenience.

The present paper describes an adaptation of a procedure for the direct titration of sulfates with barium chloride which may be used in conjunction with the sulfur lamp method (3). Titration of sulfates with standard barium chloride solution using tetrahydroxyquinone as an indicator has been discussed by Sheen and Kahler (2). Such a procedure will overcome the objection noted to the acidimetric titration which has been used heretofore with the sulfur lamp, yet retain the speed and convenience of the sulfur lamp and volumetric determinations. The accuracy obtainable by the barium chloride titration has been tested, and the results of the experiments are presented here. While the discussion is confined to the determination of organic sulfur in gas, obvious changes in apparatus will make the procedure applicable to the determination of sulfur in motor fuels (1) with an accuracy comparable to that shown for gas.

Materials and Reagents

Standard barium chloride solution, 1 ml. = 1 mg. of sulfur (7.634 grams of $BaCl_2 \cdot 2H_2O$ per liter). Standardize gravimetrically by precipitation as barium sulfate.

Sodium carbonate solution, containing 3.306 grams of the anhydrous salt per liter.

Hydrochloric acid, containing 2.275 grams of hydrochloric acid per liter. This and the sodium carbonate solution are the same as used in the acidimetric determination of organic sulfur. It is convenient to use the strength noted, although exact standardization is not necessary.

Methyl orange indicator solution.

Tetrahydroxyquinone indicator (THQ, obtained from the W. H. & L. D. Betz Laboratories, Philadelphia, Pa.).

Ethyl alcohol, ethyl alcohol denatured by formula 30 or 3-A, or isopropyl alcohol.

Procedure

For the determination of organic sulfur in gas the following procedure is now proposed.

The metered gas is burned at a rate from 14 to 28 liters (0.5 to 1 cubic foot) per hour, and the products of combustion are absorbed in sodium carbonate solution in the A. S. T. M. sulfur lamp (1, 3). At the conclusion of the test, the lamp is washed down with the smallest possible quantity of distilled water, and 3 drops of methyl orange indicator are added. The solution is neutralized with dilute hydrochloric acid which, if standardized, will give an estimate of the sulfur present. At this point the determination was concluded according to the earlier procedure.

The tan color of the acid methyl orange is discharged with a few drops of the sodium carbonate solution, and 30 ml. of ethyl or isopropyl alcohol are added (2). About 0.22 gram of tetrahydroxyquinone indicator is added, and the solution is mixed well and titrated with standard barium chloride solution. The end point is reached when the color of the solution changes from yellow to red, which is permanent with good mixing.

Calculation of Results

From the total volume of barium chloride used, 0.05 ml. is subtracted for a blank. Concentration of organic sulfur in the gas is then calculated using the following expression:

$$\frac{\text{Ml. of } BaCl_2 \times \text{strength of } BaCl_2 \text{ (in mg. of S per ml.)}}{\text{Cubic feet of gas burned (corrected to N. T. P.)}} \times 1.543 = \text{S concentration in gas in grains per 100 cubic feet}$$

Discussion

In the development of the method, several points required examination. First, it has been reported (*2*) that the CO_3^{--} ion interferes with the determination of sulfate by titration with barium chloride. The excess sodium carbonate in the absorbing solution must, therefore, be completely neutralized. It was proved experimentally that correct results were obtained only when neutralization of the excess carbonate was carried to the methyl orange end point. Neutralization of the solution containing sulfate to the phenolphthalein end point as described by Sheen and Kahler (*2*) is not sufficient when sodium carbonate is used as the absorbing solution. To keep the reddish color of methyl orange in acid solution from interfering with the red barium chloride–tetrahydroxyquinone end point, it is just discharged with 3 or 4 drops of sodium carbonate solution. Since barium chloride is neutral, there is no increase in acidity of the solution to bring back the red methyl orange color; moreover, the addition of the alcohol has a favorable effect in reducing the intensity of the methyl orange color. Numerous experiments have proved that the presence of the methyl orange offers no difficulty in recognizing the red barium chloride–tetrahydroxyquinone color.

It was desirable to perform the whole determination in the sulfur lamp absorber without intermediate transfer of the solution to other vessels. The volumes of solution usually employed in sulfur lamp determinations are larger than the 25 ml. recommended by Sheen and Kahler (*2*), and are variable because of the necessity of washing down the lamp. An effort should be made to keep the volume at a minimum, however, and it seldom need exceed 35 to 40 ml. Under these conditions, 30 ml. of alcohol have been found adequate. Absolute ethyl alcohol and isopropyl alcohol have been found to be interchangeable. It is preferable, because of the larger volume, to add about 0.22 gram of the indicator. A blank of 0.05 ml. of the barium chloride solution was necessary to give a distinct end point, and this quantity should be subtracted from the total standard solution used in a determination.

The red color obtained at the end point was very distinct, and easier to detect than the methyl orange end point in acidimetry.

In a series of preliminary tests of the procedure, sulfur was added to the sodium carbonate absorbing solution as standard sulfuric acid, instead of burning gas containing sulfur compounds. The quantity of acid added was determined acidimetrically by titrating the excess sodium carbonate with standard hydrochloric acid solution and methyl orange indicator, and the sulfate was determined by titration with standard barium chloride solution and tetrahydroxyquinone indicator. The solutions used were of the following strengths:

H_2SO_4: 1 ml. = 0.507 mg. of S (gravimetric standardization)
Na_2CO_3: 1 ml. = 0.994 mg. of S (standardized independently)
$BaCl_2$: 1 ml. = 1.0026 mg. of S (gravimetric standardization)

The experiments were performed in a sulfur lamp absorber according to the procedure outlined above, with the results shown in Table I.

Table I. Comparison of Acidimetric and Barium Chloride–Tetrahydroxyquinone Titrations of Sulfate Solutions

H_2SO_4 Taken	= S Taken	Na_2CO_3 Used	= S Found	Difference	$BaCl_2$ Used	= S Found	Difference
Ml.	*Mg.*	*Ml.*	*Mg.*	*Mg.*	*Ml.*	*Mg.*	*Mg.*
16.00	8.11	8.20	8.14	0.03	8.05	8.02	0.09
10.00	5.07	5.10	5.07	0.00	5.10	5.06	0.01
5.20	2.63	2.61	2.59	0.04	2.60	2.56	0.07
4.00	2.00	2.04	2.03	0.03	2.10	2.06	0.06
			Av.	±0.025			±0.06

From these data, it appears that the barium chloride–tetrahydroxyquinone titration gives results of ample accuracy. The average error of 0.06 mg. of sulfur is of the same magnitude as that found by Sheen and Kahler (*2*) in the determination of sulfur in oil.

Table II. Comparison of Organic Sulfur Concentrations in Gas by Three Procedures

Gas Sample	Sulfur Concentration: Acidimetrically	By $BaCl_2$–THQ	Gravimetrically
	Grains/100 cu. ft.[a]	*Grains/100 cu. ft.*[a]	*Grains/100 cu. ft.*[a]
1	10.7	9.9	10.1
2	12.4	11.9	11.5
3	13.7	13.1	13.0
4	13.3	12.6	12.6
5	13.5	12.5	12.7
6	15.0	14.1	14.0

[a] 1 grain per 100 cu. ft. = 0.0229 gram per cu. m.

As a final check, determinations were made on several gas mixtures containing varying amounts of organic sulfur compounds. Two gas samples were collected simultaneously for each test in two calibrated gas holders. After combustion and absorption of the sulfur dioxide formed in sodium carbonate solution in separate sulfur lamps, the sulfur concentration was determined in each acidimetrically. Then the solution in one absorber was titrated with the standard barium chloride solution, while that in the other was subjected to gravimetric determination of sulfur by precipitation as barium sulfate. The standard sodium carbonate and barium chloride solutions were of the concentrations noted above. About 22.5 liters (0.8 cubic foot) of gas were burned for each separate determination. The concentrations of organic sulfur found in the gas in six tests are summarized in Table II.

Excellent agreement between the results of barium chloride–tetrahydroxyquinone and gravimetric determinations is observed. The accuracy obtainable by the volumetric procedure is well within the limits generally required in these determinations.

The acidimetric determinations on these gas samples gave results on the average 0.018 gram per cu. m. (0.8 grain per 100 cubic feet) too high. Nitrates were found in the absorption solution in quantities which may at least partially account for these differences. The source of the nitrates has not yet been determined.

Summary

The use of standard barium chloride solution for volumetric determination of sulfate with tetrahydroxyquinone indicator is adaptable to the procedure for the determination of the concentration of organic sulfur compounds in gas. Instead of determining the sulfates acidimetrically in the absorption solution contained in the A. S. T. M. sulfur lamp (*3*), the sulfate may be determined directly by titration with barium chloride solution. This modification avoids errors which may arise from the presence of other acidic constituents in the combustion products of the gas, which would be determined as sulfur by the acidimetric titration.

The accuracy of the method is such that the concentration of organic sulfur in gas can be determined to within ±0.0045 gram per cu. m. (±0.2 grain per 100 cubic feet) if approximately 28 liters (1 cubic foot) of gas are burned. This accuracy is as great or greater than that given by any other procedure so far described, and is attained at no sacrifice in speed or convenience.

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A Colorimetric Method for the Determination of Ascorbic Acid

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DURING studies of the diazo reaction in urine (*13*, *14*) it was observed that ascorbic acid reduces certain diazonium salts, but not the azo color formed upon coupling. This decrement in the concentration of the diazonium ion bears a direct relationship to the diminution in the coupling color. Standardization of this relationship affords a simple, accurate colorimetric method for the determination of vitamin C in various media.

To 5.0 cc. of sulfanilamide (5.00 mg. per cent), 1.0 cc. of sodium nitrite (0.050 per cent) and 1.0 cc. of sulfosalicylic acid (20.0 per cent) were added. The solution was allowed to stand 1 to 3 minutes and 1.0 cc. of urea (1.0 per cent) was added. After 5 minutes, 10.0 cc. of a fresh 10 per cent acetic acid solution containing varying concentrations (0.100 to 0.400 mg.) of the vitamin were added. After 5 minutes, 7.4 cc. of 1-dimethylnaphthylamine solution (1.0 cc. diluted to 500 cc. with 95 per cent alcohol) were added and the solution was mixed. After 10 minutes but within 50 minutes the colors developed were compared in a colorimeter with appropriate standards, prepared by diminishing the sulfanilamide concentration and replacing the vitamin solution by vitamin-free 10 per cent acetic acid.

In the standardization work, weighed samples of the crystalline vitamin (Merck & Co., Inc.) were dissolved in 10 per cent acetic acid, and aliquots were diluted to appropriate concentrations. These solutions were prepared fresh for each series of determinations. Fresh nitrite solutions were made up daily, although these solutions are stable for at least 2 to 3 days. Merck's reagent grade sulfosalicylic acid was used, although sulfuric acid appears to be equally satisfactory. The 1-dimethylnaphthylamine (Eastman No. 1063) satisfied Marshall's requirements (*9*) for the use of this reagent in diazotization procedures. Crystalline sulfanilic amide (Winthrop) was used to form the diazonium salt, and double-distilled water was used throughout the standardization work. The blank reagents contained no reducing substances as determined by the 2,6-dichlorophenolindophenol titration of Tillmans (*16*) as modified by Harris and Ray (*5*).

The variation from a strict inverse proportion of sulfanilamide concentration and column length was determined by comparing known solutions in distilled water differing by 100 per cent in sulfanilamide concentration. Each figure in column 3 of Table I represents the average of 9 separate determinations, five or more readings being taken in each determination. The colors produced by the coupling reaction matched excellently, and the average colorimetric readings were within ±0.1 mm. This deviation is equivalent to 0.01, 0.02, and 0.05 mg. per cent sulfanilamide, the higher values being associated with the higher concentrations. These deviations are equivalent to errors of 0.8 to 1.0 per cent for readings differing by 10.0 mm.

TABLE I. COMPARATIVE DETERMINATIONS

Concentration of Standard	Concentration of Unknown	Concentration by Color Comparison	Error (±1) for Readings Differing by 10 Mm.
Mg. %	*Mg. %*	*Mg. %*	*%*
0.625	1.25	1.16	7
		1.17	
1.25	2.50	2.32	7
		2.35	
		2.32	
2.50	5.00	4.85	2
		4.95	

Under the conditions selected, vitamin concentrations of 0.100 mg. in the 10 cc. of 10 per cent acetic acid produced a color decrement of about 7 colorimeter units. At vitamin concentrations of 0.025 mg. no color decrement could be measured, and at 0.050 mg. the decrement was too small to give consistent results. Values were established at intervals of 0.050 mg. of ascorbic acid over the range 0.100 to 0.400 mg. Each figure in column 2 of Table II is an average of 9 separate determinations, five or more readings being taken in each determination. Standards were prepared in the absence of ascorbic acid by diminishing the sulfanilamide concentration. All colors were matched within 1.0 to 4.0 mm. Thus, the errors due to variations from Beer's law are less than 4 per cent. The average in each series of determinations deviated by ±0.1 to 0.2 mm., or ±0.01 to 0.10 mg. per cent sulfanilamide prevented from coupling. The highest deviations in milligrams per cent can occur only at the highest concentrations reduced.

TABLE II. SULFANILAMIDE REDUCED

Weight of Vitamin	Sulfanilamide Reduced		
Mg./10 cc.	*Mg. %*	*Mg.*	*Molecules*
0.100	1.74	0.087	1.15
	1.68	0.084	1.19
	1.67	0.084	1.19
0.150	2.24	0.112	1.34
	2.16	0.108	1.39
0.200	2.63	0.132	1.51
	2.73	0.137	1.46
	2.75	0.138	1.45
	2.76	0.138	1.45
0.250	2.91	0.146	1.71
	2.92	0.146	1.71
	2.93	0.147	1.70
	2.94	0.147	1.70
	2.95	0.148	1.69
0.300	3.27	0.164	1.83
	3.49	0.175	1.71
	3.57	0.179	1.67
	3.60	0.180	1.67
0.350	3.88	0.194	1.80
0.400	4.11	0.206	1.94
	4.13	0.207	1.93
	4.17	0.209	1.91

At concentrations of the vitamin above 0.4 mg., with an initial 10 mg. per cent sulfanilamide, the reduction of the diazonium salt is accompanied by the formation of a yellow color which makes colorimetric comparison difficult. (The formation of this color is discussed below.) The calibration curve of Figure 1 is accordingly restricted to that range of concentrations lying between 0.1 and 0.4 mg., or 0.01 and 0.04 mg. of the vitamin per cubic centimeter of unknown.

The change in slope below 1 mg. per cent of vitamin indicates an exponential graph. Over the desired range, however, the data may be plotted within the experimental error as a straight line described by the simple slope intercept expression $Y = 0.8\,X + 1$. For a series of determinations this line may be plotted and the value read directly, or more simply, a two-line nomograph may be constructed.

It has been shown that nitrites are reduced (*6*) to hydroxylamine (*8*) by ascorbic acid. In the present procedure it is not possible to test for an excess of the nitrite ion by the usual starch-iodide procedure, since the diazonium salt oxidizes the iodide. To lower the effect of possible nitrite oxidation of the vitamin, an excess of urea was added, and the reaction mixture was permitted to stand for 5 minutes. Any nitrite oxidation of the vitamin should produce a trend opposite to that observed (Table II, column 4). Experiments, run in duplicate at increasing vitamin concentrations with and without the urea treatment, showed no differences at concentrations of 0.1 mg. of vitamin. At 0.4 mg., the amount of vitamin oxidized by excess nitrite was within the

experimental error. Consequently, the influence of the nitrite ion cannot be significant.

To explain the nitrogen evolution and the yellow color formation observed by him, Barak (*2*), studying the influence of caustic upon a solution of ascorbic acid and diazotized sulfanilic acid, suggested that the primary alcohol group of the vitamin, functioning as an alcohol, was oxidized to an aldehyde, and the diazonium salt was reduced to a mixture of sodium benzenesulfonate and azobenzene—*p*, *p*″-di-sodium-sulfonate.

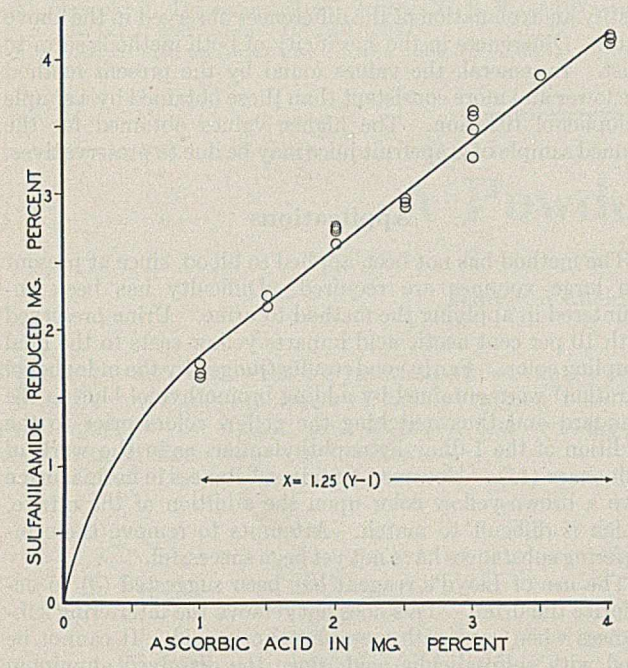


FIGURE 1

In the present study, the number of molecules of ascorbic acid required to reduce one molecule of diazotized sulfanilamide (Figure 2) increases from 1.1 to 1.9 with increasing vitamin concentrations. The reduction process appears to be instantaneous. Although the 10-minute interval required for maximum azo color development prevents an accurate time measurement, allowing periods of 1 to 30 minutes after the addition of the vitamin gave no measurable difference in the extent of diazonium reduction. Prior to the addition of the beta component, no yellow color was formed in 1 to 30 minutes at low vitamin concentration nor within 1 to 15 minutes at high concentrations. These facts suggest the following reaction mechanism wherein $(RN{=}NH)_x$ the common reduction intermediate is entirely hypothetical, R is equivalent to the *p*-sulfamidobenzene radical, and 2H is equivalent to one molecule of the vitamin:

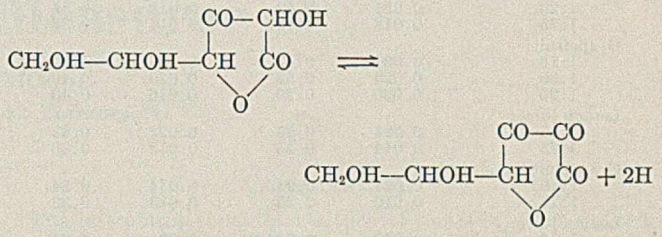
$$R\bar{N}{\equiv}N + 2H \rightarrow (RN{=}NH)_x$$

$$\begin{cases} + R\bar{N}{\equiv}N \rightarrow (RN{=}N{-}N{=}N{-}R) \rightarrow RN{=}NR + N_2 \nearrow & (1) \\ + 2H \rightarrow RNH{-}NH_2 & (2) \end{cases}$$

The absence of alkali diminishes extraneous diazo decomposition, and azobenzene-*p*,*p*′-disulfonamide (*12*), if formed, is insoluble in and imparts no discernible color to acidic solutions, although it forms yellow alkaline solutions. The tangent to the curve of Figure 2 at the origin is essentially 60° with respect to the *x*-axis. Reaction 1 requires a slope of approximately 60°. As the concentration of the vitamin increases this angle is reduced to appreciably below 30°. Reaction 2 requires a slope of slightly less than 30°.

At the concentrations studied nitrogen evolution is imperceptible. Nitrogen is evolved at higher concentrations. Measurements of this resultant would yield further insight into the reaction mechanism. In the absence of these data, and in consideration of the complexity of diazonium reduction reactions, the above mechanism must be considered tentative, since the reduction process may result from a series of concomitant reactions. For example, benzenesulfonamide formation, requiring one molecule of vitamin per molecule of diazonium salt, or interaction of ascorbic acid with the hydrazine, involving three moles of the vitamin per mole of diazonium ion, may occur. The curve of Figure 2 does not appear to be influenced by air oxidation, since solutions of the vitamin are stable for longer periods of time than those used.

The simple reversible reaction indicated is not possible, since there is no time factor involved in the reduction process.



Two series of experiments were run in duplicate at vitamin concentrations of 1 and 4 mg. per cent in the 10 cc. of 10 per cent acetic acid. Increasing periods of time, from 1 to 30 minutes, were allowed to elapse before the addition of the 1-dimethylnaphthylamine. At 1 mg. per cent of the vitamin a slight yellow color was formed within 30 minutes. A similar but deeper color was obtained at 4 mg. per cent. (When no 1-dimethylnaphthylamine was added the yellow color intensified considerably on longer standing.) The decrement in the final coupling color in all sixteen experiments was precisely that obtained in the standard procedure. The formation of the yellow color is therefore due to subsequent reactions.

Borsook *et al.* (*3*) demonstrated that above a pH of 4, dehydroascorbic acid undergoes a nonoxidative, irreversible change, and the initial product was provisionally assigned the structure 2,3-diketo-*l*-gulonic acid. With increasing pH, lactone hydrolysis is followed by a second and third oxidative change. These are not measured in the present method. Borsook observed that 10 mg. per cent of dehydroascorbic acid at a pH of 7 gave rise to a brown-yellow

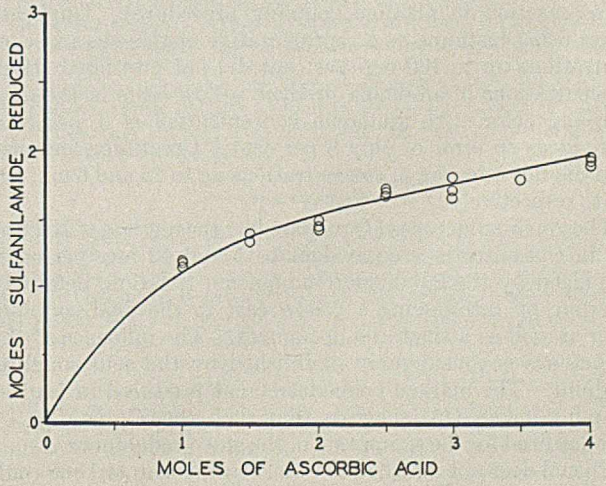


FIGURE 2

color after several hours, and the products responsible for this color did not reduce 2,6-dichlorophenolindophenol. The substances producing the yellow color in the present work can be the same as those observed by Borsook only if lactone hydrolysis of the dehydroascorbic acid can occur at pH values below 4. This does not seem likely from Borsook's work. In accordance with the suggested reaction mechanism, the yellow color may result from hydrazone formation (*11*).

TABLE III. COMPARISON OF METHODS

Sample and Dilution	Indophenol Diluted	Indophenol Undiluted	Diazo Method Diluted	Diazo Method Undiluted
Orange juice:				
1:15	0.035	0.53	0.026	0.39
1:20	0.024	0.48	0.020	0.40
1:25	0.018	0.45	0.015	0.36
Orange juice:				
1:15	0.036	0.54	0.025	0.38
1:20	0.021	0.42	0.018	0.36
1:25	0.018	0.45	0.014	0.36
Grapefruit:				
1:15	0.031	0.47	0.026	0.39
1:20	0.026	0.52	0.020	0.40
1:25	0.020	0.50	0.016	0.40
Lemon juice:				
1:15	0.024	0.36	0.022	0.33
1:25	0.014	0.35	0.012	0.30
Grapefruit, canned:				
1:10	0.024	0.24	0.034	0.34
1:25	0.010	0.25	0.013	0.33
Lemon juice:				
1:10	0.033	0.33	0.036	0.36
1:25	...	..	0.014	0.35
Lemon juice:				
1:25	0.012	0.30	0.014	0.35
After 4–5 hours:				
1:25	...	..	0.014	0.35
Orange juice:				
1:15	0.026	0.39	0.029	0.44
After 4–5 hours:				
1:15	...	..	0.025	0.38
1:25	0.016	0.40	0.017	0.42
After 4–5 hours:				
1:25	...	..	0.015	0.38

As indicated by the work of Martius and von Euler (*10*), cysteine does not interfere in the present method in concentrations up to 10 mg. per cent (given in terms of the concentration of the extraneous reducer in 10 cc. of 10 per cent acetic acid). Borsook has shown that sufficiently high concentrations of glutathione reduce dehydroascorbic acid, that cysteine is one-half as efficient as the tripeptide in this respect, and that this property is characteristic of the thiol group. In this method concentrations of 20 mg. per cent cysteine do not cause variations in the presence of 1 to 4 mg. per cent ascorbic acid.

Barak (*1*) has shown that imidazoles give a pseudo-phenol diazo reaction in alkaline coupling procedures. Duplicate series using histidine as a representative of this class in concentrations up to 100 mg. per cent did not interfere. High concentrations of ammonia produce yellow casts in the final coupling color. An ammonia concentration of 1 per cent introduces an error of only 8 per cent. Creatinine and uric acid did not interfere in concentrations up to 25 and 6 mg. per cent, respectively.

The gluco-reductones (*4*) prepared by the method of Kertesz (*7*) in concentrations equivalent to 5 and 12 mg. per cent, as judged by the 2,6-dichloroindophenol titration, definitely interfere by introducing a yellow cast to the final coupling color as well as a tinctorial decrement. The influence of the reductones is considerably diminished by the acid coupling medium. The marked color decrement produced in neutral coupling media (*13*) suggests that such procedures may be standardized for the estimation of the gluco-reductones.

Phenol does not interfere in concentrations up to 1 per cent nor tyrosine up to 20 mg. per cent. Hydroquinone produces pronounced errors at 5 mg. per cent. The specificity phase of this work has not yet been completely elucidated.

In Table III each figure given for the indophenol method represents the average of 4 to 6 titrations. The data for the diazotization method represent duplicate determinations upon the same fruit juices preserved in 10 per cent acetic acid. Two or three determinations were performed upon the same fruit juice at increasing dilution. Multiplying the concentration by the dilution gives a good indication of the consistency of the method.

The present method is too new, and data are too limited to justify an explanation of the differences observed in the above data. Differences in the specificity of both methods seem to exist. In general, the values found by the present method are lower and more consistent than those obtained by a simple indophenol titration. The higher values obtained for the canned sample of grapefruit juice may be due to preservatives.

Applications

The method has not been applied to blood, since at present too large volumes are required. Difficulty has been encountered in applying the method to urine. Urine preserved with 10 per cent acetic acid imparts yellow casts to the final coupling color. Fairly good results (judged by the indophenol titration) were obtained by adding bromothymol blue to the standard and thus matching the yellow colors prior to the addition of the 1-dimethylnaphthylamine, as in the work of Shinohara (*15*). However, certain substances in normal urine give a brown-yellow color upon the addition of the nitrite, which is difficult to match. Attempts to remove these interfering substances have not yet been successful.

The use of Lloyd's reagent has been suggested (*3*) to decolorize the urine. This does not remove the interfering substances when used with metaphosphoric acid. It cannot be used with sulfosalicylic acid, since the dissolved aluminum forms a red chelated coördination complex of the alizarin S type. Addition of caustic does not precipitate hydrated aluminum oxide from this complex, but changes the color to a greenish yellow. Similar results were obtained with refined silica. Sulfosalicylic acid thus appears to be a delicate reagent in such cases.

Summary

The reduction of diazotized sulfanilamide by ascorbic acid in acidic media has been studied. Stoichiometric relationships suggest that the products are largely phenylhydrazine-*p*-sulfonamide and azobenzene-*p,p'*-disulfonamide. The reduction process is not reversible. The yellow color formed upon long standing is the result of secondary reactions involving the resultants of the initial reduction process.

A quantitative method for the estimation of ascorbic acid has been devised. A calibration curve has been established empirically. From this curve the concentration of ascorbic acid, X, in the unknown may be read directly from the concentration of the diazotized sulfanilamide reduced, Y. That portion of the curve to which the method is applicable may, within the experimental error, be plotted as a straight line satisfying the expression $X = 1.25\,(Y - 1)$.

Specificity studies have shown that cysteine, tyrosine, histidine, creatinine, ammonia, phenol, and uric acid do not interfere. Hydroquinone and the gluco-reductones interfere. This interference is markedly reduced by coupling in acid media.

Analyses of a series of fruit juices gave satisfactory results as compared with the standard 2,6-dichlorophenolindophenol method. Slight differences, apparently due to specificity differences, exist.

Acknowledgment

Thanks are due Solomon Weintraub, pathologist, and Jesse G. M. Bullowa, visiting physician, for their kind interest in this work. The assistance of Harold W. Grossman and Sidney Recht is greatly appreciated. This study was in part supported by the Metropolitan Life Insurance Company and the Baruch gift.

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A Powder Measurer

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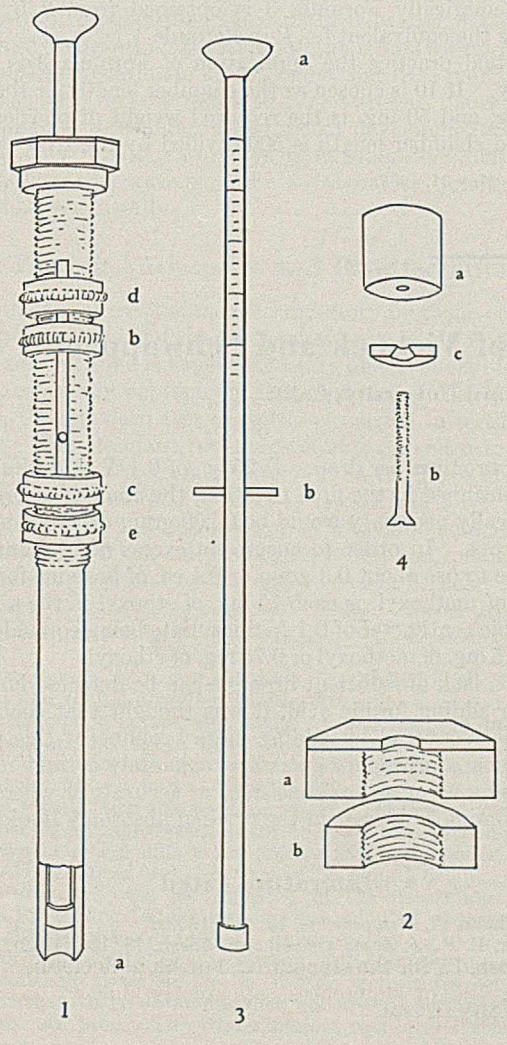
THE powder measurer is designed to measure, rapidly and fairly accurately, powders of widely varying bulk in small amounts by weight, and fill them into cylindrical vials of small diameter or other containers.



Construction

The instrument consists of a tube of metal or other suitable material about 12.5 cm. long and 5 mm. in inside diameter, and a plunger bearing at its lower end a cork washer tightly fitting the lumen of the tube. The lower end of the tube (Figure 1) is cut off at right angles and abruptly tapered on the outside with a rounded bevel to a sharp edge on the inside, *a*. The upper part of the tube is provided with a double slot about 3.5 cm. long and 2 mm. wide, extending downward from a point about 2.5 cm. below the top. The outside of the tube is threaded through its upper 6.5 cm. and provided with two check collars, *b*, *c*, and their locking nuts, *d*, *e*. The top of the tube is provided with a hexagonal cap, *a* (Figure 2), perforated with a central hole snugly fitting the shaft of the plunger, and its lock nut, *b*, threaded to fit the outside of the tube. The lower 6 cm. of the tube is thinned to a wall thickness of about 1 mm. The outer surface of this part of the tube is highly polished, as is also its entire inner surface.

The plunger (Figure 3) consists of a shaft of metal or other suitable material, about 1.5 cm. longer than the tube, and of slightly smaller diameter than its lumen. The upper end is provided with a cap or thumb rest, *a*, about 1 cm. in diameter.

The shaft is drilled and provided with a removable pin (the check pin, *b*), its two ends projecting so as to fit into the slot of the tube when the plunger is in place. When the end of the plunger is flush with the lower end of the tube the check pin is 1 or 2 mm. from the bottom of the slot. The upper part of the shaft is provided with a millimeter scale reading downward from a 0 mark—the point on the shaft opposite the top of the hexagonal cap when the end of the plunger is flush with the end of the tube—and so can be used to measure the variable length of the chamber at the end of the tube for all positions of the plunger.

The lower end of the shaft (Figure 4) is fitted with a perforated cork washer, *a*, which tightly fits inside the tube and is secured to the shaft by a bolt, *b*, and metal washer, *c*. The latter is about 4 mm. in diameter, just narrow enough to clear the walls of the tube yet broad enough to lend sufficient support to the cork. It is drilled and reamed to countersink the head of the bolt and make it flush with the washer.

Operation

The plunger, with its check pin removed, is inserted in the tube and thrust downward until the lower metal washer is flush with the lower end of the tube. Keeping the plunger in this position, the check pin is passed through the slot and forced into its hole in the shaft of the plunger, and the lower check collar is raised to contact the check pin and locked in position. The plunger is next drawn up to indicate the required reading on the scale, and the upper check collar is screwed down to contact the check pin and locked in position. The instrument is then ready for use with all powders of the same bulkiness or specific powder number. The filler is thrust through the powder held in a narrow cylindrical jar, and firmly pressed against the bottom three or four times or until the chamber of the tube is completely filled.

The powder is then ejected into the required container by forcing down the plunger. In this way the instrument can be made to deliver repeatedly 50 mg., for example, with a variation of not more than 1.3 per cent from the mean and with great rapidity.

A fresh setting of the lower check collar and its accompanying 0 setting of the hexagonal cap are rarely required—only when a worn cork washer is replaced by a new one of different thickness. Fresh settings of the upper check collar are required for each change of specific powder number.

Determining Specific Powder Number

The specific powder number is a measure of the bulkiness of the powder and is defined as the ratio of the weight of the packed powder to the weight of an equal volume of water at maximum density. It is thus the specific gravity of the combination of air and powder when the powder is packed as tightly as possible. Maximum packing is generally attained by three thrusts through the powder—more only if the powder is unusually fluffy. To determine the specific powder number the upper check collar of the measurer is screwed up out of the way, and the plunger is drawn up to a point on the scale which it is estimated will give the proper chamber length to deliver a convenient amount of powder. The chamber is then filled by an appropriate number of thrusts into the powder mass, and the powder picked up by the filler is ejected onto the pan of a balance and weighed. The specific powder number, P, may be calculated from

$$P = \frac{W_1}{l_1 \pi r^2} \qquad (1)$$

where W_1 is the weight of the powder, l_1 is the length of the chamber as indicated on the scale, and r is the radius of its cross section, assuming the chamber to be a perfect cylinder.

Different species of pollen afford interesting examples of specific powder numbers. Two species seldom have exactly the same powder number, yet those of closely related species are generally nearly the same:

Name	Powder Number
Tall ragweed, *Ambrosia trifida*	0.45
Short ragweed, *Ambrosia elatior*	0.48
Timothy, *Phleum pratense*	0.725
Bermuda grass, *Cynodon dactylon*	0.75
Sweet vernalgrass, *Anthoxanthum odoratum*	0.71
Rocky Mountain yellow pine, *Pinus scopulorum*	0.375
Colorado fir, *Abies concolor*	0.375

The specific powder numbers are highly characteristic and may even be used in checking the identities of powders. When the specific number is known, any desired weight of powder may be easily and rapidly measured out by the powder measurer. The length of the chamber or setting of the shaft scale, l_2, is found from

$$l_2 = \frac{W_2}{P \times \pi r^2} \qquad (2)$$

where P is the specific powder number, W_2 the weight of powder required, and r the radius of the cross section of the tube.

If the instrument is to be used only to measure out stipulated weights of different powders, it is not necessary to determine their specific powder numbers nor to measure the internal diameter of the tube; it is only necessary to make one trial weighing of each powder, using an arbitrarily chosen chamber length, and from this correct the chamber length for all subsequent measurements of this or any other required weight. The corrected chamber length is the trial length multiplied by the quotient of the required weight over the trial weight. This may be expressed:

$$l_2 = \frac{l_1 \times W_2}{W_1} \qquad (3)$$

Mathematically Formula 3 is obtained from 2 by substituting the equivalent for P in Formula 1.

In actual practice the application of Formula 3 is rapid and easy. If 10 is chosen as the chamber length for the trial weighing, and 50 mg. is the required weight of powder, the corrected chamber length is 500 divided by the trial weight.

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Determination of Alkoxyl by the Method of Vieböck and Schwappach

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IN ATTEMPTING to apply the Vieböck and Schwappach modification (*3*) of the Zeisel determination of methoxyl, difficulty was encountered in spite of the fact that the method has given excellent results in the hands of others (*1*, *2*).

According to the directions of Vieböck and Schwappach, the addition of formic acid to destroy the excess bromine should give a light-colored solution. In the authors' blank runs this was the case, but when a sample was analyzed, the solution turned darker on the addition of formic acid. Although Vieböck and Schwappach state that if the bromine color is not rapidly destroyed an insufficient amount of acetate is present, use of a larger quantity of acetate did not remedy the difficulty.

After considerable experimentation it was found that insufficient bromine was present originally to oxidize all the iodine monobromide to iodic acid. All previous directions state that 6 to 7 drops of bromine should be added for a 20- to 50-mg. sample, the size of sample to be chosen so that a convenient amount of 0.1 N thiosulfate will be consumed. The authors found that a medicine dropper having an approximately 0.7-mm. opening delivered an average of 0.023 gram of bromine per drop. If 25 cc. of 0.1 N thiosulfate are to be consumed in the final titration, the amount of bromine theoretically necessary would be 0.200 gram or about nine of these drops. In order to ensure an excess of bromine it is desirable to use about 0.3 gram or 0.1 cc. of bromine for each 10 mg. of methoxyl or each 15 mg. of ethoxyl in the sample, each cubic centimeter of 0.1 N thiosulfate being equivalent to about 0.5 mg. of methoxyl or 0.75 mg. of ethoxyl.

Since a lack of sufficient bromine can be detected immediately on adding formic acid, it was thought that such runs might be recovered by adding more bromine at this point. While such a procedure gives approximately correct results, they are by no means as consistent or accurate as when sufficient bromine is present in the original absorbing liquid.

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Recent Developments in Methods of Testing Germicides

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This is a review and discussion of recent developments in the testing of antiseptics and disinfectants, as well as an attempt to evaluate suggested modifications of present standard methods and proposed new tests. The trend towards more general use of practical testing of germicides is commended, since such tests are valuable not only in showing actual value of germicides under practical conditions of use but also in supporting the use of laboratory tests. The importance of practical and clinical tests and their possible use in standard methods and in research are emphasized. The recent tendency to test the reaction of germicides on tissues and the value of a toxicity index for antiseptics are discussed. The limitations of some of the proposed new procedures and the promising possibilities of others are considered from the viewpoint of possible use as standard methods for testing germicides.

THERE has been considerable interest in methods of testing antiseptics and disinfectants since publication by the U. S. Department of Agriculture in 1931 of the official methods of test employed by the Food and Drug Administration in the testing and control of these products. The methods of test which were described by the author from 1925 to 1929 (*14, 15, 19, 20*) were incorporated in U. S. Department of Agriculture Circular 198 (*28*) and officially designated as the Food and Drug Administration Methods of Testing Antiseptics and Disinfectants in 1931. Since that time much further work has been done in this field. The present paper reviews and attempts to evaluate these recent developments, including not only recent improvements in laboratory methods of testing these preparations, but also laboratory, practical, and clinical tests generally.

Testing Antiseptics and Disinfectants

Among the present standard laboratory methods of testing antiseptics and disinfectants, probably of most importance at the present time is the recent study on the effect of culture media, especially peptone, on the resistance of *Staphylococcus aureus*, the standard test organism employed in testing antiseptics. The standard resistance of this organism to phenol when tested by the technic employed in the Food and Drug Administration method is that it is not killed by 1 to 60 phenol at 20° C. in 5 minutes nor by 1 to 80 phenol at 37° C. in 5 minutes. When culture media, broth, and nutrient agar are made as described by the author and in Circular 198, the resistance of this test organism seldom varies from this standard of resistance when a suitable peptone is employed.

Peptone made by Armour and Co. is specified in the F. D. A. method and should always be used. For some reason, however, the Armour peptone specially made for this purpose during the past few years has failed to give cultures which uniformly met this standard of resistance. In fact, Meyer and Gathercoal (*9*) and Vicher, Meyer, and Gathercoal (*30*) were unable to secure uniform results with this peptone in their studies on the phenol resistance of *Staphylococcus aureus* and as a result recommended to the National Formulary Committee that a somewhat weaker standard be adopted for use in the new National Formulary. This weaker standard was incorporated in the description of the method for testing Liquor Antisepticus adopted as standard by National Formulary VI (*10*).

It has been shown by the author (*23*) that the use of such weak cultures gives results in testing germicides which are different than those obtained when cultures of normal standard resistance are used. The whole subject was later restudied by the Antiseptics Committee of the National Association of Insecticide and Disinfectant Manufacturers (*25*) and it was proved that media made from regular Armour's peptone gave cultures of normal resistance, whereas the specially purified peptone which had been made especially for this purpose gave weak cultures. Incidentally Burlingame and Reddish (*4*) restudied the effect of ten different brands of peptone on the resistance of *Staphylococcus aureus* and proved again that Armour's peptone is the most satisfactory for media used in the testing of antiseptics and disinfectants. As a result of this study arrangements were made with the Armour Laboratories whereby each lot of peptone which is set aside for use in testing antiseptics and disinfectants will first be tested and approved by this Antiseptics Committee (*24*) and then submitted to the Food and Drug Administration before it is made available for use. This tested and approved peptone will always give cultures of normal standard resistance of the test organisms employed in these tests. The importance of this is obvious.

Another recent development had to do with the laboratory method of testing antiseptic lozenges. The author (*19*) recommends the wet filter paper method, which is also specified as official by the U. S. Department of Agriculture (*28*) for testing "Solid Soluble Antiseptics: (*a*) Lozenges, tablets, etc."

However, this class of preparations has often been tested by the method used for liquid antiseptics, first dissolving the lozenge or tablet in water. The justification for this, apparently, is that this method for testing liquid antiseptics is specified for "soluble and liquid antiseptics" and since most lozenges are soluble in water the method has erroneously been used for water-soluble tablets. Because of the liberty taken with this test by many, the author made a special study of the germicidal activity of lozenges containing antiseptics, using both these laboratory methods together with extensive practical tests (*21*). It was found that those antiseptic lozenges which kill *Staphylococcus aureus* by the standard F. D. A. wet filter paper method will kill very large numbers of bacteria when the lozenge is dissolved in the mouth, reducing their numbers to a significant degree. If, on the other hand, the lozenge does not contain sufficient antiseptic to pass this severe test but does pass the test for liquid antiseptics, it does not kill significant numbers of bacteria when dissolved in the mouth. The results of these practical tests constitute sufficient proof, if any were needed, that the standard F. D. A. wet filter paper method should be used for solid soluble tablets and that the method employed for liquid antiseptics is not a proper test for this purpose.

Recent studies have been made on bacterial reduction in the mouth by means of oral antiseptics and these results compared to those obtained by laboratory tests by the standard method for liquid antiseptics (*18*). It was found that Liquor Antisepticus N. F. IV, which passes the F. D. A. test for liquid antiseptics, reduces the bacterial count of the mouth and throat 96 to 98 per cent when used as a mouth wash and gargle. This is additional proof that the F. D. A. standard laboratory test for liquid

antiseptics is satisfactory for indicating the germicidal activity and effectiveness of such preparations when used under practical conditions.

Phenol Coefficient Test

The phenol coefficient test has been employed for determining the germicidal efficiency of disinfectants for thirty-five years, and has served a very useful purpose. One of the uses made of the phenol coefficient of phenol-like disinfectants is for calculating the dilutions to be used in practice. It is generally recognized that a dilution of such disinfectants which is 20 times the phenol coefficient will be equal to 5 per cent carbolic acid when used under practical conditions. This simple means of calculating the proper dilution of phenol-like disinfectants for practical use was recently submitted to test by Varley and Reddish (*29*) on a large series of such disinfectants of various phenol coefficients. It was found that these compounds when diluted to 20 times their respective phenol coefficients, regardless of what this figure might be, are of sufficient germicidal strength to kill very large and even exaggerated numbers of disease-producing bacteria within a short time under practical conditions of use. In fact, as was expected, such dilutions of these disinfectants were just as germicidal under these practical conditions as 5 per cent carbolic acid. This is additional proof that the phenol coefficient of phenol-like disinfectants is a suitable measure of the practical value of such compounds and that the factor "20 times the phenol coefficient" is a proper means of calculating the dilutions for general use in practice.

These studies in which the results of laboratory and practical tests are correlated are important. The practical tests supplement the laboratory tests, and the results of such comparisons supply additional proof that these standard laboratory tests are adequate for the purpose of indicating the practical value of the preparations so tested. As a result of such studies standard laboratory tests can be employed with confidence.

During the past few years the phenol coefficient test has been widely misused. Although developed for determining the germicidal efficiency of disinfectants which are chemically related to phenol, it has lately been employed for other compounds, for compounds insoluble in water, and for antiseptics of all kinds. This test, as pointed out by the author (*16*, *22*), should be limited to those preparations which are to be used on inanimate objects and to water-soluble compounds, and should never be used for testing antiseptics. These limitations of this valuable test should be recognized by all who are interested in this field.

Sterilization of Surgical Instruments

It is to be expected that present laboratory methods will be improved from time to time and that new and different tests will be proposed as the need arises. Especially during the past seven years, various chemicals have been recommended for the sterilization of surgical instruments in place of heat sterilization. This so-called "cold sterilization" has the advantage over heat sterilization in that the cutting edge of such instruments is not injured. The F. D. A. method now specified for testing solutions used for treating surgical instruments makes use of *Staphylococcus aureus* as the test organism. Usually this method is adequate, since ordinarily such instruments are not heavily contaminated with pathogenic spore-forming microörganisms. When these preparations are recommended for complete sterilization, as is usual within the past seven years, solutions must kill all microörganisms present, including spores (*12*). There is, then, a need for a standard laboratory method for testing sterilizing solutions in which spore-forming organisms will be employed.

Preliminary steps have already been taken towards the development of such a method (*26*). Surgical instruments have first been contaminated with all kinds of skin organisms, including some that form spores, by exposing the instruments to the air, handling in the hands, rubbing on dirty clothing, floors, etc. The contaminated instruments were then plated in nutrient agar and the numbers of spore-forming organisms counted. Instruments similarly contaminated were then treated with a solution of 3 per cent formaldehyde in 85 per cent alcohol and were found to be completely sterilized within 5 minutes at room temperature. When the number of spores ordinarily found on contaminated instruments was increased a hundred fold on similar instruments, this solution of formaldehyde in alcohol was found to sterilize such instruments completely within 10 to 20 minutes at room temperature. The use of one hundred times the number of spores usually found on contaminated instruments is considered a sufficient margin of safety for a laboratory test. This method when perfected should be found satisfactory for testing germicides recommended for the sterilization of surgical instruments.

Athlete's Foot

The need for another standard laboratory test has also become apparent during the past seven years. Because of the increased prevalence of epidermophytosis (athlete's foot), many fungicides are now recommended for the treatment of this infection, but there is no accepted laboratory test which might be used for testing such preparations. Two or three methods have been employed by different laboratories engaged in testing germicides but none has found general acceptance. A method which appears to be suitable has been used by the author for the past two years and practical and clinical tests indicate that it is satisfactory for this purpose (*5*).

The following test organisms are employed: *Trichophyton rosaceum*, *Trichophyton rubrum*, *Trichophyton interdigitale*, and *Epidermophyton inguinale*.

Each organism is streaked over the entire surface of Sabouraud's agar in 9-cm. Petri dishes, using a 5-day culture of each organism, and inoculating it by means of a sterile dry cotton swab, each organism being streaked on separate plates. These plates are then incubated at room temperature for 5 days, at the end of which time the agar cultures are cut into 1-cm. squares. The fungicide to be tested is then poured over the surface of the culture so as entirely to flood the plate. At the end of 5, 15, and 30 minutes a square of culture and agar is removed and placed in 10 cc. of sterile broth. The excess fungicide is then washed out of the matted culture by shaking the tube lightly for 5 minutes. At the end of this time the block of culture is removed from the broth and the culture is spread over the surface of a sterile plate of Sabouraud's agar. These plates are then incubated at room temperature for 3 weeks and observed for growth.

An effective fungicide should kill these test organisms within 5 minutes. This may seem an arbitrary figure, but experience has shown that fungicides which kill these organisms within 5 minutes by this test are effective in the treatment of "athlete's foot" as proved by clinical test, and that preparations which do not pass this test in 30 minutes are not effective under practical conditions of use. The standard time period should be between these two points and it is suggested that 5 minutes be used as a margin of safety. Another margin of safety is the large numbers of organisms used in the test and the use of four test organisms instead of one. Present indications are that this test will prove satisfactory.

Recent Developments

During the past few years many new procedures have been proposed for testing the germicidal activity of antiseptics and

disinfectants. Some are promising, while others have proved disappointing.

Probably the most disappointing is the method suggested by Allen (*1*), which is supposed to simulate practical conditions to a large degree and to evaluate the actual clinical value of antiseptics more accurately than do our present standard tests (*2*). For various reasons the method has not been found acceptable and has not been employed by bacteriologists in this country. A careful study by Lewis and Rettger (*8*) has shown that the test is inaccurate and unreliable, and that little significance may be attached to the results obtained by its use. Since it does not simulate practical conditions and has the disadvantages mentioned, this method offers no promise as an additional test or even a supplementary test for germicides.

One of the most interesting and promising recent suggestions for testing germicides is that of Salle *et al.* (*27*). It is generally recognized that different antiseptics vary considerably in tissue toxicity; in fact, toxicity tests have been suggested as supplementary tests for antiseptics from time to time. Salle and his associates, however, have made the definite suggestion that such preparations can best be evaluated by means of a toxicity index. This figure would represent the ratio between the highest dilution of germicide required to prevent the growth of embryonic chick heart tissue and the dilution required to kill the bacterial test organisms. Much valuable information can be obtained regarding the suitability of antiseptics for different purposes by determining the toxicity index and this method should and no doubt will be widely used.

Considerable interest has recently been shown in methods of testing antiseptic ointments. Making use of the standard F. D. A. agar plate method, Bryan (*3*) suggests the use of a mercury ointment coefficient as a means of comparing the bacteriostatic activity of antiseptic ointments. Since there is no standard of comparison in our present standard method (*17*), such as the phenol coefficient test for disinfectants, the adoption of a mercury ointment coefficient is highly desirable. The use of ammoniated mercury ointment, U. S. P., is suggested by Bryan and seems suitable as a standard for comparison, and should be generally employed for this purpose. Such a standard of comparison would tend to raise the quality of such preparations and this is not only desirable but necessary.

Husa and Radin (*7*) have stimulated considerable interest in this subject by their study of the variations in ointment bases on the antiseptic value of phenol ointments. Vicher, Snyder, and Gathercoal (*31*) have also shown that other factors, such as size of particles of the active ingredient, calomel, affect greatly the antiseptic value of calomel ointment. Prout and Strickland (*13*) have studied the effect of fatty and nonfatty ointment bases on antiseptic activity. Other studies in this field are planned which will contribute information on the correlation of results of laboratory and clinical tests on antiseptic ointments and will be especially helpful in interpreting results of laboratory tests in terms of practical values.

Recently some effort has been made to get away entirely from laboratory methods of testing germicides and to use clinical and practical testing instead. Hunt (*6*) has become an exponent of this method of determining the actual value of antiseptics when used under conditions of practical use. He suggests the application of antiseptics to cutaneous lesions in mice as a means of testing germicidal value of such preparations. By this means the therapeutic value of antiseptics may be evaluated by testing them directly on artificially infected tissue. While such information is valuable, it is extremely difficult if not impossible to standardize such technic so that concordant results may be obtained by different investigators. For this and other reasons Hunt's method offers little promise as a standard method of testing germicides, but should be found valuable for research in therapeutic studies in this field.

Salle (*27*) and Nye (*11*), on the other hand, retain the well-standardized laboratory methods and in addition make use of tests on tissue toxicity. This is a step in the right direction and offers far more promise than strictly clinical tests, at least for general use. Future work on methods of testing germicides should retain as much as possible of the desirable features of present standard laboratory methods but make use of such supplementary practical and clinical tests as may give additional desirable information. A combination of laboratory and practical tests will very probably give sufficient information for the proper evaluation of the efficiency of antiseptics and disinfectants.

Summary

During the past seven years many new procedures have been proposed. There is a trend towards the use of practical testing of germicides, useful in substantiating results obtained by means of laboratory tests and also possibly supplementing present standard methods. The tendency to test germicides for tissue reactions is emphasized and the use of a toxicity index for antiseptics is discussed. The importance of practical and clinical tests and their possible use in standard methods and for research is considered in the light of present knowledge of the subject.

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Oxygen Pressure Aging

Improved Equipment

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An oxygen pressure-aging apparatus is described, which presents the following improvements: (1) small stainless-steel, quick closing jacketed pressure vessels which are easily removable from the system for repair purposes and can be operated singly or in series; (2) an electrically heated, constant-temperature system which can be adjusted to maintain a constant temperature in the pressure-aging vessel at 70° C., and is readily adjustable to a wide temperature range, depending on the liquid used for the heat transfer; (3) a valve which automatically closes the oxygen supply to the pressure vessel if the safety releases, conserving oxygen and preventing loss of tests in other pressure vessels attached to the same oxygen supply; and (4) a simple, easily operated safety release.

SINCE the introduction of the oxygen pressure-aging test by Bierer and Davis (*1*), prevailing standard conditions for the test have been 70° C. (158° F.) and 300 pounds per square inch oxygen pressure. Various types of equipment have been used; usually the equipment has consisted of a pressure vessel immersed in a constant-temperature water bath to which is connected an oxygen supply. In the majority of instances the equipment has been difficult to operate and maintain for several reasons:

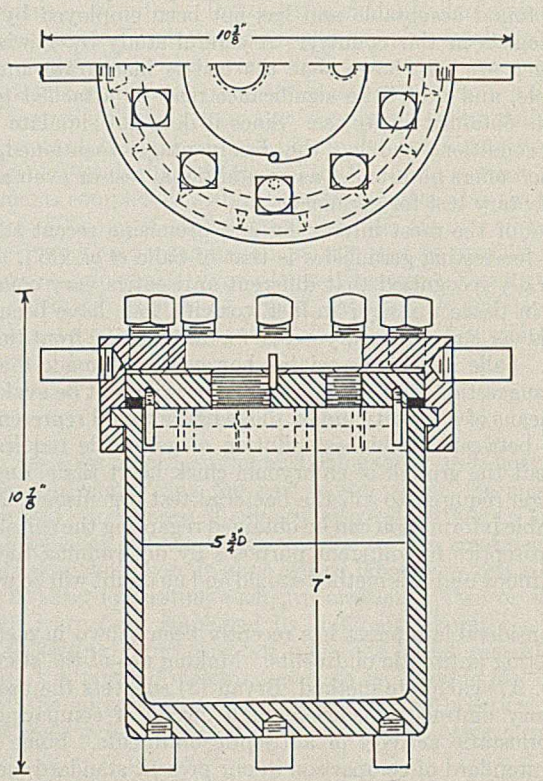


FIGURE 2. PRESSURE VESSEL BEFORE JACKETING

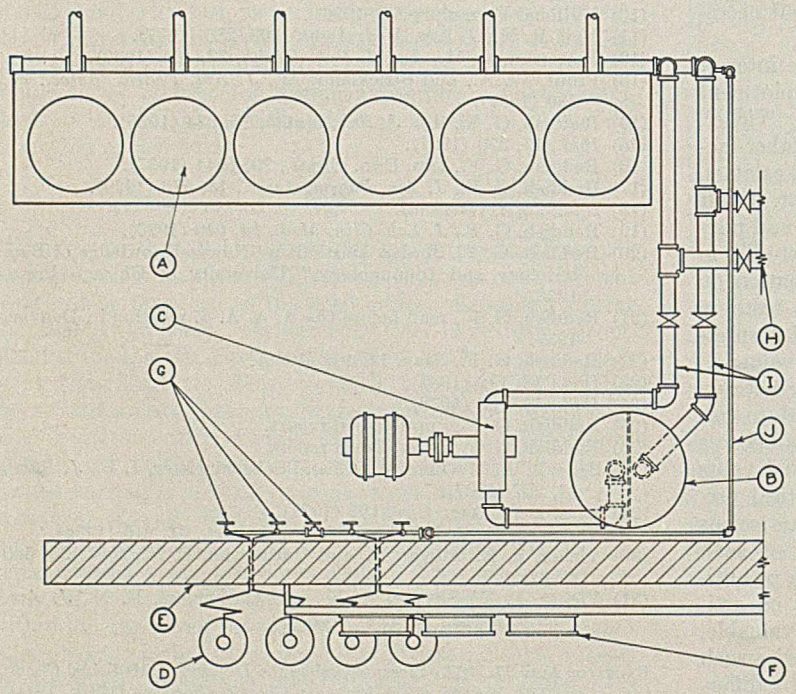


FIGURE 1. INSTALLATION DIAGRAM

Immersion of pressure vessels in a water bath which made handling difficult. Corrosion was a continuous source of trouble, causing "freezing" of cover bolts and making it difficult to obtain a leakproof oxygen seal between cover and vessel. This caused loss of oxygen.

Each time the pressure vessel was removed from the bath it was necessary to disconnect the oxygen supply and make the connection again when the test was started. This also caused loss of oxygen.

If more than one pressure vessel was connected to the oxygen supply and a safety released, the entire oxygen supply was exhausted.

The original pressure vessels were relatively large. Since the use of age resistors on a large scale, smaller units have been desirable in order to decrease migration of age resistors and eliminate erroneous results.

Some of these operation difficulties were outlined by Ingmanson and Kemp (*2*), who also emphasized the importance of temperature control to obtain reproducible results.

It is the purpose of this paper to describe an improved oxygen pressure installation which avoids some of these difficulties.

Installation

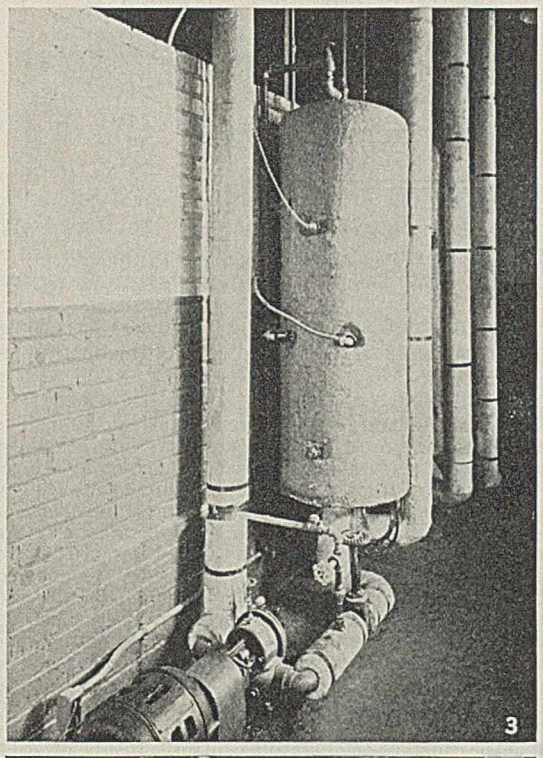
Figure 1 is a chart showing one of two duplicate installations.

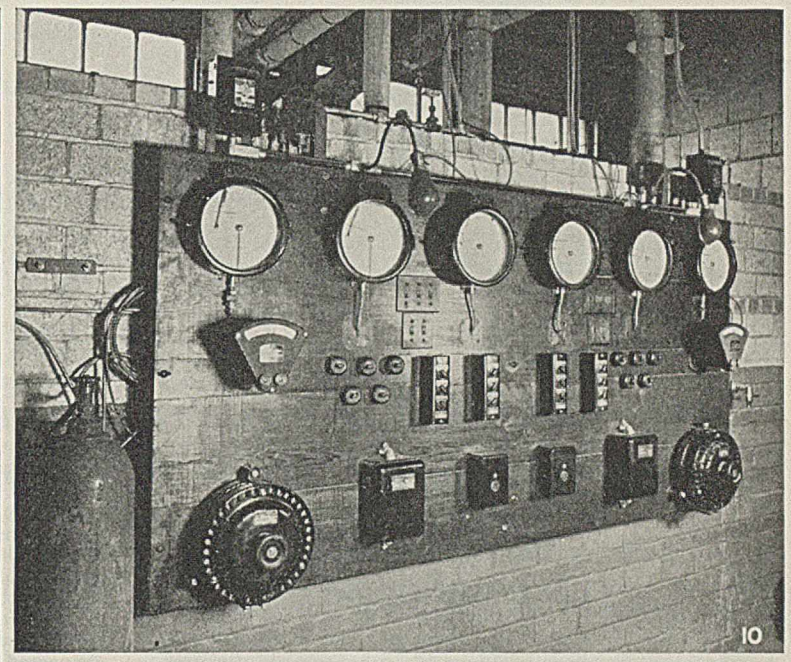
A crossover, *H*, connects the two circulating systems, so that if one of the constant-temperature supply tanks or circulating pumps must be repaired the entire oxygen system can be operated from the other supply. The pressure vessels, *A*, are jacketed and mounted on a steel table. The heat-transfer medium at present is water, which is maintained at constant temperature in tank *B* and circulated around the pressure vessels by circulating pump *C* through pipes *I*. The oxygen supply tanks, *D*, are on the opposite side of a brick wall, *E*, from the aging equipment. The instrument panel, *F*, is adjacent to the oxygen supply, so that the operator can read the instruments and shut off the oxygen pressure or temperature controls while on the opposite side of the brick wall from the pressure vessels. The oxygen supply is connected through reducing valves *G* and pipe *J* to the pressure vessels.

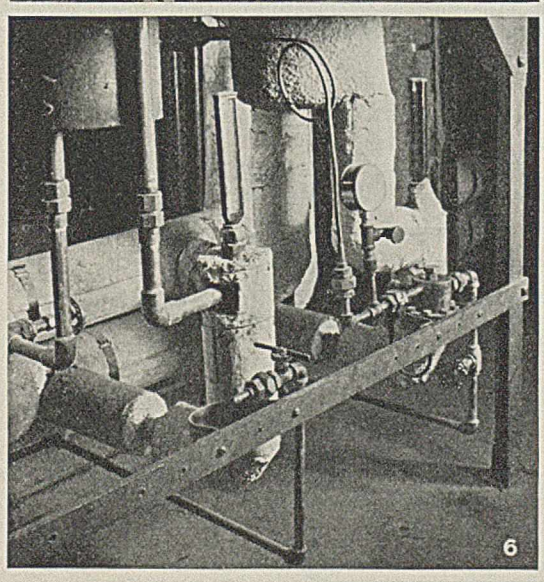
The pressure vessels are of two types:

1. Steel, inside diameter 5 inches and 11 inches high, jacketed so that a heat-transfer medium may be circulated around the vessel. The covers are fastened on with ten bolts.
2. Stainless-steel, 5.75 × 7 inches high (Figure 2 is a drawing of the pressure vessel before jacketing), jacketed so that a heat-transfer medium may be circulated around the vessel. The cover consists of a circular disk which rests on a scaling ring and is held in place by a slide ring. An eighth turn of the slide ring locks it in place over the flange of the pressure vessel and then by means of ten set screws mounted through the ring, all impinging on the cover, a leakproof seal is easily obtained.

Figure 3 is a photograph of the heating system, showing how the electric immersion heaters are installed. The cir-







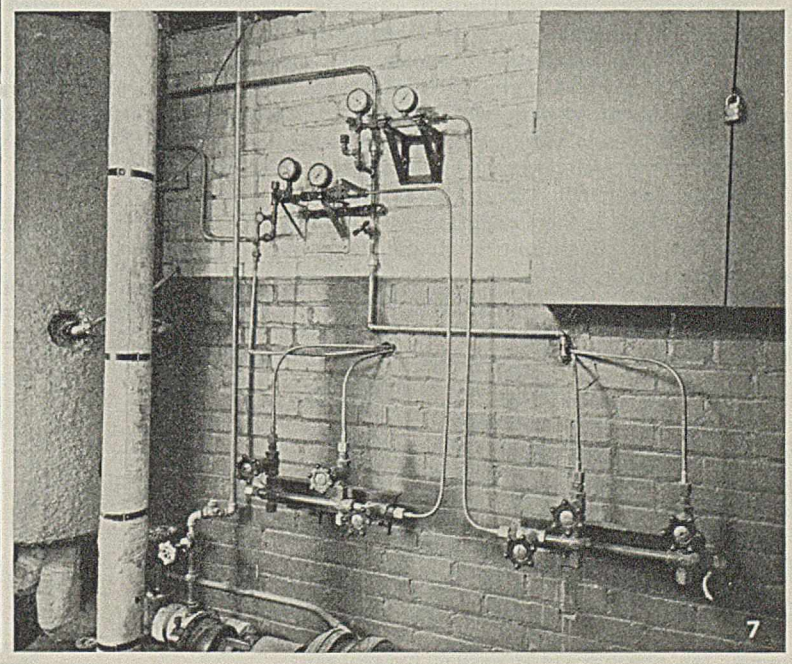


FIGURE 3. HEATING SYSTEM
FIGURE 6. PRESSURE VESSEL IN PLACE

FIGURE 7. REDUCING VALVES
FIGURE 10. INSTRUMENT PANEL

culating pump and motor are shown in the lower left-hand corner. Figure 4 is a drawing of the heating tank.

The water returns from the jacketed pressure vessel through pipe *A*, is forced up past heaters *B* and over baffle plate *C* down past temperature controller *D* and heaters *E*, through pipe *F* and circulating pump to the pressure vessels. Recording thermometers in pipes *F* and *A* indicate the temperature of the water before entering the pressure vessel jacket and upon return to the constant-temperature supply tank. To date the differential between these two recordings has been less than 0.5° C. (0.9° F.). The temperature bulb, *G*, actuates an automatic cutoff which is installed in the immersion heater circuit. This cutoff is adjusted to 77° C. (170.6° F.) when the operating temperature is 70° C. (158° F.) and is necessary in order to protect the apparatus in case the circulating system stops.

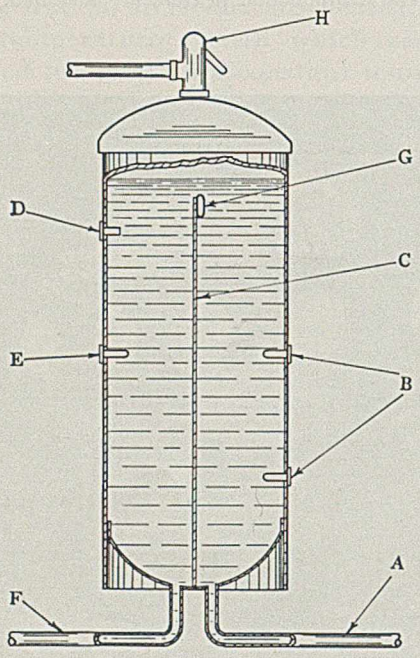


FIGURE 4. HEATING TANK

Figure 5 is a drawing and Figure 6 a photograph of a pressure vessel mounted in place. In Figure 5 the oxygen connection with safety mounting is shown at *A* and the water connections at *I*. The thermometer serves to show whether or not the water is circulating around the individual pressure vessel. It is possible to remove any individual pressure vessel very easily without interfering with the remainder of the system by closing the valves to the circulating system line and to the oxygen supply line, and disconnecting the unions.

The oxygen pressure is supplied from the tanks in Figure 1 through the reducing valves shown in Figure 7.

Between each pressure vessel and the oxygen supply line there is an automatic cutoff valve shown at *B* in Figure 5 and in detail in Figure 8. The oxygen supply from the reducing valve enters the automatic cutoff valve through line *A*. The valve is actuated by a diaphragm, *D*, and by opening valve *C* the oxygen is released to the pressure vessel through line *G* and the pressure on both sides of the diaphragm is equalized through *F* and *B*. When the pressure is equal on both sides of the diaphragm, spring *H* opens valve *E;* valve *C* is then closed and if there is only a small leak through the pressure vessel system oxygen can feed through valve *E;* however, if pressure builds up in the pressure vessel and the safety releases, then the pressure is released through *F* on one side of the valve and the diaphragm immediately closes valve *E*. These valves operate so rapidly that it is impossible to determine on the pressure-recording chart the time at which a safety has released.

Figure 9 illustrates the safety release—a disk of stainless-steel sheet mounted between tin disks in a compression seal. When the system is operating at 300 pounds per square inch the thickness of the disk is adjusted so that it releases at 350 pounds per square inch.

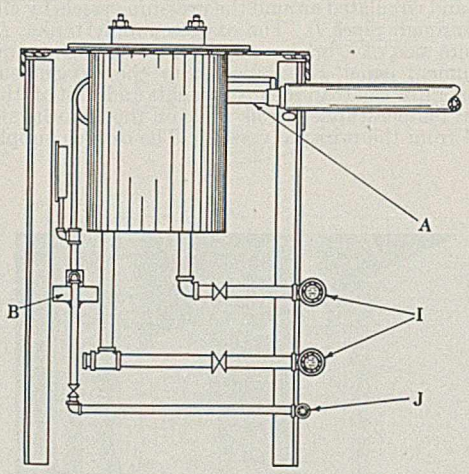


FIGURE 5. PRESSURE VESSEL IN PLACE

The instrument panel shown in Figure 10 has two complete sets of instruments to record and control the operations of the two aging units.

Across the top of the panel are six recording instruments. The two pressure recorders are at the extreme ends; between these are the two pairs of temperature recorders connected to the return and outgo lines from the constant-temperature tanks. Below the pressure recorders are the temperature controllers for the constant-temperature tanks. These controllers actuate the heaters illustrated at *B* in Figure 4. The upper row of the two sets of snap switches controls the heaters which are connected through the temperature controllers; the lower pair of the two sets controls the booster heaters, *E* (Figure 4). The other two pairs of switches can be adjusted so that heaters *B* (Figure 4) are connected separately or in any combination through the rheostats at the lower corners of the instrument panel to the thermometer controller or so that the heaters are connected direct to the thermometer controller. This makes possible a very accurate adjustment of the constant-temperature control. Just below each set of temperature recorders are lights in parallel with the heaters

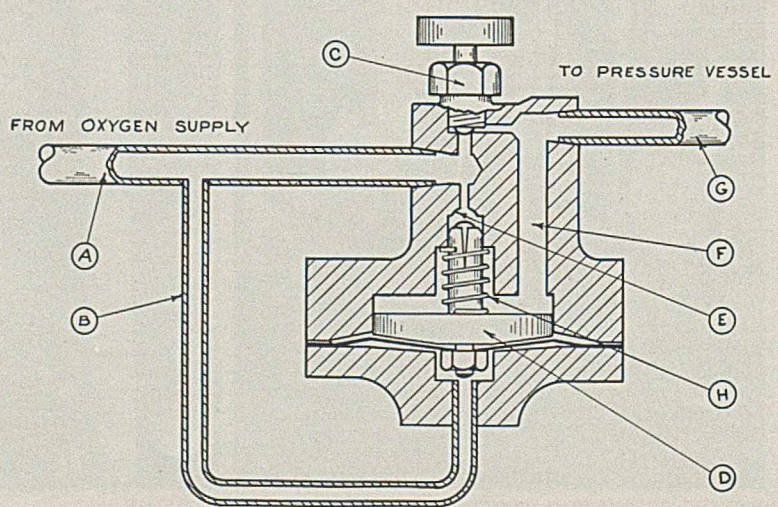


FIGURE 8. AUTOMATIC CUTOFF VALVE

to show which heaters are in operation. Above the instrument panel and adjacent to the main supply line switches are the automatic cutoff switches which connect with bulb *G* in the constant-temperature tank.

Temperature inside Pressure Vessels

The original plans for the equipment specified that the pressure vessels be bolted to the metal tables, using a gasket to insulate the pressure vessel from the table. After the installation was made according to these plans the following temperatures were obtained inside the 5 × 11 inch pressure vessel:

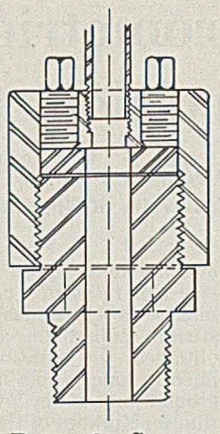


FIGURE 9. SAFETY RELEASE

Position inside Pressure Vessel *Inches from top*	Temperature ° *C.*	Water Temperature ° *C.*
1	68.5	70
3	68.5	70
4	69.0	70
6	69.5	70
7	70.0	70
10	70.0	70

Similar readings inside the 5.75 × 7 inch stainless-steel vessels were low and more variable. In order to correct this variation within the pressure vessel, the tables were covered with Celotex and the pressure vessels then mounted in place.

A wooden top was then fitted over the Celotex and around the lids of the pressure vessel covers. Covers of wood and Celotex were made to place over the pressure vessels and temperature readings inside the 5.75 × 7 inch stainless-steel vessels were as follows:

Position inside Pressure Vessel	Temperature ° *C.*	Water Temperature ° *C.*
1 inch from top	69.5	70
1 inch from bottom	69.5	70

With these data available it is possible to adjust the temperature of the circulating water to maintain 70° C. (158° F.) inside the pressure vessels.

Aging studies making use of the flexible facilities of this improved oxygen pressure-aging apparatus will be presented later.

Acknowledgment

Acknowledgment is made to N. E. Raber of the B. F. Goodrich Engineering Department, who designed the automatic cutoff valve (Figure 8).

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Determination of Formaldehyde in Dilute Solutions and in the Presence of Interfering Substances

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A FLUID pharmaceutical preparation, which at the time of analysis contained about 0.003 per cent of formaldehyde, could not be even roughly analyzed for its formaldehyde content by the ammonia (*2*), cyanide (*1*, *5*), hydrogen peroxide (*3*), and iodine (*6*) methods. This was due in part to the flavoring agent, one ingredient of which was oil of cinnamon (aldehyde). The deep red color of the preparation prevented the use of procedures depending upon production of colors. On attempting to isolate the formaldehyde by distillation, it was found that the small amount of formaldehyde present was reduced to practically zero.

Satisfactory results were obtained with a modification of the silver method (*4*) used with mixtures of known formaldehyde content. This modification has also the advantage that reducing sugars do not interfere.

Procedure

Exhaust the aqueous or aqueous alcoholic fluid with ether–petrolic ether (1 + 2) to remove flavor, etc. Four to five extractions, each with one-half volume of solvent, are usually sufficient. To 10-cc. aliquot, add in rapid succession 100 cc. of 0.1 *M* silver nitrate, 1 cc. of hydrochloric acid (37 per cent), and 3 cc. of sodium hydroxide (25 per cent). Whirl once after each addition. Finally whirl 10 minutes for good contact. Filter through paper and wash until chloride free. Pour warm nitric acid (1 + 3) onto precipitate to dissolve all reduced silver. Wash with hot water and titrate with 0.1 *N* ammonium thiocyanate and ferric alum. $2\,Ag = 1CH_2O$.

A determination can be done in about 30 minutes. The average percentage reproducibility observed is of the order of two units in the third decimal place.

A mixture was made of 10 cc. of the sample under investigation plus 10 cc. of an aqueous 0.20 per cent formaldehyde, newly made up from about 37 per cent stock and for the purpose of introducing all like interfering factors, also shaken out with the ether–petrolic ether. This mixture analyzed 0.097 per cent; the actual content was calculated as 0.101 per cent (the average of 0.003 and 0.20 per cent).

The aqueous 0.20 per cent formaldehyde made from about 37 per cent stock analyzed 0.19 per cent formaldehyde. After extracting with ether–petrolic ether, it still showed 0.19 per cent, while the Association of Official Agricultural Chemists cyanide method gave 0.009 per cent in both cases.

An approximately 0.2 per cent solution of acetaldehyde, a little freshly made silver chloride, and a slight excess of sodium hydroxide turn the silver chloride a light bluish gray after about one minute, whereas 0.2 per cent formaldehyde, under the same conditions, turns the silver chloride black immediately.

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RECEIVED March 28, 1938.

An Apparatus for Electrometric Titrations of High Precision

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THE apparatus described below was designed to meet the demand for a convenient titration apparatus suitable for all kinds of titrations. The special stirring device was found to eliminate the difficulties encountered with ordinary stirrers in foaming solutions. This apparatus during two years' use has proved to be very suitable.

The titration vessel (Figure 1) is pear-shaped and has a total volume of about 125 cc. The pear form makes it possible to use from 20 to 100 cc. of solution for a titration. On the upper surface, the vessel has five interchangeable ground joints. For measurements with glass electrodes, the MacInnes type of electrode used is held in the central joint by means of a paraffined cork stopper; for hydrogen-electrode measurements, the central joint is used for the gas inlet and outlet. Calomel and other electrodes, burets, etc., are carried by the peripheral joints. These joints and their accessories have glass hooks and are held together by rubber bands.

The stirring device is shown in Figure 2. The titration vessel with attached accessories is fixed to the stirring device by an easily detachable metal clamp, *A*, around the central joint. This clamp, on the other hand, is mounted on two vertical rods, *B*, in such a way that it can be moved vertically and fixed by screws, *C*, in a suitable position. The vertical rods, *B*, are fixed to the upper part of a cross slide, *D*, the lower part of which is screwed to the aluminum U-rod, *E*, which is fixed across the thermostat vessel. By means of an adjustable eccentric, *F*, and the cross slide, *D*, an electric motor gives the titration vessel a rotatory movement in the horizontal plane. The radius of the eccentric and the motor speed are adjusted to give the desired radius and speed of rotation. Stirring is obtained by the rotation, which makes the electrodes rotate relative to the liquid. A separate stirrer is superfluous and although a very effective stirring is thus obtained, the titration vessel moves perfectly steadily; consequently long burets need only be held by their interchangeable ground joint at the lower end and there is no fear of breaking the thin membranes of the glass electrodes.

The design of the lower end of the burets is shown in Figure 3. Below the stopcock, the bore is capillary, about 0.5 mm. in internal diameter. If the tip only is capillary, light precipitates in the solution may enter the tip and lie beneath the stopcock. When the bore is capillary all the way up to the stopcock, any

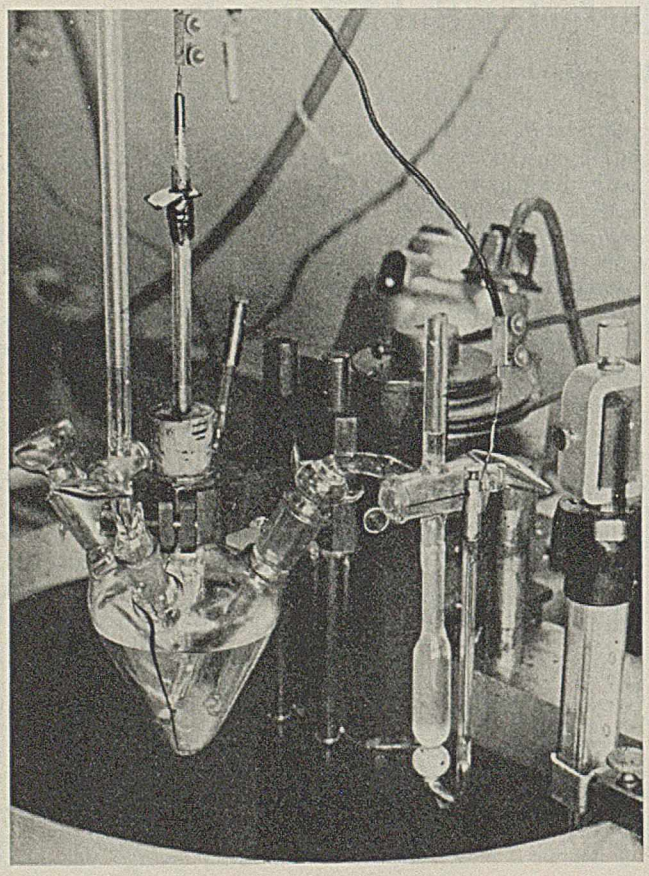

FIGURE 1

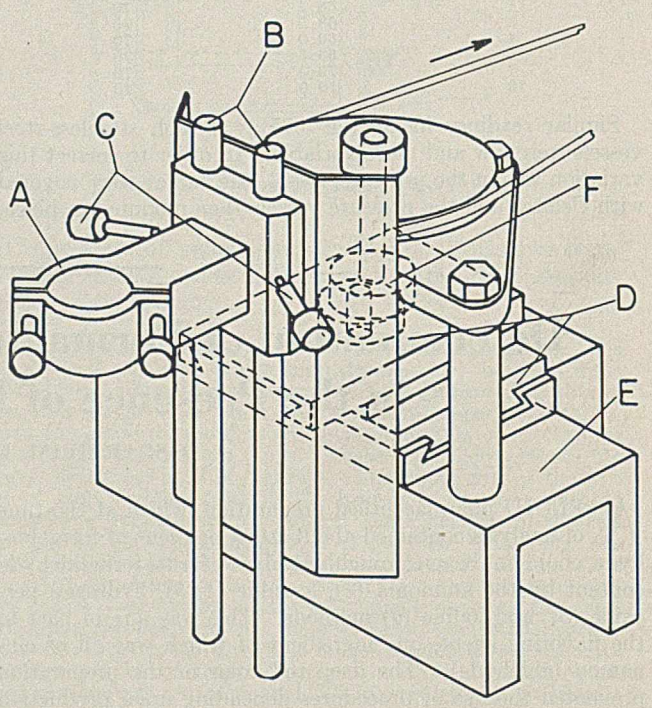

FIGURE 2

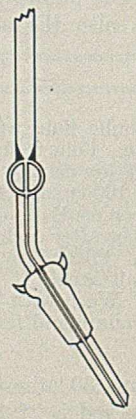

FIGURE 3

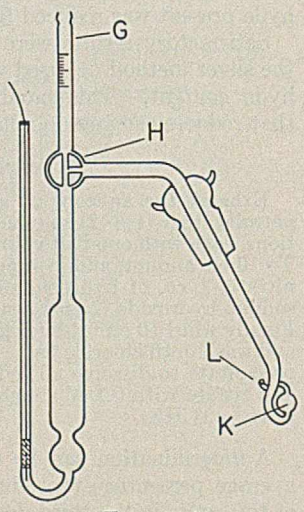

FIGURE 4

precipitates which have entered the buret tip can easily be washed out.

The type of calomel electrode used is shown in Figure 4. The goose-neck type of liquid junction, described by Clark (*1*), has proved to be the most suitable for an apparatus of this kind. The liquid junction is made by applying gentle suction at *G* with stopcock *H* slightly open and *L* dipping into the solution to be examined. The solution flows into the small bulb, *K*, and a sharp junction with the heavy potassium chloride solution can be formed in the middle of bulb *K*. This procedure is facilitated by a calibration on tube *G*. The capillary, *L*, has a length of about 8 mm. and an internal diameter of 1 mm. There is no detectable diffusion of potassium chloride into the solution in the vessel during the time required for an experiment, when using this form of liquid junction. Agar-agar bridges, on the other hand, allow fairly large amounts of salt to diffuse into the solution to be titrated.

An aluminum vessel serves as an oil thermostat. The oil used is a mixture of equal parts of transformer oil (Shell K 2) and white spirit. This mixture does not smell or evaporate and has about the optimal viscosity. All metal parts are earthed. No further screening is necessary, and glass electrodes give perfectly steady potentials even when stirring.

The freedom from rubber connections makes the apparatus very suitable for titrations in organic solvents. Furthermore, the absence of any external stirrer simplifies titrations which have to be carried out in an inert gas atmosphere.

The photograph in Figure 1 shows the apparatus ready to be lowered into the oil bath for an experiment. All glass parts are made from Pyrex or Jena glass. A microapparatus has been constructed on the same lines, taking from 2 to 10 cc. of solution to be examined.

Literature Cited

(1) Clark, W. M., "Determination of Hydrogen Ions," 3rd ed., p. 301, Baltimore, Williams & Wilkins Co., 1928.

RECEIVED April 26, 1938.

Determination of Sulfur in Some of the More Common Alloys

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THE copper chloride–perchloric acid method (*2*) for sulfur is applicable to certain ferroalloys as well as to many nonferrous alloys.

Brass

Bureau of Standards Sample No. 63 may be considered representative. Its percentage composition is: S 0.06, Cu 78.05, Pb 9.74, Sn 9.91, Sb 0.55, As 0.19, Zn 0.48, Fe 0.27, and P 0.62. The Bureau of Standards method is based on the preliminary separation of tin oxide, the removal of copper and lead by electrodeposition, and the removal of nitric acid by hydrochloric acid and heat. The proposed method is more rapid, requires much less manipulation, and is less difficult. The results indicate the same order of precision, but are 0.01 per cent higher in value.

PROCEDURE. Transfer 5 grams of brass to a 600-cc. beaker and cover with 500 cc. of potassium-copper chloride solution. Maintain the solution at about 90° C. as on a steam bath. Mechanical stirring is preferred. Keep the solution covered as much as possible.

When all copper has dissolved, filter the warm solution through a fast filter paper, and wash with hot water. Remove the paper from the funnel, place it in the beaker, cover with strong bromine water, and agitate with a glass rod. Add 10 cc. of zinc oxide–nitric acid solution and 8 cc. of perchloric acid. Heat the beaker to destroy the paper and to drive out the nitric acid and the excess perchloric acid. When perchloric acid begins to condense at the top of the beaker, remove the beaker from the hot plate and allow to cool.

Dissolve the residue, usually solid, in water, dilute to about 100 cc., and boil to remove chlorine. Filter off any insoluble matter on paper, and wash with hot water. Dilute the filtrate to 200 cc. and precipitate sulfates with barium chloride. The weight of barium sulfate divided by 5 and multiplied by 0.1373 gives the weight of sulfur found per gram.

REAGENTS: 500 grams of $(KCl)_2 \cdot CuCl_2 \cdot 2H_2O$, 100 cc. of hydrochloric acid, and 2000 cc. of water. Sift 200 grams of zinc oxide into 1 liter of concentrated nitric acid.

RESULTS: 0.072, 0.071, and 0.070.

Nonferrous Alloys

The same procedure has been applied to pure copper and to its alloys of tin, lead, zinc, iron, and aluminum; to various types of monels; and to nickels, cobalts, and nickel-cobalt alloys. These latter dissolve rather slowly.

Ferromanganese

Using Bureau of Standards No. 68 (S, 0.014 per cent) as a test sample, it was found that the copper chloride solution must be added cold, after which the procedure is as usual for ordinary steels. Results: 0.014, 0.013 per cent. Manganese metals seemed to contain not more than 0.003 per cent of sulfur.

Ferromolybdenum

Lundell (*1*) used a hot tube method, or an aqua regia solution method. Apparatus for the first is not usually available, and for the second method the advantages of the copper chloride method apply (*2*). The only modifications to be applied to the above procedure are:

A smaller sample is used: 3 grams.

Agitation of the alloy in copper chloride solution must be continued until nearly all the iron and molybdenum are dissolved.

In the third paragraph of the procedure, the insoluble matter contains molybdic acid and is washed with hot 0.5 per cent (by volume) hydrochloric acid instead of distilled water.

With these extra precautions not more than 0.0002 gram of molybdenum was found in any barium sulfate precipitates of ferromolybdenum. Converting this value to barium molybdate and calculating to sulfur, no error was found in reporting the result as pure sulfur (to two significant figures). Results: No. 30747: 0.11, 0.11, 0.11, 0.12. No. 4626: 0.25 (*1*), 0.22, 0.23.

The results obtained with brass and ferromolybdenum were checked by Owen Gates, U. S. Navy Laboratory, Munhall, Pa.

Literature Cited

(1) Lundell, Hoffman, and Bright, "Chemical Analysis of Steel," p. 484, New York, John Wiley & Sons, 1931.
(2) Silverman, Louis, IND. ENG. CHEM., Anal. Ed., 7, 205 (1935).

RECEIVED April 21, 1938.

An Electronic Voltage Regulator

With Supplementary Circuit to Supply Low Voltages

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ELECTRONIC voltage regulators have been known to communication engineers for several years, but apparently none has been described in any publication available to chemists. A simple circuit with limited voltage range for use with a moisture tester is described by Working (*1*), but the circuit shown in Figure 1 gives a much wider voltage range, and at the same time improves the voltage regulation.

Theory of Operation

To secure voltage regulation, an approximately fixed voltage above the negative side of the line is established by means of a gas-discharge tube (a neon glow lamp with resistor removed from the base, or a type 874), in series with current-limiting resistors. This voltage is applied to the cathode of a tube of high amplification, such as type 6J7. A bleeder across the output which is to be regulated is tapped at a point which will be approximately 3 volts negative to the fixed voltage when the output is at the voltage desired, and this tap is connected to the grid of the 6J7. Any change in output voltage is thus amplified and is applied to the grid of a power tube, as a type 6L6, placed in the positive line to carry the entire output current.

Assuming this simple circuit, an increase in input voltage tends to increase the output voltage, and thus the grid voltage of the 6J7. The increased plate current of this tube lowers the voltage on the grid of the 6L6, thus preventing more than a very slight rise in output voltage. If very precise measurements are made, the gas-discharge tube will be found to have increased slightly in voltage, and altogether for a 10-volt increase in input voltage the output voltage will increase perhaps 0.1 volt under favorable conditions; much more than that if the tubes are operating outside their range of high sensitivity.

The foregoing assumes a constant screen voltage for the 6J7, but if this voltage be taken from a tap on the resistor which supplies the gas-discharge tube, it will increase with a rise in input voltage and further reduce the plate current of the 6L6. By using a resistor with an adjustable tap the screen voltage may be adjusted to balance exactly, over a limited range, the tendency of the output voltage to change in the same direction as the input. However, if the screen voltage is set at about 100 volts for maximum sensitivity of the 6J7, it will ordinarily overcompensate for voltage changes in the input. Accordingly a second gas-discharge tube is used, N_2 (Figure 1), considerably reducing the fluctuations in screen voltage.

Circuit Details

The components specified under Figure 1 were chosen to be capable of supplying 60 milliamperes at 343 volts as required by the titrimeter described by Working (*2*), with line voltage fluctuations up to ±13 per cent. However, if the transformer output is only 500 volts, the regulator is operating near its upper limit at this rating, and accordingly the bleeder, R_4, R_5, R_6, is chosen to have a fairly high resistance in order to put no unnecessary current drain on the output. If this circuit is to be used to supply an instrument using a very small current during part or all of its operation, this will require

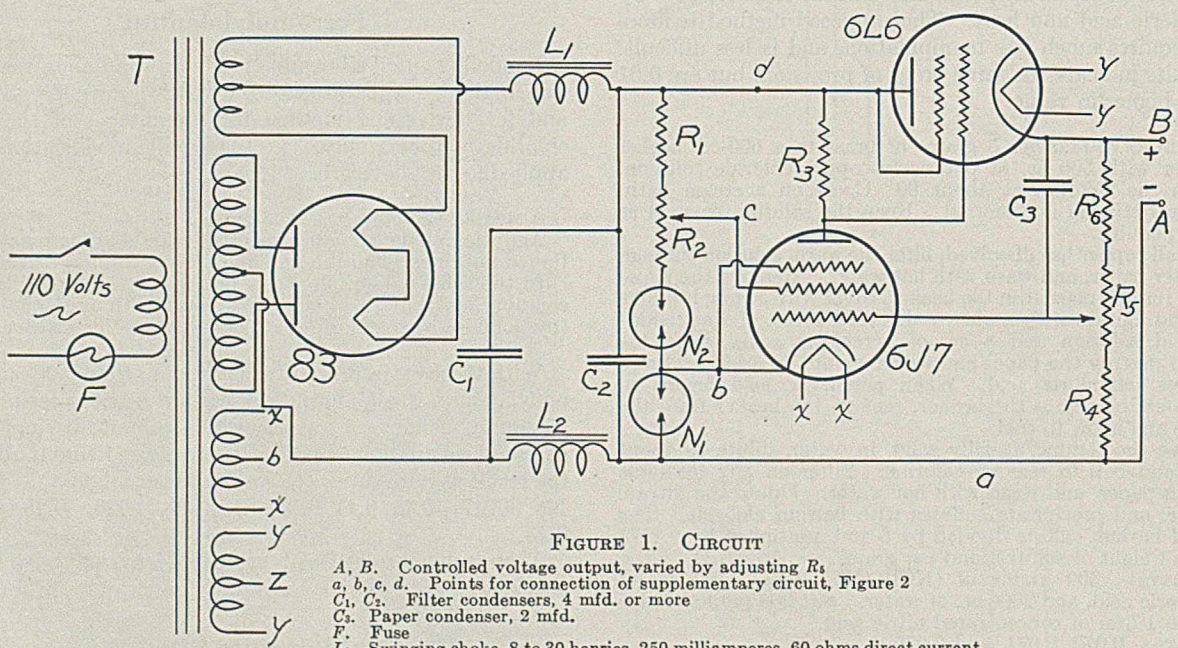

FIGURE 1. CIRCUIT

A, *B*. Controlled voltage output, varied by adjusting R_5
a, *b*, *c*, *d*. Points for connection of supplementary circuit, Figure 2
C_1, C_2. Filter condensers, 4 mfd. or more
C_3. Paper condenser, 2 mfd.
F. Fuse
L_1. Swinging choke, 8 to 30 henries, 250 milliamperes, 60 ohms direct current
L_2. Filter choke, 20 henries, 200 milliamperes, 100 ohms direct current
N_1, N_2. Neon glow lamps, 2 or 3 watts, with resistor removed from base
R_1. 35,000 ohms, 10 watts
R_2 5000 ohms, 10 watts with adjustable tap
R_3. 2 megohms
R_4. 50,000 ohms
R_5. 100,000-ohm potentiometer
R_6. 200,000 ohms

operating the 6L6 tube very close to its cutoff, and better regulation will be secured by introducing an additional bleeder, or reducing the resistance of R_4, R_5, R_6. The output voltage may be reduced to about 135 volts by adjusting R_5, while for small currents the maximum voltage is about 400.

A power transformer with a rating of 200 milliamperes or more should be chosen to assure a low voltage drop. A voltage output greater than 550 is permissible if R_1 is increased to limit the current through it to about 10 milliamperes; rectifier tubes of higher voltage rating than the 83 should probably also be substituted in such a case.

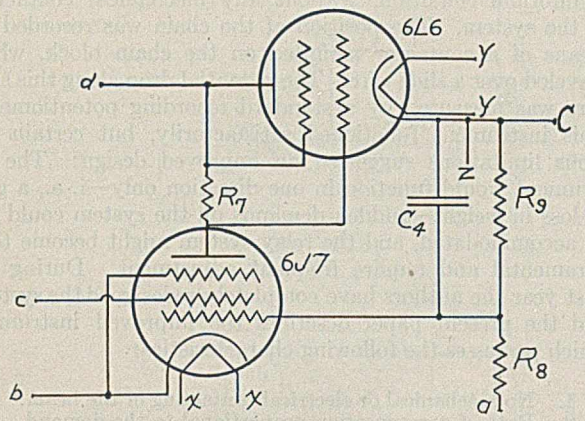

FIGURE 2. SUPPLEMENTARY CIRCUIT

C. [Output, controlled with respect to *A* (Figure 1), so that the voltage between *C* and *B* may be adjusted to any value from zero to about 200 volts
C_4. Paper condenser, 2 mfd.
R_7. 2 megohms
R_8. 1500 ohms, 10 watts
R_9. 2250 ohms, 10 watts

If it is preferred to use a type 874 voltage-regulator tube instead of the neon glow lamp, N_1, the resistance in series should be reduced to give a current of about 15 milliamperes, and R_4 and R_6 changed to 75,000 ohms each. The minimum voltage obtainable will be increased about 30 volts. It is ordinarily better to eliminate N_2 altogether than to use a type 874 tube in place of it.

The 6L6 tube was chosen because the power transformer used was supplied with 6.3-volt filament windings, and the separate cathode of the 6L6 tube allows the output tube of the supplementary circuit for low voltages (Figure 2) to operate from the same filament winding. Type 2A3 is more frequently used, and will pass up to about 75 milliamperes with slightly less voltage drop. For greater output than this, the 6L6 has a slight advantage, and its indirectly heated cathode has a decided advantage if it is essential to filter out as much alternating current hum as possible. The 6J7 was chosen to match the metal 6L6, but a 6C6 would give identical results.

For most purposes it is merely necessary to connect the screen of the 6J7 to such a point on R_2 as to give a voltage between 90 and 100 volts above the cathode, and if preferred, R_2 may be a fixed resistor of 4000 ohms and the screen connected between it and R_1. However, if especially accurate regulation is necessary, the voltage control should be put into operation with its load connected, and the screen voltage adjusted until artificial changes in input voltage cause no change in output voltage. It will be necessary to provide a very sensitive means of detecting changes in the output voltage. In this laboratory, the adjustment has been made so that an increase or decrease of 15 volts in the input voltage changes the output less than 1 millivolt, the smallest change which the author could detect. For this accuracy, filter condensers C_1 and C_2 should be at least 8 microfarads each. If the special conditions of the circuit require for this adjustment a screen voltage much above 100 volts, tube N_2 should be omitted, R_2 increased to about 10,000 ohms, and the screen voltage adjusted as outlined above. If the screen voltage is too high, output voltage will drop on increase of input voltage, and vice versa.

The circuit as shown is designed to correct accurately for line voltage changes at a fairly constant output current drain; a 20-milliampere increase in output drain may cause a 20-millivolt voltage drop. This can usually be corrected by replacing N_2 by a 5000-ohm resistor, and increasing the resistance of L_1 and L_2 or introducing a series resistor of 500 to 1000 ohms immediately before or after L_1. It will also be better to use a type 5Z3 tube instead of the 83V, or two type 81 tubes if it is necessary to use a transformer of higher voltage to produce the necessary output voltage. With these changes, an increase in output drain will lower the screen voltage of the 6J7 and thus tend to hold the output voltage constant. If this compensation is not sufficient for the requirements, the screen of the 6J7 should be connected to a separate bleeder between the negative line and the plate of the 6L6, which may consist of a 100,000-ohm resistor connected to the negative line, a 100,000-ohm potentiometer for screen voltage adjustment, and a 300,000-ohm resistor connecting this to the positive line. Such a circuit can be adjusted to hold the output voltage constant within less than 1 millivolt with a change in drain of 50 milliamperes, but there will be some overcompensation for changes in line voltage. This will usually not be more than 2 to 4 millivolts for each volt change in line voltage.

If an electronic voltage control is to supply current to a device that will be damaged by a brief over-voltage, a switch should be provided to delay the application of voltage after turning on the apparatus until the cathode of the control tube has had time to heat to operating temperature. This is especially important when a 2A3 output tube is used, as it will pass current for some time before a 6J7 or 6C6 warms up sufficiently to control the voltage.

Low Voltages

The lowest voltage obtainable from a circuit of the type just described is ordinarily from about 90 to 140 volts, depending on the voltage delivered from the rectifier and the current drawn from the output. A lower voltage may be obtained by means of a voltage divider if the current required is constant, but such a device is useless if the drain fluctuates, and if more than a few milliamperes are required temperature changes in the resistors may lead to troublesome voltage changes. But if the circuit shown in Figure 2 is connected to Figure 1 at the indicated points, *a*, *b*, *c*, *d*, *x*, *y*, *z*, the voltage between *C* and *B* can be adjusted to any value from zero to about 200 volts, which is well above the minimum available without this addition. The bleeder, R_8R_9, carries sufficient current to allow a drain up to 30 milliamperes between *C* and *B;* if a larger current is required, an additional bleeder may be connected from *A* to *C* to permit an output up to about 175 milliamperes.

If the voltage control is to be used both with and without the supplementary circuit, the latter should be supplied with a switch to disconnect its filaments and its plate connection at *d*.

Literature Cited

(1) Working, E. B., *Cereal Chem.*, 15, No. 4 (July, 1938).
(2) Working, E. B., IND. ENG. CHEM., Anal. Ed., 10, 397 (1938).

RECEIVED April 8, 1938. Contribution No. 56, Department of Milling Industry.

An Electronic Recording Analytical Balance

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IN THE course of photoelectric studies on the properties of fine suspensions, certain optical difficulties indicated the desirability of supplementing the optical measurements with sedimentation data on the same system. Recording balances for this purpose have been devised, notably by Oden (*2*, *4*) and Svedberg and Rinde (*5*). Many other problems, such as studies on desiccants, rate of reaction, etc., may be solved most conveniently with an instrument of this type. While the groundwork (*1*) has been laid for this problem, the extraordinary progress in electronics has provided means for an entirely new and improved solution.

The original instrument which was developed about a year ago (demonstrated at the meeting of the New York Section of the AMERICAN CHEMICAL SOCIETY, December 10, 1936, *3*) consisted of a standard chainomatic balance in which the chain mechanism was driven by a motor. A small target mounted on the balance pointer intercepted a sharply focused beam of light. A conventional photocell amplifier system operated a set of relays controlling the restoring motor. The balance was therefore continuously maintained in the balanced equilibrium condition, without any mechanical contacting of the system. The position of the chain was recorded by means of a contactor mounted on the chain block, which traveled over a slide wire. The potential drop along this slide wire was measured by a standard recording potentiometer. This instrument functioned satisfactorily, but certain obvious limitations suggested an improved design: The instrument would function in one direction only—i. e., a gain or loss in weight—sudden demands on the system could not be accommodated, and the relay system might become temperamental and require frequent adjustment. During the past year the authors have completely redesigned the system, and the present paper describes the improved instrument which possesses the following characteristics:

1. No mechanical or electrical contacting of the beam.
2. Rate of compensation proportional to the demand.
3. "Antihunting" circuit to prevent overshooting and tendency to oscillate about the equilibrium position.
4. Directional discrimination. The balance will respond instantly to an increase or decrease in weight without hesitation or "nervousness."
5. The recording is linear and direct-reading, requiring no extended computation from the record.
6. The time axis is linear and accuracy is assured, as the recorder is driven by a synchronous motor and will therefore record time as accurately as a Telechron clock. A choice of speeds is available.
7. The sensitivity is adjustable, so that one division on the record may be set at exactly 10, 1, or 0.1 mg. This is an electrical adjustment and necessitates no mechanical adjustment of the balance proper.
8. The over-all sensitivity is from 20 to 50 times greater than the normal sensitivity of the balance proper, owing to the very precise photoelectric scanning of the pointer since 0.04-mm. displacement of the latter is sufficient to energize the compensating motor.
9. Remote indication and automatic control are inherent features.
10. The instrument utilizes standard components—i. e. a magnetically damped chainomatic balance, a recording potentiometer, and readily available electronic parts. No elaborate mechanical or instrumental construction is involved.

A general view of the instrument is given in Figure 1. A standard relay rack provides the framework, the upper portion of which supports the balance. Below this is located the restoring motor. The remaining space is occupied by the recording-potentiometer amplifier and associated equipment.

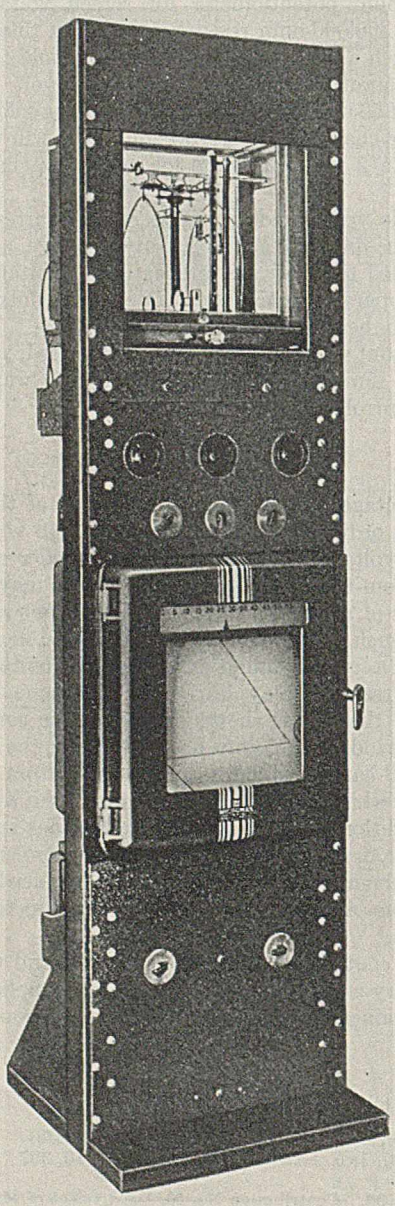

FIGURE 1

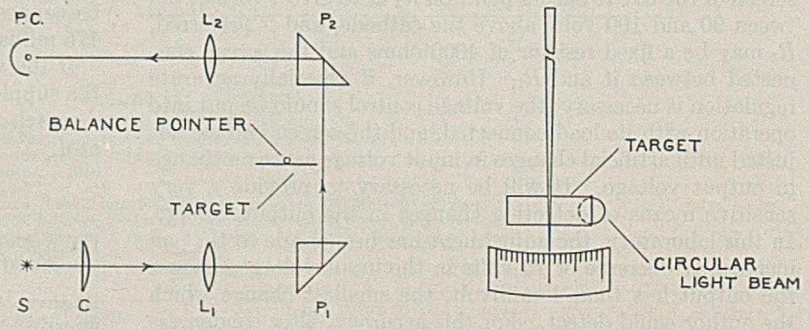

FIGURE 2

All necessary controls and pilot lamps are accessibly located on the panels.

The optical parts are located on the left wall and floor of the balance case. The light source and photocell are housed in an enclosed and thermally insulated case which replaces the glass window on the left side of the balance case. The arrangement is shown in Figure 2.

the pointer will therefore increase or decrease the amount of light reaching the photocell. The use of this large light beam permits directional discrimination and proportional response when combined with the appropriate photocell-amplifier circuit. In the older instrument a sharply focused beam was intercepted and provided abrupt on-or-off control.

The source is a 20-watt single-coil filament lamp operated from a Ward Leonard constant-current transformer. A circular stop defines the contour of the light beam.

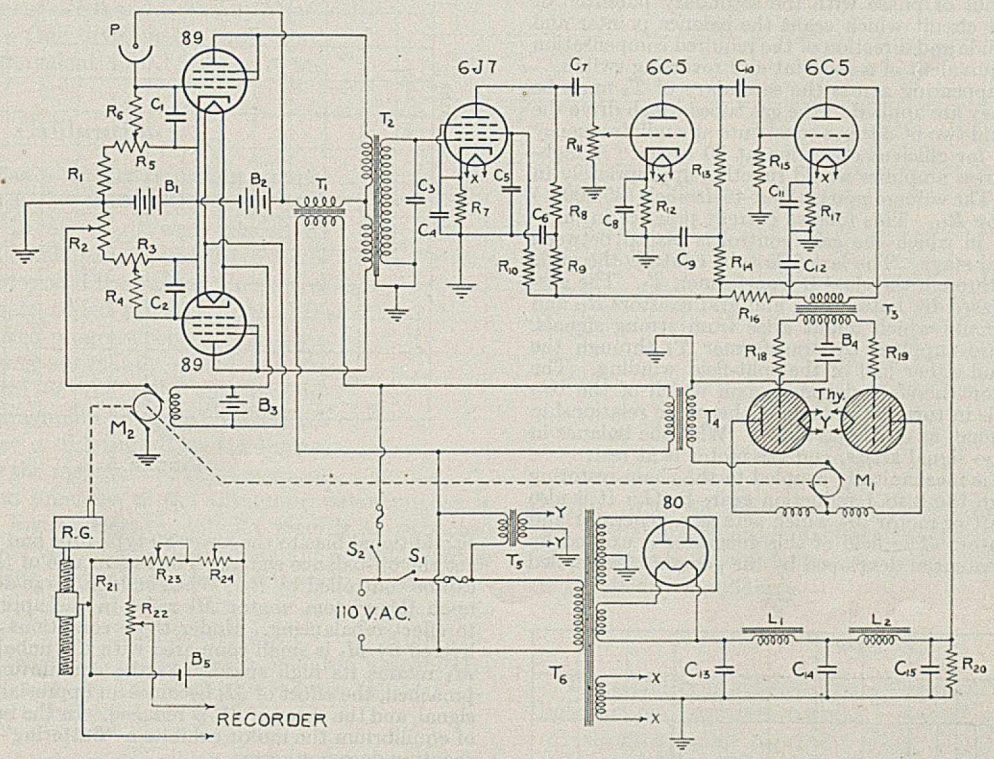

FIGURE 3. CIRCUIT

B_1, B_2. 45-volt B batteries
B_3. 6-volt storage battery
C_1, C_2. 0.01-mfd., 1500 volts, oil-filled
C_3. See text
C_4. 25 mfd., 10 volts, electrolytic
C_5. 0.1 mfd., 600 volts, paper
C_6, C_9, C_{12}. 2 mfd., 600 volts, paper
C_7, C_{10}. 0.5 mfd., 600 volts, paper
C_8, C_{11}. 50 mfd., 25 volts, electrolytic
C_{13}, C_{14}, C_{15}. 8 mfd., 600 volts, electrolytic
L_1. 22 H, Thordarson type T1892
L_2. 42 H, Thordarson type T7430
M_1. Electrical Specialties Co. split-field series motor
M_2. Bodine 1/75 horsepower direct current shunt motor with 8 to 1 gear reduction

R_1. 25,000 ohms, 1 watt
R_2, R_3, R_5. 25,000-ohm potentiometer
R_4, R_6. 5 megohms, 0.5 watt
R_7. 2200 ohms, 1 watt
R_8. 0.5 megohm, 1 watt
R_9. 50,000 ohms, 1 watt
R_{10}. 3 megohms, 1 watt
R_{11}. 1 megohm, volume control
R_{12}. 6000 ohms, 1 watt
R_{13}. 0.1 megohm, 1 watt
R_{14}. 10,000 ohms, 1 watt
R_{15}. 1 megohm, 1 watt
R_{16}, R_{18}, R_{19}. 10,000 ohms, 1 watt
R_{17}. 1000 ohms, 1 watt
R_{20}. 25,000-ohm voltage divider, 25 watts

R_{21}. 6-ohm slide wire
R_{22}, R_{23}. 200-ohm General Radio Type 314A
R_{24}. 5-ohm General Radio Type 314A
T_1. Universal output transformer, Thordarson type R107
T_2. Symmetrically wound interstage shielded transformer, Thordarson type T9005
T_3. Single pole to P. P. grids, Class B driver transformer, Thordarson type T5289
T_4. 110 volts to 110 volts at 0.5 ampere
T_5. 2.5 volts at 12 amperes
T_6. Power transformer, 680 volts at 55 milliamperes, 6.3 volts at 1.5 amperes, and 5 volts at 2 amperes

The condensing lens, C, forms a parallel beam on lens L_1. By means of prism P_1 a circular patch of light is brought to a focus in the plane of the small target which is mounted on the balance pointer. Prism P_2 and lens L_2 direct the beam to photocell $P.C.$ The optical components are arranged to offer the minimum interference to the normal handling of the balance.

The relative position of the light beam and target is shown on the right of Figure 2. In the balanced condition one-half of the circular light beam is covered by the target. A slight motion of

The circuit shown in Figure 3 may be resolved into five parts based upon their respective functions. In the upper left the two type 89 tubes, photocell P, and associated circuit form the detecting and discriminating element. Its function may be understood from the following:

Let R_3 be adjusted until the plate current of the lower 89 attains its normal value. If photocell P is illuminated with the half-

intercepted beam, the photocurrent flowing through R_6 will drive the grid of the upper 89 toward positive potentials. R_5 is adjusted to compensate for this until the plate current is equal to that of the lower 89. Since transformer T_1 applies an additional but alternating potential to the plates of both tubes, under this balanced condition the symmetrical pulsations in the two primary windings of transformer T_2 cancel and no potential appears at the secondary terminals. If now the light intensity at P increases or decreases, the plate current of either the upper or lower 89 predominates and cancellation in the primary of T_2 no longer occurs; consequently a potential will appear at the secondary of T_2 proportional to the change in light intensity and either in phase or 180° out of phase with the secondary potential of T_1. This photocell circuit which scans the balance pointer and decides the magnitude and direction of the required compensation is the electronic equivalent of a rheostat and reversing switch.

The potentials appearing across the secondary of T_2 must be amplified before they are applied to the gas tubes which drive the motor. The 6J7 and two 6C5 tubes constitute an audiofrequency amplifier designed for efficient operation at 60 cycles. A high-quality public address amplifier would function appropriately in place of this unit. The voltage gain is about 45 decibels (35,000×) and is controlled by R_{11}. This follows current practice in high-quality amplifiers, in which the gain control is placed between the first and second stage. The last 6C5 tube controls the grids of the gas triodes through the class B transformer, T_3. The gas-triode grids are biased by battery B_4, and grid resistors R_{18} and R_{19} limit grid currents which might arise from strong signals. Plate potentials are supplied by transformer T_4 through the motor armature and either half of the split-field winding. The direction of rotation therefore depends upon which of the two tubes fires, and this in turn depends upon the phase relationship of the incoming signal, as described above. With the balance in true equilibrium, no signal arises, and the motor is at rest.

The motor, M_1, is mechanically coupled to the chain-restoring mechanism through the 8 to 1 reduction gear, R. G. It is also coupled to the shaft of motor M_2 which acts as a generator and antihunting regulator. The field of this generator is excited by battery B_3. The potential developed by the generator is applied as additional bias to the lower 89 type tube and tends to counteract the off-balance signal. The magnitude of this compensation can be controlled by R_2. When sudden, large demands are made upon the system, motor M_1 races in the appropriate direction to effect rebalancing. Under these conditions the potential delivered by M_2 is small compared with the unbalanced signal, and M_1 retains its high speed. As the equilibrium position is approached, the effect of M_2 becomes an appreciable fraction of the signal, and the speed of M_1 is reduced. In the immediate vicinity of equilibrium the motor exhibits a "fluttering" action and overshooting does not occur.

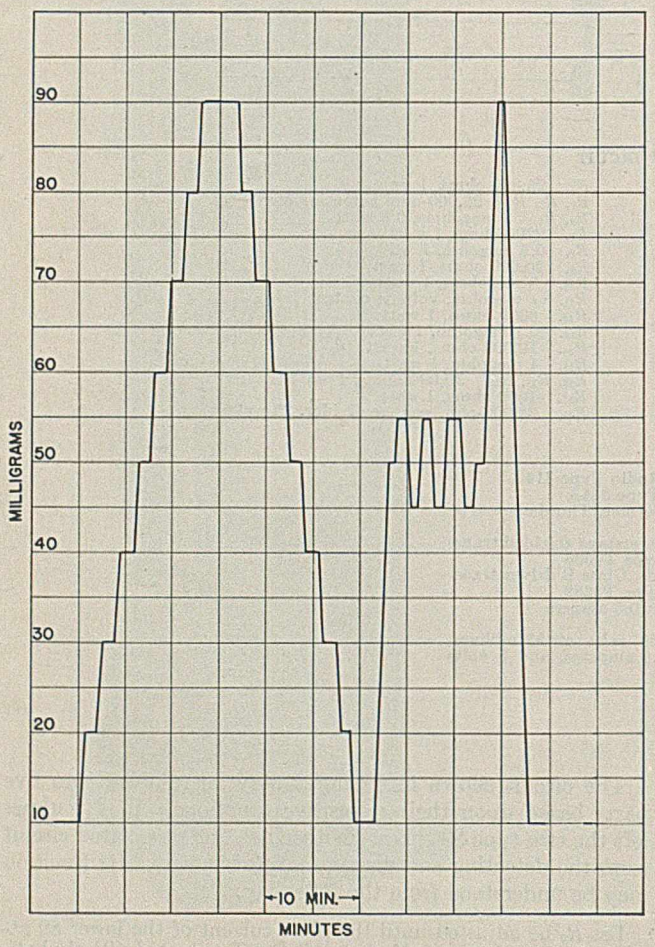

FIGURE 4

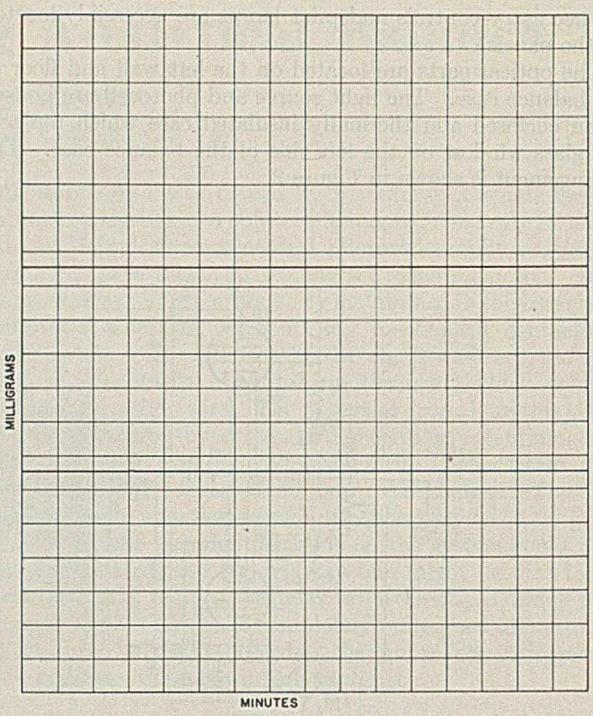

FIGURE 5

The chain-restoring mechanism is shown in the lower left. The helical feed screw supplied with the balance was replaced by one with smaller pitch—i. e., 20 threads per 2.5 cm. (1 inch). The block driven by this screw carries a hook for the free end of the chain and also a sliding contact moving over the uniform slide wire, R_{21}.

The position of this block, which carries the conventional vernier, establishes the instantaneous value of the weight of the sample. To record this and subsequent positions, the sliding contact picks off potentials from the slide wire which is fed by battery B_5 and network R_{22}, R_{23}, and R_{24}. The voltage divider, R_{22}, has two functions: It is equivalent to the end coils of a Kohlrausch slide wire and eliminates uncertainties in the exact positioning of the extremities of the wire, and it enables one to adjust the resistance of the network to the critical external damping resistance required by the recorder galvanometer.

The power supply shown in the lower right (Figure 3) requires no particular explanation, as it follows standard practice. Line fuses are included for protection and red and green pilot lamps (not shown in the circuit) are used to indicate filament and plate excitation of the gas triodes. The controls which appear on the panels (Figure 1) are those necessary for initial adjustment and operation. They include in addition to the switches and pilot lamps detector-discriminator-unit controls R_3, R_5; antihunting control R_2; amplifier gain control R_{11}; and recorder network R_{22}, R_{23}, R_{24} (for adjusting scale weights on recorder paper).

It has also been found convenient to include pilot lamps in the field circuit of M_1. These are located on the panel immediately under the balance case. The continuous "blinking" of either lamp indicates the direction of compensation which is taking place, no matter how small it may be.

The use of batteries may be considered objectionable, since in the main the instrument is operated from the power line. However, with the exception of B_3 which is not at all critical, all batteries deliver negligible current and their life is practically equal to the shelf life. Furthermore, the stability and reproducibility of the balance are in no wise affected by the potential of these batteries, with the possible exception of B_5. With little

additional difficulty this may be checked automatically from time to time, just as the working cell of the recorder is periodically monitored.

To those unfamiliar with electronic circuits, the arrangement may seem very complex. Aside from the detector discriminator unit, which is new, standard practice is followed and any circuit expert would understand it at a glance.

The recorder is a Leeds and Northrup Micromax Model S. One division on the recording paper is equivalent to 0.1, 1.0, or 10 mg.

Calibration

The performance of the recording balance is illustrated in Figure 4. In this case, carefully adjusted 10-mg. loads were placed on the pan and the balance was permitted to readjust itself and record the value. Successive 10-mg. loads were added up to a total of 90 mg., then removed progressively, and finally a single weight was alternately added and removed as shown on the right side of Figure 4. The maximum recorded deviations are of the order of 0.1 mg. This is a limitation imposed by the recorder and not a measure of the maximum precision of the automatic balancing, as is shown below. Figure 5 shows stability records at two constant loads. Over very long periods (days) the recorded values may show a drift which indicates the necessity of rebalancing the net-work, R_{22}, R_{23}, R_{24}, just as in standard potentiometer practice. The balancing system, however, is not subject to such fluctuations and will remain balanced indefinitely. This, of course, assumes the absence of gross thermal disturbances of the balance proper.

Reproducibility and Sensitivity

The precision of recording is limited by the total space covered on the record and the exactness of rebalancing greatly exceeds that which can be recorded conveniently. Successive automatic weighings of an approximately 20-gram load (20.050 grams) were as follows: 20.0500_5, 20.0500_0, 20.0501_0, 20.0500_5, 20.0500_5, and 20.0500_0. In this series of measurements, the balance was thrown off balance and allowed to readjust itself. When automatic compensation was completed, the weight was read from the column and accompanying vernier. No differences that could be estimated on the vernier could be detected. The sensitivity is therefore about ± 50 micrograms for a 20-gram load. Simple calculation showed that a 0.02-mm. displacement of the pointer would suffice to start the compensating motor. The high sensitivity is therefore due to the very delicate scanning system. No claim is made for the absolute value of the weights listed as it was of no interest at this point.

Accuracy

It is obvious that the high reproducibility and sensitivity will have little to do with the accuracy of weighing, which will be governed by the reliability of the chain. Indeed this is the present limitation. It is always possible to calibrate the chain with artificial standards and apply empirical corrections. With more difficulty the chain may be adjusted bit by bit until it is uniform. Either procedure is entirely unnecessary if one is content with ordinary sensitivity and accuracy, but where inordinate sensitivity is available it becomes of interest to see from what direction further improvements must come. With all the limitations of present-day chains, they are probably preferable to electromagnetic balancing schemes where an empirical relationship between current and weight is practically mandatory.

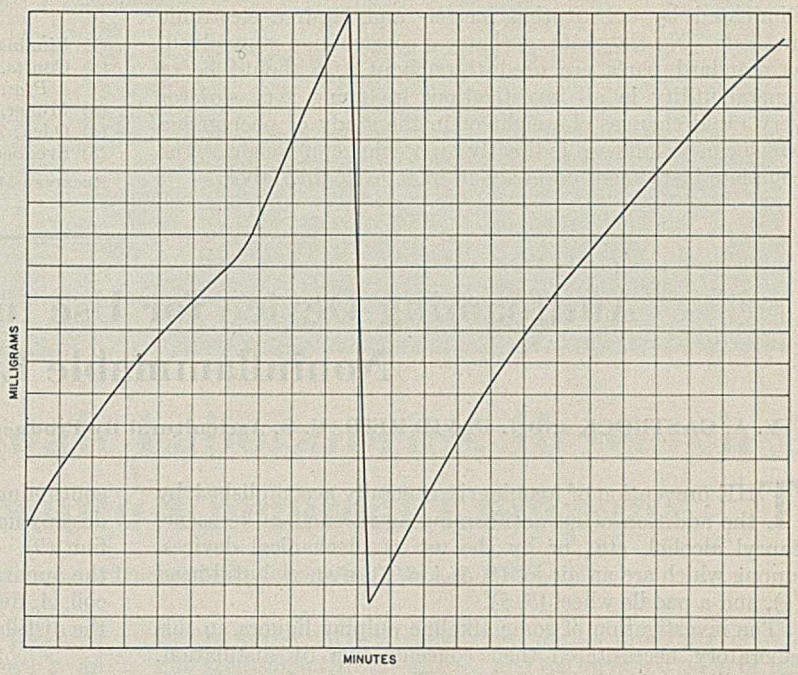

FIGURE 6

Stability

The stability is independent of the small fluctuations in the light source (constant-current transformer). The question of vibration arises naturally, since motors are mounted in the assembly. Although these are mounted on sponge rubber bases, there is a very faint but perceptible tremor in the system. Careful investigation of this point has led to the conclusion that these high-frequency, symmetrical tremors are in no wise objectionable but may possibly contribute to the establishment of a "dynamic" equilibrium. Gross asymmetric shocks, occasioned by heavy vehicles or the slamming of doors, are of course as detrimental here as with any sensitive balance.

Line voltage variations have no influence on stability and can only cause changes in sensitivity and speed of response. Inasmuch as both factors are far in excess of requirements, such effects are negligible.

Range

With the present arrangement, weight changes of ± 100 mg. can be accommodated without manual attention. The range can be extended by the use of a heavier chain with little sacrifice in sensitivity. When changes greater than 100 mg. are encountered an additional 100-mg. weight can be placed on the pan; the chain block will race to the zero position and then resume its normal course. This is illustrated in Figure 6, in which a particularly rapid weight increase is recorded. An automatic mechanism may be provided to release 100-mg. weights as required (*1*, *4*).

Summary

An electronic recording analytical balanc is described which is direct-reading, rapid, and sensitive. All mechanical or electrical contactors, relays, and the like have been

eliminated by substituting inertia- and lag-free electronic methods. No elaborate mechanical construction is involved, as standard parts are used throughout. It should be of general utility in all investigations involving very slow or very rapid changes of weight or in the study of phenomena which can be followed indirectly by a change of weight. The complete instrument costs about $500, excluding labor.

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RECEIVED March 12, 1938.

Antifoaming Device for Use in Concentration of Noninflammable Liquors

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THE prevention of foaming is generally accomplished by the addition of an antifoaming agent such as kerosene, capryl alcohol, etc., or by the use of mechanical devices, among which are an air jet (*2*, *3*, *4*, *6*, *7*), a special still-head (*1*), and a paddle wheel (*5*, *8*).

The investigation of some alkaline pulping liquors, in this laboratory, necessitated their concentration by distillation. These liquors foamed profusely. The commonly used antifoaming agents were unsatisfactory because they were inefficient, their presence interfered with subsequent chemical examination of the residue, and the volatile agents were lost by distillation. Fanto (*2*) used a current of cool inert gas to break the bubbles by condensation of the enclosed vapor, but stated that sometimes the liquid was carried over mechanically, so that it was necessary to redistill.

Certain evaporators of commercial size control foaming by continuing the heating surfaces above the liquor level, thus destroying the foam by contacting and vaporizing the liquid film (*9*). An improvement on this action is made use of in the apparatus shown in Figure 1, whereby the heat from the coil, *A*, disrupts the foam bubbles without contact and allows the distillation to proceed at a rapid rate without carry-over.

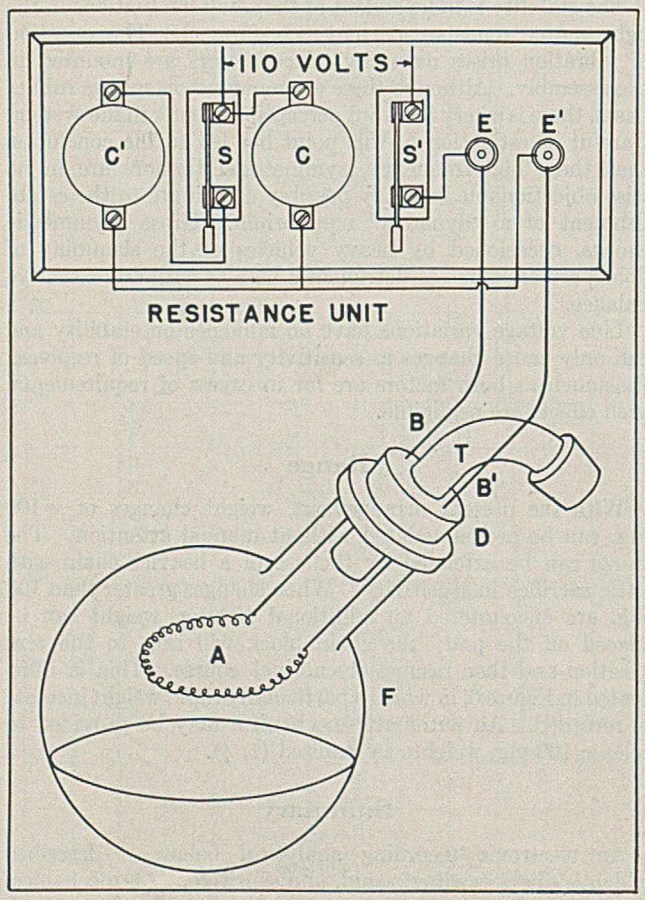

FIGURE 1. DIAGRAM OF APPARATUS

Apparatus and Operation

The assembled apparatus is shown in Figure 1. The foam-breaking coil, *A*, consists of 88 cm. of No. 22 gage (0.64-mm.) Nichrome wire, in the form of a helix, fastened to No. 16 gage (1.3-mm.) copper wire leads, *B* and *B'*. The leads pass through the stopper, *D*. Coil *A* must be placed low enough to avoid undue heating of the flask wall above it. The power is 110-volt alternating current, suitably controlled by a resistance unit or transformer.

The resistance unit used is made by connecting two 500-watt heating coils, *C* and *C'*, mounted in porcelain sockets, with switches, *S* and *S'*, so that one or both may be used. Switch *S'* controls the unit. The temperature of coil *A* is higher when switch *S* is closed than when it is open. The binding posts, *E* and *E'*, are convenient for connecting the copper leads, *B* and *B'*. Material for the unit costs about one dollar.

Flask *F* should have a capacity of 2 liters or more, providing sufficient room to place the hot wire, *A*, at least 4 cm. from the glass above it. The liquid should be heated to incipient boiling before the current is turned on. Ordinarily, only coil *C* is used as the wire, *A*, need not be at red heat. However, the wire should be hot enough so that the bubbles burst at least 1 cm. from the wire, in order to prevent the wire from becoming coated by material which would then dry and burn. The temperature of wire *A* may be varied by changing its length or gage, or by changing the resistance of the coils, *C* and *C'*. Condensate from tube *T* should not drop on the hot wire. Once started, the distillation proceeds at a rapid rate without further attention.

Discussion and Conclusions

The antifoaming device described is limited in use to the distillation of noninflammable substances. It has proved successful in the laboratory distillation and concentration of alkaline pulping liquors that foam excessively and should prove useful in the distillation of many aqueous foam-producing solutions. There appears to be no reason why it should not be applicable to large-scale apparatus.

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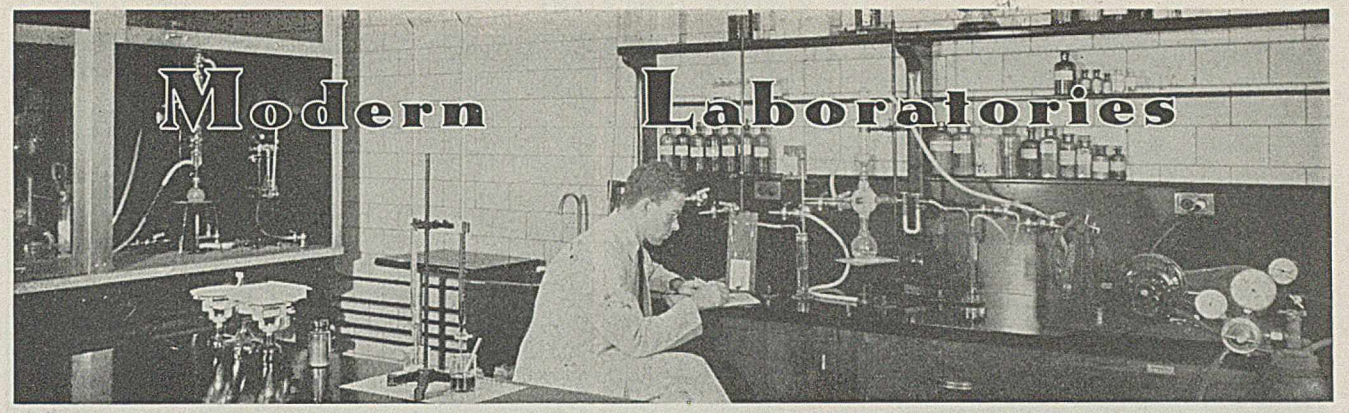
RECEIVED May 26, 1938.



Thomas and Hochwalt Laboratories, Research Division of Monsanto Chemical Company

MARY B. MOSHIER, Thomas and Hochwalt Laboratories, Dayton, Ohio

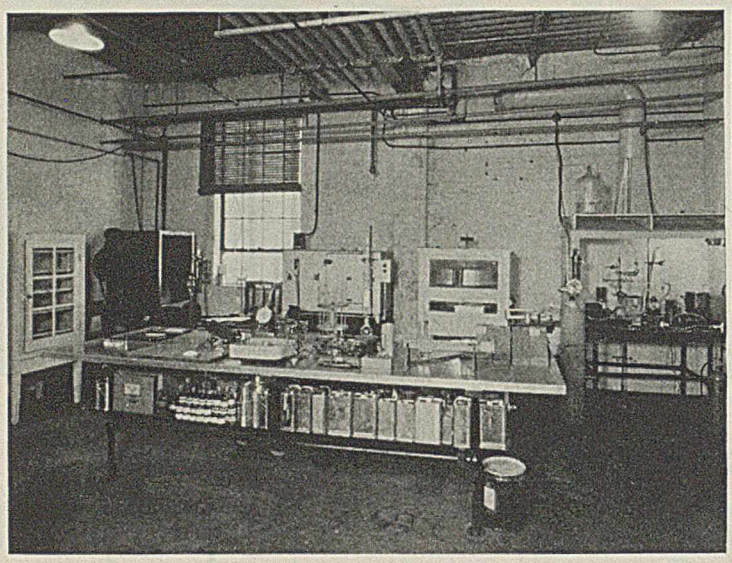
THE Thomas and Hochwalt Laboratories, a Research Division of the Monsanto Chemical Company, is an ideally situated and well-planned unit at the outskirts of Dayton, Ohio. The quiet, almost rural setting, free of the distractions usually coincident with urban or industrial surroundings, is an excellent site for a chemical research laboratory. The main part of the building was erected in 1929 as the then new home of the Thomas and Hochwalt Laboratories, Incorporated. It was designed by Coles and Coleen, architects of Chicago. In 1936, upon the merger of the laboratories with the Monsanto Chemical Company, a harmonious addition was made to the building under the supervision of Douglas Lorenz, a Dayton, Ohio, architect.

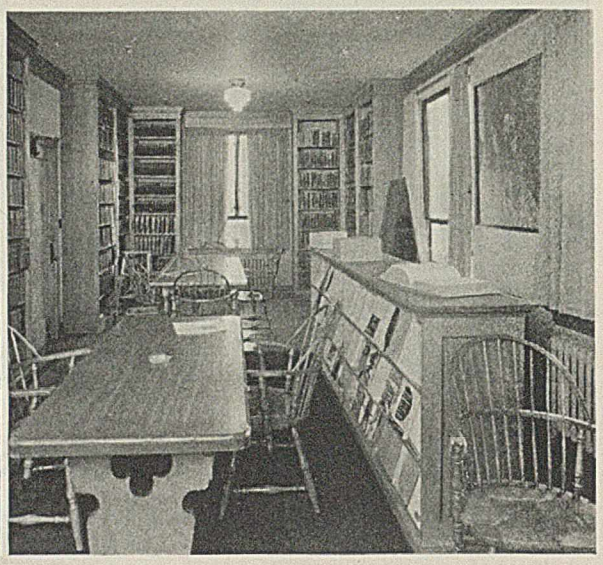
The Main Building

The structure, including the added wing, provides 12,200 square feet of floor space. The front section, comprising air-conditioned, sound-proofed offices and library, is of two stories. The remainder of the building is a one-story, L-shaped structure which houses fourteen laboratories, an instrument room, an office for chemists, a sample room, a stock room, a wash room for apparatus, locker room, dining room, and kitchen. The latter, together with the dining room, is located at the end of the new addition, thus providing for outside accessibility to the kitchen and for quiet seclusion of the dining room. Corridors placed between the administrative section and the laboratories, and between the new wing of the laboratory and the old section, diminish extensive fire hazard.

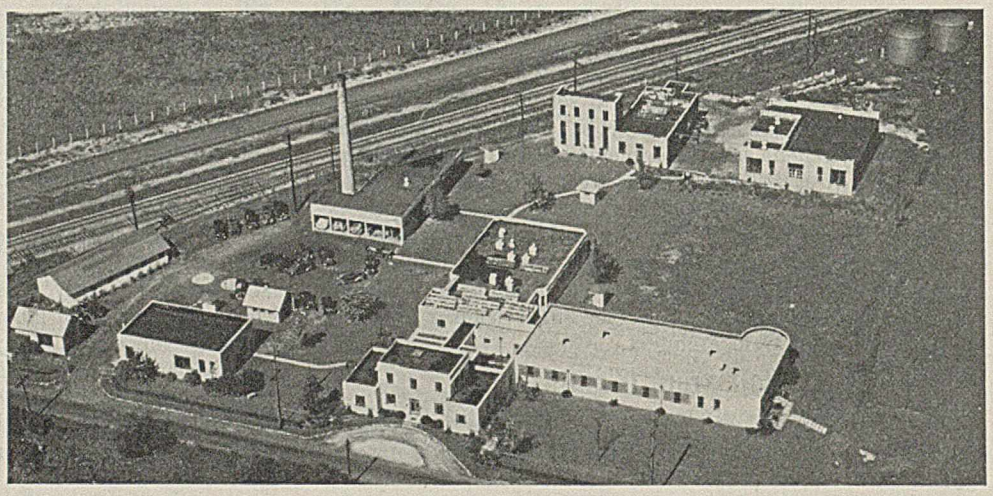
As can be seen in the accompanying layout of the plant, the floor space afforded by each laboratory is somewhat varied. Such variation is desirable in a research laboratory where the type of problem determines the space required for its execution. As a rule, not more than two men are assigned to each laboratory; generally, these two men work on the same problem. Although each laboratory is provided with desks, the chemists' office and the library afford additional privacy. The spaciousness of each laboratory makes possible the retention of assembled apparatus from one job to another. Except in two large laboratories, the benches are arranged around the sides of each room in order to prevent possible obstruction of light. Treatment of the tops of the benches with an acid-proof stain has been found to give satisfactory protection against chemicals. The use of wood tops instead of a harder surface has also been found to cut down on breakage of glassware.

Although the entire plant is equipped with a sprinkler sys-





COATINGS LABORATORY AND LIBRARY



AERIAL VIEW OF LABORATORIES

tem, each laboratory is provided with a shower placed over sinks, as a further safety measure against fire or chemical injury. Fume hoods are found in each laboratory. Because the fan for each hood is mounted on the roof of the building, quietness in operation is attained. Whenever possible, additional precautions against poisonous gases are taken—for instance, in several of the laboratories the Mine Safety Appliance carbon monoxide alarm is used.

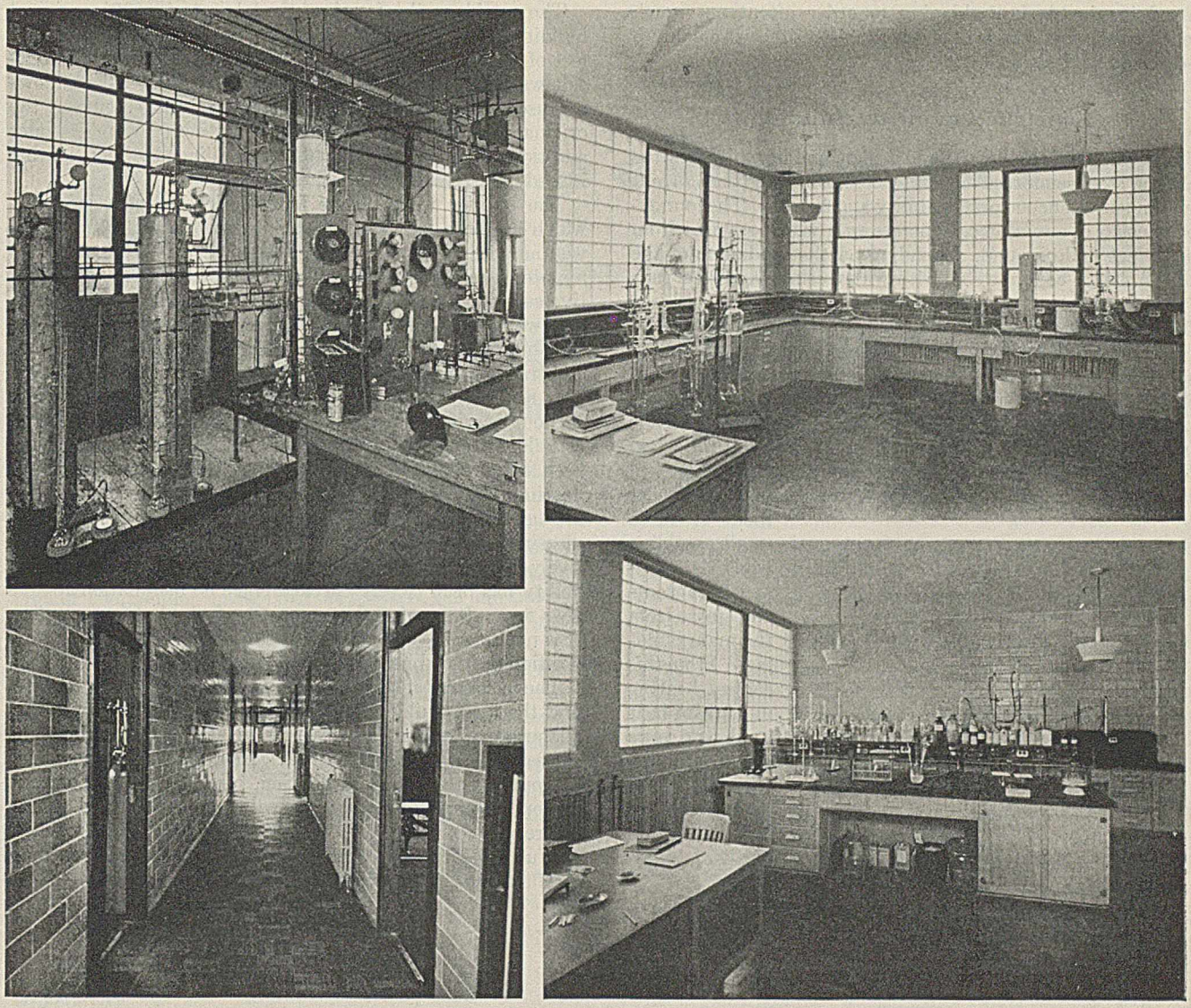
Each laboratory is an individual unit, fitted with the necessary equipment to carry on research of a specific nature. Ample closet and shelving space enables the storing of each man's apparatus and supplies within his own laboratory. Sinks are plentifully supplied, but washing of all apparatus and equipment is done in the centrally located washroom by a man employed for that purpose.

Since Monsanto's interests are broad, the type of research carried on necessitates almost every type of chemical equipment and apparatus. Balances and other equipment which require special care, such as gas-analysis apparatus, Podbielniak fractionating columns, constant-temperature baths, and pH determination outfits are kept in the instrument room. Instruments used for the evaluation of special compounds are found in the materials-testing laboratories.

Except in a few cases, however, the evaluation of materials synthesized in the laboratories is not conducted at the Dayton unit. The greater part of the work deals with organic and inorganic synthesis in the laboratory and its application and control in pilot-plant work. Hence in most laboratories is found that type of equipment which is generally associated with research in synthesis. The study of gaseous reactions, necessitating the use of rheostats for controlling the temperature of the reaction chamber, flowmeters, thermocouple switches, and wash towers, for instance, is conducted in one of the laboratories; another laboratory is equipped for high-pressure work. Equipment for the study of thermal conditions of reactions—for example, calorimetric determinations of heats of reactions—is provided for in the analytical laboratory, where is also found other apparatus used in determining data necessary for the elucidation and control of organic and inorganic reactions.

The Library

Since chemists at the laboratories realize that adequate chemical literature is as important as is equipment and other working facilities, the library at the laboratories has been carefully fostered. Begun by Charles A. Thomas and Carroll A. Hochwalt some twelve years ago, it has had a phenomenal growth and now carries a current subscription list of some eighty American and European chemical journals. Although housing facilities do not permit the binding of each journal, all are kept on file in steel stacks in a room especially designated for that purpose. A great many of the more important journals are bound, however, and complete files of these, including the American, British, and German abstracting journals, are in the library. An especially designed magazine rack is used for the current journals. Besides indispensable reference literature like Beilstein, Landolt-Bornstein,



PILOT PLANT, CORRIDOR, AND TWO LABORATORIES

Richter, Mellor, etc., there is a good collection of monographs and publications on fields of especial interest. A file of catalogs and technical literature is kept in the library as well as an extensive file of American and foreign patents.

The Dining Room and Kitchen

Because the laboratories are not located in an industrial section of the city, a dining room and kitchen were deemed indispensable and were accordingly incorporated into the new addition to the building. The dining room accommodates sixty people. A decorative scheme has been carried throughout, and it is air-conditioned and sound-proofed. All kitchen equipment is of stainless steel.

The Pilot Plants

Besides the main structure, three smaller buildings afford facilities for pilot-plant work and materials testing. With due regard to fire protection these are spaced at least 100 feet apart from the main building and from each other. They provide a total of 8800 square feet of additional floor space. Here are found units for studying vapor-phase reactions and a unit for studying gaseous reactions at high pressure. Opportunity is given for the study of other reactions on a pilot-plant scale. Electrically operated controls are used as are automatic temperature controls, indicating thermocouples, and precision gas meters. A 2200- to 220-volt transformer ensures ample power for the operation of the pilot plants. Here, too, are found such auxiliary equipment as assay furnaces and small gas muffles and electric muffles, allowing a wide range of temperature control. An optical pyrometer is used for measuring the temperatures of these furnaces. For large-scale fractionation, there is a twenty-plate column of approximately 20-gallon capacity. Similarly, in the protective coatings laboratory, housed in a separate building, is found such apparatus as temperature baking ovens, spray booths, grinding mills, kettles, weatherometer, and other equipment which is ordinarily found in laboratories of this kind. Provision is made for local exposure of panels on roofs.

The Machine Shop

It has been found that very often a novel piece of equipment needed can best be made to specifications in the laboratories. For this reason, one of the smaller buildings houses a machine shop which is in charge of an able mechanic who is constantly kept busy at making some piece of laboratory or pilot-plant equipment designed by a chemist. Although hearkening back to old "glass-blowing" days, the laboratory machine shop has

proved invaluable in fashioning to order oftentimes much needed equipment, the lack of which might otherwise hold up research on a problem.

Construction of the Building

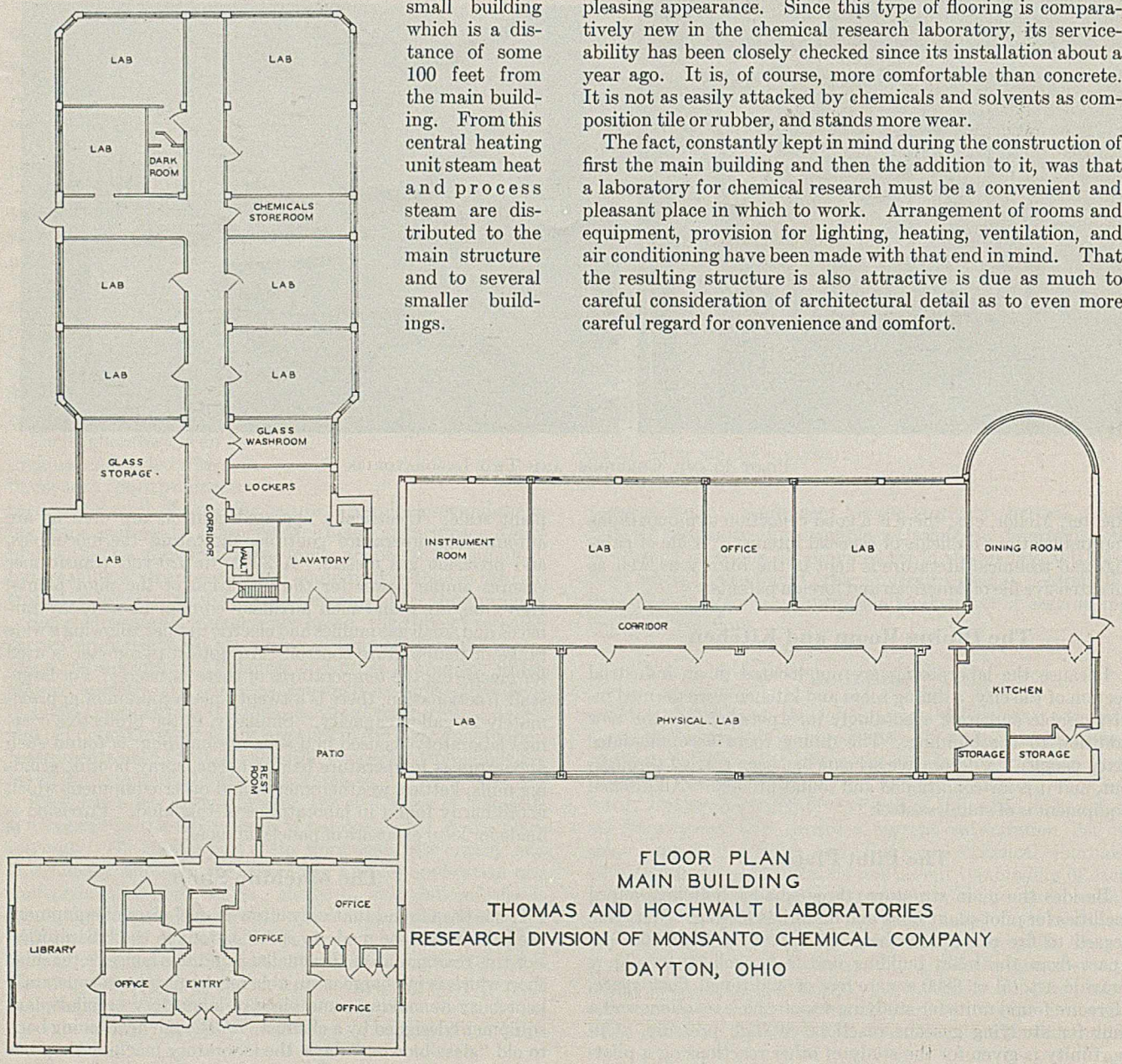
The structure is modernistic in design, and its white-painted walls of steel-reinforced concrete conform with the current trend in simultaneous practicability and charm. The foundation consists of a reinforced concrete wall which extends from 5 to 7 feet underground, depending on the grade-line of the site. A 4-inch concrete slab with wire meshing and steel rods is used for flooring throughout the building. The roof is a 2.5-inch concrete slab laid on rib lath over Truscon joists. It is covered with layers of pitch, felt, and composition roofing. Suspended ceilings with 4 inches of insulation are used. Copper flashings and down spouts afford adequate roof drainage.

A concrete tunnel, 6 feet wide and 6.5 feet high extends throughout the length of both the main building and the wing. This is used for all gas, water, and steam piping, for electrical conduits, and for air-conditioning compressors. The boiler room is in a small building which is a distance of some 100 feet from the main building. From this central heating unit steam heat and process steam are distributed to the main structure and to several smaller buildings.

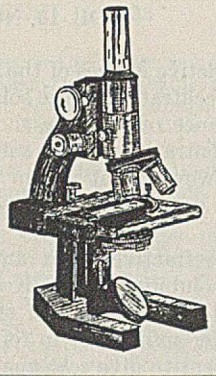
The outside walls of each of the laboratories in the original building have steel sash windows reaching from within 4 feet of the floor to the ceiling. In the new addition the outside walls are of glass bricks. Here small steel sash windows are used for ventilation. An attractive patio between the original building and the more recently added wing provides additional light. Throughout the building, indirect lighting is used for artificial illumination.

The interior walls of the laboratories are of glazed tile, which has been found to provide maximum light diffusion as well as general serviceability, and to require a minimum of maintenance expenditure. The partitions in the administrative section of the building, on the other hand, are gypsum tile, which is plastered and painted to harmonize with the birch millwork. In the laboratories, Kalamein doors, frames, and transoms are used as an added measure of fire protection. The flooring throughout has been planned to give service without sacrificing appearance. In the offices and library, cork floor in color to harmonize with the light birch scheme, has been laid on the concrete slab used as the foundation for all the flooring. Walnut wood blocks cemented to the concrete slab are used in the laboratories, and present an exceptionally pleasing appearance. Since this type of flooring is comparatively new in the chemical research laboratory, its serviceability has been closely checked since its installation about a year ago. It is, of course, more comfortable than concrete. It is not as easily attacked by chemicals and solvents as composition tile or rubber, and stands more wear.

The fact, constantly kept in mind during the construction of first the main building and then the addition to it, was that a laboratory for chemical research must be a convenient and pleasant place in which to work. Arrangement of rooms and equipment, provision for lighting, heating, ventilation, and air conditioning have been made with that end in mind. That the resulting structure is also attractive is due as much to careful consideration of architectural detail as to even more careful regard for convenience and comfort.



FLOOR PLAN
MAIN BUILDING
THOMAS AND HOCHWALT LABORATORIES
RESEARCH DIVISION OF MONSANTO CHEMICAL COMPANY
DAYTON, OHIO



Microchemistry

Microtechnic of Organic Qualitative Analysis

Determination of Solubility

FRANK SCHNEIDER AND D. GARDNER FOULKE, Department of Chemistry, Trinity College, Hartford, Conn., and School of Chemistry, Rutgers University, New Brunswick, N. J.

IN A PREVIOUS paper (*2*) the authors presented the general technic for the determination of the solubility of organic compounds, using both the *schlieren* cell and the capil lary tube. In the present paper an extension and elaboration of these technics are given.

The determination of the solubility of a substance in various solvents is of increasing importance, since it is used not merely for classifying the original substance in certain groups as done by Kamm (*3*) and Shriner and Fuson (*7*) but also for the subdivision of groups as described by Mulliken and Huntress (*6*). It must be kept in mind, however, that a purely qualitative test is not sufficient and that the variety of solvents employed excludes some otherwise simple and obvious methods. For example, while the dissolving of a crystal in a liquid such as water can readily be observed on a microscope slide or under a lens, the semiquantitative results required cannot be obtained, and working with highly volatile liquids such as ether is obviously out of the question. Centrifuge cones cannot be used because of their comparatively large volume which necessitates the use of a large sample in order to obtain the solute-solvent ratio. Linde and Gardner (*5*) use a slide with a cylindrical well in semimicrowork, the slide being illuminated by a special lamp. The quantities of material used, are much larger than usually considered in microwork.

TABLE I. RESULTS WITH CAPILLARY METHOD (LIQUIDS)

Substance	Water				Ether				5% HCl				5% KOH				H_2SO_4			
	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d
Acetaldehyde	1	25	0	+	1	25	0	+	1	20	0	+	2	30	0	+	1	25	0	*
Methyl acetate	2	55	0	+	1	10	0	+	1	10	0	+	1	15	0	+	2	20	0	+
Acetone	2	20	0	+	2	10	0	+	3	25	0	+	1	25	0	+	2	20	0	+
Aniline	1.5	40	1	−	1	10	0	+	7	50	0	+	2	30	2	−	3	30	P	..
Dimethylaniline	2	55	2	−	1	20	0	+	5	75	1	+	3	55	3	−	3	30	0	+
Benzaldehyde	2	50	2	−	1	20	0	+	1	25	1	−	1	25	1	−	2	50	0	+
Benzene	2	50	2	−	2	30	0	+	3	60	3	−	5	60	5	−	3	35	3	−
n-Butyl alcohol	3	90	0.25	−	1	25	0	+	1	25	0.2	−	1	30	0.2	−	6	30	0	+
tert-Butyl alcohol	2	50	0	+	2	38	0	+	1	25	0	+	1	30	0	+	5	50	0	+
Allyl alcohol	3	20	0	+	1	20	0	+	3	60	0	+	1	30	0	+	2	55	0	+
Anisole	2	30	2	−	1	25	0	+	1	25	0.5	−	3	75	2	−	1	25	0	+
Benzyl alcohol	3	90	2.5	−	1	20	0	+	2	50	2	−	1	30	1	−	1	20	P	..
2-Methyl-2-butanol	1	24	0	+	1	28	0	+	2	50	0	+	2	45	0	+	5	50	0	+
Carbon tetrachloride	1	20	1	−	1	25	0	+	1	20	1	−	1	20	1	−	1	20	1	−
Chloroform	1	20	1	−	1	28	0	+	1	20	1	−	1	20	1	−	1	20	1	−
Cinnamaldehyde	2	27	2	−	2	40	0	+	1	20	1	−	1	20	0.9	−	1	22	0	+
o-Cresol	2	50	2	−	1	20	0	+	2	50	2	−	1	10	0	+	1	25	0	+
Epibromohydrin	3	62	3	−	1	22	0	+	2	47	2	−	2	42	2	−	2	20	0	+
Ethyl phenyl ketone	2	55	1.9	−	1	26	0	+	1	20	1	−	1.5	22	1.3	−	1	10	0	+
Quinoline	2	50	2	−	1	10	0	+	1	27	0	+	1	20	1	−	1	20	0	+
Cyclohexanol	2	52	1.5	−	1	25	0	+	1	25	1	−	3	45	3	−	2	30	0	+

a = mm. solute b = mm. solvent c = mm. undissolved solute d = + soluble, − insoluble * = much charring P = precipitate

TABLE IA. RESULTS WITH CAPILLARY METHOD (SOLIDS)

	Water			5% HCl			5% KOH			H_2SO_4		
	a	b	c	a	b	c	a	b	c	a	b	c
1,2-Diphenyl-1-propene	0.2	5	R	0.2	5	R	0.2	4.5	R	0.2	6	R
Palmitic acid	0.2	5	R	0.2	5	R	0.3	7	S	0.2	6	R
Sodium benzenesulfonate	0.1	2	S	0.2	4	S	0.1	2	S	0.2	6	R
β-Naphthol	0.2	5	R	0.1	5	R	0.2	5	S	0.5	10	S
Phenol	0.2	3.2	S	0.1	2	S	0.3	3.2	S	0.2	4	S
Uric acid	0.2	5.0	R	0.2	5	R	0.2	6	S	0.3	5	S
Trinitrotoluene	0.1	2.5	R	0.1	2.6	R	0.2	6	R	0.2	7.5	R
2,4-Dinitrotoluene	0.1	5	R	0.1	5	R	0.1	5	R	0.2	7.5	R
Phthalimide	0.2	5	R	0.2	6	R	0.2	5	S	0.2	4	S
Anthracene	0.2	8	R	0.4	10	R	0.2	8	R	0.3	8	R
Maltose	0.2	5	S	0.2	5	S	0.1	3	S	..	..	..

a = mg. solute b = mg. solvent (density × volume) c = R, residue, insoluble; S, no residue, soluble

TABLE II. REFRACTIVE INDEX OF SATURATED AQUEOUS SOLUTIONS

Substance	Pure Substance	Saturated Solution	Water	Δn_D	Solubility of Substance in 100 Parts of H_2O
Quinoline	1.6283	1.3345	1.3325	0.0020	Slightly soluble
o-Toluidine	1.5728	1.3350	1.3325	0.0025	1.5
Ethyl ether	1.3515	1.3355	1.3325	0.0030	7.5
Methyl alcohol	1.3288	1.3288	1.3325	0.0037	∞

TABLE III. SENSITIVITY OF SCHLIEREN DETECTION

Substance	Δn_D[a]	*Schlieren*
Quinoline	0.0020	−
o-Toluidine	0.0025	−
Ethyl ether	0.0030	+
Methyl alcohol	0.0037	+
Sucrose (aqueous solution)	0.0020	Very faint
Sucrose (aqueous solution)	0.0011	−
Hydroquinone (aqueous solution)	0.0050	+
α-Naphthylamine (aqueous solution)	0.0025	Very faint

[a] Δn_D is the difference in refractive index of the fluid and static samples.

The two procedures found applicable by the authors are the capillary and the *schlieren* methods.

Capillary Method

This method was described in the first paper at this series (*2*). In the case of solids it has been found advisable to determine at once whether or not the substance is soluble in the maximum amount of solvent permissible under the definition of what constitutes "soluble" without determining the exact solvent-solute ratio of a saturated solution. That is, the sample of the substance was weighed out in the capillary and then 25 times as much solvent was added. If a residue remained after mixing, the substance was designated as "insoluble," if none remained undissolved, "soluble." Although the volume of the solid cannot be determined by the length of solid layer in the capillary, a diminution in the volume on adding additional solvent can be determined accurately. Results obtained using this method are given in Tables I and IA.

The capillary method is recommended for all determinations in which a semiquantitative result is desired. For very rapid determinations of solubility in which the analyst wishes to know only whether the substance is soluble according to the definition of Kamm (*4*)—i. e., 1 part in 25 parts of the solvent—the *schlieren* method is to be preferred. The technic has been modified considerably from that described previously (*2*) and hence will be given in detail.

Schlieren Method

The appearance of *schlieren* depends upon the fact that the fluid and static samples (*1*) differ in refractive index. If, as in this case, the samples differ by the presence of the solute in one, the appearance of *schlieren* will depend on the degree to which the amount of solute will change the refractive index of the solvent. If refractivity is regarded as an additive property, the change in refractive index will depend on two factors: (1) the difference in the refractive indices of the solvent and the solute; and (2) the concentration of the solute. Theoretically, therefore, the appearance of *schlieren* should not be a measure only of the concentration of the solute. The following experiments show, however, that within certain limits *schlieren* can be used as a direct measure of solubility.

Several compounds with a high refractive index and a solubility in water below the limit solubility set by Kamm (*4*)—that is, 4 per cent—were selected. Solutions of these compounds might be expected to differ sufficiently in refractive index from that of the pure solvent to give *schlieren* despite their low solubility. Several other compounds with a refractive index very close to that of water but with a solubility above the limit were also used. These would not be expected to give *schlieren* unless in very high concentrations. The refractive index at 18° C. of saturated solutions of all these substances was determined by means of an Abbe refractometer. The results are shown in Table II.

From Table II it is evident that if a method of observing *schlieren* which is sensitive to differences of refractive index of not less than 0.0025 is used, it should be possible to distinguish between "soluble" and "insoluble" substances by the use of *schlieren*.

In order to reduce the sensitivity of *schlieren* detection to this value, the usual "*schlieren* microscope" or the "visual method" of Emich (*1*) cannot be used. Emich states that the sensitivity of the microscope method of observation is Δn_D 0.00005 and that of the visual method, Δn_D 0.0001. He also suggested the use of test tubes with a reduction in sensitivity to Δn_D 0.0005. The authors have found by experiment that if a simple glass tube 4 mm. in inside diameter, 6 mm. in outside diameter, and about 60 mm. long sealed at one end, is used in place of the usual *schlieren* cell or the one mentioned in the first paper of this series (*2*) and the method of illumination is changed (discussed later), the sensitivity of the *schlieren* observation is reduced to the desired value. Results of some experiments in which water was used as solvent are shown in Table III.

TECHNIC FOR LIQUIDS. The substance to be tested is used as the fluid sample. The *schlieren* tube is filled with the solvent to

TABLE IV. SOLUBILITY OF ORGANIC SUBSTANCES

Substance	H_2O	Ether	5% HCl	5% NaOH	H_2SO_4	Solubility Group *Schlieren* method	Solubility Group Kamm's method
Solids							
Anthranilic acid	−	+++	+++	+++	..	III	III or IV
Acetanilide	−	++	(+?)	−	..	VII	VII
Benzoic acid	−	++	....	+++	..	IV	IV
Benzidine	−	++	+++	−	..	III	III
β-Naphthol	−	+++	−	+++	..	IV	IV
Phthalimide	−	+	−	+++	..	IV	IV
Phenol	+++	+++	+++	+++	..	I	I or III
Sodium benzenesulfonate	+++	−	+++	+++	..	II	
Sucrose	+++	−	+++	+++	..	II	II
Resorcinol	+++	+++	++	+++	..	I	I
Trinitrotoluene	−	+	−	++	..	IV	IV
Uric acid	−	−	−	++	..	IV	IV
Liquids							
Ethyl acetate	++	+++	+	−	++	I or V	I or V
Ligroin (b. p. 90–120°)	−	+++	−	−	−	VI	VI
Acetic anhydride	+++	++	++	+++	..	I	I
Ethyl ether	+		(+?)	−	++	I or V	I or V
Aniline	(+?)	+++	+++	−	..	III	III
Dimethylaniline	−	+++	+++	−	..	III	III
Quinoline	−	+++	+++	−	..	III	III
Ethyl alcohol	+++	++	++	+++	..	I	I
Glycerol	+++	−	+++	++	..	II	II
Benzyl alcohol	−	+++	−	−	Ppt.	V	V
Nitrobenzene	−	+++	−	−	..	VII	VII
Acetophenone	−	+++	−	−	++	V	V
Acetone	+++	++	++	++	..	I	I
Acetic acid	+++	++	+++	+++	..	I	I
Toluene	−	+++	−	−	−	VI	VI
Ethyl bromide	−	+++	−	−	−	VI	VI

− No *schlieren*.
(+?) Very faint *schlieren* (regarded as negative).
+, ++, +++ *Schlieren* observed.

serve as the static sample. The fluid sample is drawn into a capillary pipet varying in diameter from 0.5 to 1.0 mm. The more viscous the substance, the wider the capillary pipet should be. The *schlieren* tube is held so that the illumination is obliquely downwards, as obtained, for example, by holding the tube below the (horizontal) edge of a lamp shade, or below a horizontal crossbar of a window. The illumination should not be too intense. When only very small amounts of substance are available, the fluid sample may be introduced, as suggested to the authors by A. A. Benedetti-Pichler, into the static sample by first absorbing it in a very small piece of porous tile which has been fixed in a platinum wire and then dipping the tile into the static sample. In the case of inert solvents such as water or ether, a piece of ashless filter paper may also be used.

TECHNIC FOR SOLIDS. A saturated solution of the solid substance is first prepared by placing a drop of the solvent in a depression of a spot plate and adding the solid until some remains undissolved. In the case of solvents which are pure substances, a saturated solution may also be prepared by making up a solution on the spot plate and allowing it to evaporate until a crust forms. The clear solution is taken up in a capillary pipet and used as the fluid sample. In the case of solvents which are already solutions, such as 5 per cent hydrochloric acid and potassium hydroxide, a control consisting of several large drops of the solvent is placed in an adjacent depression of the spot plate and is allowed to stand until the fluid sample has been taken up in the pipet. Then the control is used as static sample. In this way differences in refractive index between the static and fluid samples which might result from partial evaporation and consequent concentration of the solvent solution are avoided. The same technic as used for liquids is followed from this point on.

The *schlieren* method of determining the solubility of organic substances was tried on 28 compounds. The results are given in Table IV. These results indicate that the *schlieren* method of determining solubility places each compound in the correct group according to Kamm. The method has the advantages of great simplicity and speed.

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RECEIVED June 8, 1938. This is the second paper in this series. For previous article, see reference (2).

Microscopical Determination of Potassium with Naphthol Yellow S

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Potassium may be determined qualitatively in the presence of ammonium by means of naphthol yellow S. Ammonium, cesium, lithium, magnesium, and sodium ions do not react with the reagent to form insoluble crystalline salts. Cupric, lead, rubidium, and silver ions form precipitates and may mask the microscopical test for potassium.

RECENTLY Clark and Willets (*4*) have reported the use of a new reagent, naphthol yellow S (2,4-dinitro-1-naphthol-7-sulfonic acid), for the qualitative macrodetection of potassium. Since the ammonium ion does not interfere with this test, it was decided to investigate the possibilities of using this reagent in the determination of potassium by microscopical methods.

One of the best micromethods for the detection of potassium ion uses chloroplatinic acid (*8*). However, the ammonium ion (as well as rubidium, cesium, and tellurium) forms with the reagent insoluble crystals which are isomorphous with those of potassium (*2*). The perchloric acid (*5*), bismuth sulfate, and tartrate tests (*8*) also serve for potassium-ion determination, but the salts formed are isomorphous with the insoluble ammonium salts. Thus, in all these tests the ammonium ion must be removed from solution before proceeding with the test for potassium.

Solutions Used

The naphthol yellow S used for the test reagent was thrice recrystallized from aqueous solution. The original material had previously been utilized for the preparation of 2,4-diamino-1-naphthol-7-sulfonic acid according to the method of Lauterbach (*7*). The product when further recrystallized from hydrochloric acid yielded the trihydrate mentioned by Knecht and Hibbert (*6*). The physical constants could not be used to determine the purity, as the literature merely records (*6*) a melting range of 140–150° C. with decomposition occurring at 175° C. The product used melted between 148° and 149.5° C. Although Clark and Willets (*4*) reported a greater sensitivity with 2 and 5 per cent solutions, the reagent solution used in this laboratory was more dilute (an aqueous one saturated at 20° C.) because of the difficulties involved in using warm solutions in microtests. When a drop of the test solution (with no further dilution) was placed on a slide it was found that with a room temperature of 23° C. one had to wait 13 to 17 minutes before crystals, discernible under the microscope, formed.

In testing for sensitivity, solutions varying in potassium-ion concentration from 0.05 to 40 mg. per ml. were prepared from recrystallized reagent grade potassium chloride.

In observing the effect of related ions on the reagent and of the presence of foreign ions on the potassium-ion determination, solutions of C. P. sodium chloride, lithium bromide, ammonium chloride, cesium chloride, rubidium chloride, silver nitrate, lead nitrate, magnesium acetate, and cupric nitrate, containing 40 mg. per ml. of the cation were prepared and used.

Procedure

In determining the sensitivity of the test, one drop of the test solution containing the cation studied was placed on a slide by means of a platinum loop. A similar drop (approximately 0.04 ml.) of the reagent was placed next to the test drop and the two were brought together by means of a sharpened platinum wire probe. For the foreign ion effect one drop of the solution containing the foreign ion was added to the drop containing the potassium ion before addition of the reagent.

Sensitivity

When the reagent solution is added to a drop of solution containing potassium ion, well-defined acicular and fiberlike crystals form which may be single, although predominantly occurring as bundles (Figure 1). These crystals are characteristically different from the fine, individual unclumped fibers appearing upon slow evaporation of a drop of the reagent solution. Although Clark and Willets report that the reagent yields a positive macrotest with a potassium-ion concentration of 0.39 mg. per ml. (the precipitate taking 5 hours to form), it was found that a concentration of 7.5 mg. per ml. was required to yield a positive microscopical test immediately upon addition of the reagent. This eliminates possible error due to crystallization of the free acid from solution. Obviously, although smaller concentrations may be detected, it is inconvenient to await extremely long periods of time in microtesting. Using the capillary pipet method suggested by Benedetti-Pichler and Spikes (*1*), and a 0.5 per cent reagent solution, and permitting the mixed drop to stand for 3 minutes, the limit of identification was found to be 1.9 micrograms and the limiting proportion of ammonium to potassium, 200 to 1.

FIGURE 1. POTASSIUM SALT (× 100)

FIGURE 2. RUBIDIUM SALT (× 100)

Effect of Foreign Ions

Neither sodium, lithium, ammonium, magnesium, nor cesium ions yielded crystals of any sort upon addition of the reagent. Rubidium, on the other hand, formed sharply defined acicular fiberlike crystals primarily in the form of bundles. Few individual crystals were formed (Figure 2). However, the great similarity in color, size, shape, and form between rubidium and potassium crystals makes it practically impossible to distinguish between the two. The presence of magnesium, ammonium, and the other alkali metal ions, with the exception of rubidium, has no visible effect upon the potassium crystallization. Semimicrotests were also made to determine whether any of these foreign ions had any macro-effect upon the precipitate. Clark and Willets indicate that the presence of sodium causes a darkening of the flocculent yellow potassium precipitate.

Half-milliliter portions of the 20 mg. per ml. potassium-ion solution were placed in a series of 3-ml. centrifuge tubes. To one of these tubes were added 0.5 ml. of distilled water and 1.5 ml. of the reagent solution. Half-milliliter portions of the foreign ion solutions were added to the other tube before addition of the reagent. The solutions were well stirred with a platinum wire and then permitted to stand for 10 minutes. Precipitation occurred much more rapidly in those solutions containing the added ions. The tubes were placed in a microcentrifuge and spun at 3600 r. p. m. for 5 minutes. Visual comparison showed no difference in volume or color of the precipitates formed.

In a further study it was found that silver, lead, and cupric ions also yielded typical crystalline precipitates with naphthol yellow S. These crystals form rapidly. Lead yields smaller, shorter, and thicker fibers as well as small nodules and spherulitic bundles. Cupric copper slowly forms large bundles of needlelike crystals, appearing yellow-green in color under crossed nicols. The potassium is easily distinguishable from the crystals of these three metals.

As is to be expected, a slight concentration of a strong acid prevents the formation of the potassium precipitate. One drop of 6 *N* hydrochloric, nitric, or sulfuric acid is sufficient to prevent precipitation from 1 ml. of the 20 mg. per ml. potassium test solution. However, as much as 1 ml. of concentrated acetic acid has no effect, the precipitate forming as rapidly as from a neutral solution. Work is under way to determine the exact pH range over which the test is valid, as well as the effect of other ions. Because rubidium forms a very insoluble salt with the reagent while cesium does not, it is believed that the reagent may be used to advantage to distinguish between the two. Chamot and Mason (*3*) say that the differentiation is not easy (between rubidium and cesium), since in most tests dependence must be placed upon differences in solubility alone. It is the author's intention to determine how well this test may be used for such a differentiation.

The foreign anions studied here were chosen because they are the ones most apt to interfere in the determination of potassium.

Summary

Using an aqueous solution of naphthol yellow S saturated at 20° C. in the microscopical determination of potassium, the limit of concentration has been found to be 7.5 mg. per ml. Permitting 3 minutes for crystallization and using a 0.5 per cent reagent solution, the limit of identification is 1.9 micrograms and the limiting proportion of ammonium to potassium is 200 to 1.

Magnesium, sodium, lithium, ammonium, and cesium ions do not form insoluble compounds with the reagent and

do not affect the test. Rubidium forms crystals similar to those of potassium. This test therefore fails in the presence of rubidium. Although silver, lead, and cupric ions yield crystals characteristically different from those of potassium, these ions may tend to mask its presence.

Small amounts of free strong acids prevent the formation of the insoluble salts, although appreciably large amounts of acetic acid have no effect. Neutralization of the acidic solutions by means of potassium-free sodium hydroxide permits the use of the test.

Acknowledgment

The author would like to express his appreciation to C. E. Coates for his criticism and suggestions.

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Melting and Boiling Points on a Micro and Macro Scale under Various Pressures

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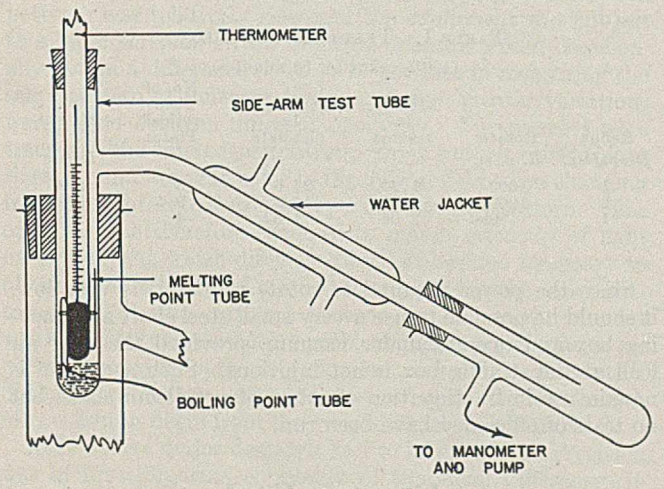
A SIMPLE apparatus, shown in the accompanying diagram, has been found very useful for determination of melting, boiling, and freezing points.

The apparatus consists of two side-arm test tubes and a water jacket which need not be sealed on. A Thiele tube is used to avoid the necessity of stirring. The liquid bath is paraffin oil, and the source of heat is gas (microburner), alcohol, or electrical resistance wire, as described by Bergstrom (*1*). Cleaning is done with alcohol and ether applied with a medicine dropper.



Determinations may be made on either a micro or a macro scale, and the results, without temperature correction, are in close agreement with values given in the literature.

The micro boiling point is determined by a modification of the Shriner and Fuson method (*2*). Place a drop or two (0.05 to 0.1 ml.) of liquid at the bottom of the tube. Attach a small inverted melting point tube to the thermometer bulb which is lowered until the open end of the capillary is below the liquid surface. Heat. Remove the flame when a steady stream of bubbles is expelled from the capillary. When the liquid rising in the cooling capillary is level with the outside liquid (vapor pressure equals atmospheric pressure) the boiling point is reached.

The micro boiling and melting points of a solid may be determined in the same operation by placing some crystals at the bottom of the tube and lowering the thermometer into the molten solid after the melting point has been taken.

Acknowledgment

The author wishes to express his appreciation to Abraham Mazur of The City College of the College of New York for his valuable aid and criticism.

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RECEIVED March 17, 1938.

Microdetermination of Arsenic

The article on "Microdetermination of Arsenic" [IND. ENG. CHEM., Anal. Ed., 10, 226 (1938)] suggests that the following information might be of assistance to laboratories which have had trouble in the selection of zinc suitable for the analysis. After trying several brands and adding several things to the solution of the acid, this laboratory secured satisfactory results by using "zincum metallicum puriss. chemisch. rein. in bacillis 8 mm., pro anal.," of E. Merck, Darmstadt, Germany.

N. V. LIJM- EN GELATINEFABRIEK "DELFT"
DELFT, HOLLAND

A New Type of Semimicro Fractionating Column

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A LARGE number of fractionating devices for use on 1 to 10 cc. of liquid have been described in the last decade. Among the simpler and more practicable may be mentioned those of Cooper and Fasce (*1*), Weston (*4*), and Craig (*2*).

In an attempt to devise a column showing high efficiency, large throughput per hour, and low tendency to slugging, a number of columns using rotating members as packing were tested. The form finally adopted has a metal band rotating at about 1000 r. p. m. in place of the usual packing or indentations. The complete apparatus for use at atmospheric pressure on solutions of 1 to 10 cc. is shown in Figure 1.

The length of the condenser was 10 cm. (4 inches), that of the column proper 37.5 cm. (15 inches). The column was a length of Pyrex tubing of 6-mm. inner diameter, to which the heating jacket was sealed. Nichrome wire wrapped around the jacket was used to provide approximately adiabatic conditions in the column. The boiler was placed on an asbestos board provided with a hole slightly smaller than the bulb and heated with a microburner. Asbestos cord was used to provide partial insulation of the portion of the boiler above the asbestos board.

The assembled apparatus was tested with a 2.52 mole per cent solution of carbon tetrachloride in benzene and with a mixture of 1 cc. of methanol and 1 cc. of water.

Table I shows the results obtained with the band rotating at 1000 r. p. m. Using the refractive index–mole per cent data and curves of Zawidzki (*5*) and the methods of calculation outlined by Walker, Lewis, and McAdams (*3*), between 15 and 16 theoretical plates for the 37.5-cm. (15-inch) column and still head combined were obtained. Since the empty column with same still head showed only 4 plates, the still head evidently could not have added more than 2 to 3 plates and the spinning column must have shown at least 13 plates for its 37.5 cm. (15 inches) of length or almost one plate per inch under total reflux. An interesting and somewhat unexpected fact developed was that the column did not reach equilibrium with this mixture in less than 1 hour of total reflux. Equally unexpected was the fact that its tendency to slugging is so low that the 6-mm. inner diameter column could be operated with a reflux above 2 drops per second, although, as the results show, the efficiency was lowered somewhat by such a high reflux rate.

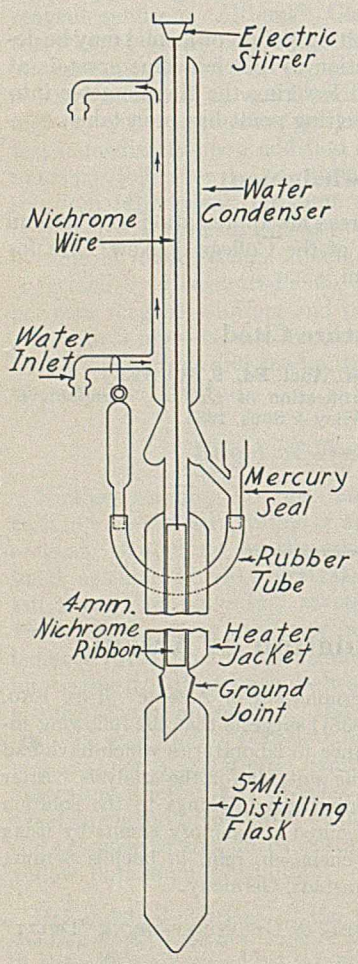

FIGURE 1. APPARATUS

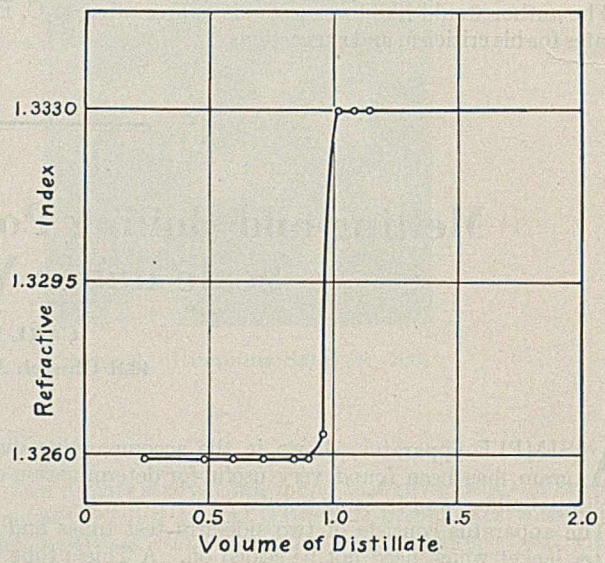

FIGURE 2. DISTILLATION OF 10 CC. OF METHYL ALCOHOL IN 10 CC. OF WATER

Figure 2 shows that the relatively simple methanol-water mixture can be cut in 80 minutes to yield only 1 cut of 0.06 cc. that is not practically pure methanol or water.

TABLE I. TESTS FOR EFFICIENCY

(100% reflux for 80 minutes)

Reflux	Rotator	Mole Per Cent CCl_4 In residue	Mole Per Cent CCl_4 In distillate	No. of Theoretical Plates	H. E. T. P.
Drops/sec.	*R. p. m.*				*Inches*
1	1000	1.96	26.6	15	1.0
2	1000	2.01	14.2	9.5	1.5
1	0	2.04	4.82	4	3.8

Since the power required to rotate the band is very low, it should be possible to use a very small steel shaft and packing box and operate under vacuum, provided the slow air leak at the stuffing box is not injurious. Such a column is now in use in fractionation of esters of petroleum acids, but no tests of efficiency have been run.

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A New Microphotometer

For Analyzing X-Ray Diffraction Patterns of Raw Cotton Fiber

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AT NO PERIOD during the history of the cotton industry in America has there been a greater demand than at the present time for information concerning the properties and characteristics of raw cotton fiber and their effects on the spinning qualities. These demands come from cotton breeders, buyers, manufacturers, and consumers and are due in large part to the increased production of foreign cottons and to competition of artificial fibers.

An attempt is being made in the Cotton Utility and Standards Research Section of the Bureau of Agricultural Economics to determine the most reliable measures of cotton quality and to develop more rapid methods for evaluating the known factors. Tensile strength is considered to be one of the more important factors affecting quality in cotton fibers. The strength may be determined by the "Chandler bundle method" (*1*) or any other method which takes into account the cross-sectional area of the fibers, but reliable methods so far developed are slow and laborious.

The object of this series of studies is to develop a more rapid method for estimating or measuring the tensile strength of raw cotton. The application of x-ray technic to this problem was first undertaken by members of the staff of the Bureau of Agricultural Economics, in informal coöperation with Wayne A. Sisson (*3*), who was at that time working at the University of Illinois as a senior fellow of the Textile Foundation and a collaborator of the Bureau of Agricultural Economics. These studies showed a relatively high degree of correlation between certain dimensions of the x-ray diffraction patterns and the Chandler strength of raw cottons.

The method used by Sisson to measure the x-ray diffraction patterns was relatively slow and the equipment was subject to a large personal error. A single operator would occasionally obtain a difference of 10 to 15 per cent in measuring the same pattern at different times, and even greater variations were found between different operators. These errors were traced to the microdensitometer, which among other things showed a difference in color in the optical field when changing from the lightest to the darkest part of the x-ray pattern. The operator was therefore required to match intensity of light on two objects which differ in color. It became necessary to obtain a more practical method for measuring the x-ray patterns, and it was apparent that no commercial instrument was suited for this particular problem. The present paper reports the progress of the investigation, including a description of the instrument developed for measuring the tensile strength of raw cotton fibers from the x-ray diffraction patterns.

At least five major demands had to be satisfied. Precision was of first importance because of the small differences in intensity to be measured. Speed of operation was also of primary importance, since it is necessary to measure a large number of patterns in standardizing the method. It was desirable to make the instrument flexible, so that modifications could be made. The instrument must be simple and easily operated by an ordinary laboratory technician. A slight difference in cost could be sacrificed in order to get these characteristics, but the price must not be prohibitive. With these characteristics in mind, the simplest arrangement that gave promise of success was chosen.

Description of Microphotometer

A microphotometer has been developed which consists essentially of a constant light source, a simple optical system for limiting the illumination to a very small section of the pattern, a means of measuring the light transmitted by this section, and a method of moving the desired portion of the pattern into the optical path.

Figure 1 shows a side view of the microphotometer which was designed by the authors and built in the shops of the Bureau of Agricultural Economics. Except for the instrument panel on the front (Figure 2), which is of aluminum alloy metal, and the top cover of brass plate, the main case is constructed of plywood. The photoelectric cell, *a*, of Figure 1 (Photox cell made by the Westinghouse Electric and Manufacturing Company) is located directly below the photo-exciter lamp, *b*, and the light is focused through a slit in the top cover of the box. The film is placed on the mechanical stage, *d*, between the lamp and the photocell, so that it just clears the raised section of the cover in which the slit is located. The film can be rotated or moved back and forth, to bring any portion of the pattern under the light beam. The photoelectric cell is connected to a galvanometer, *g*, of the reflecting type (sensitivity 0.0024 microampere per mm.).

Figure 2 shows the translucent galvanometer scale across the end of the box which is illuminated by a spot of light from lamp *c* (Figure 1). A cross hair is placed in front of the lamp in such a position that its image is formed on the scale.

A 3-candle-power, 6-volt, 0.5-ampere automobile tail-lamp bulb was found to be satisfactory for a photo-exciter lamp. The lamp voltage is controlled by means of two rheostats of 10 and 100 ohms resistance connected in parallel.

The housing for the photo-exciter lamp is constructed of aluminum alloy and has a ventilator in the top to prevent overheating in case a large lamp is necessary. The lens system consists of two plano-convex lenses with diameters of 24.5 mm. each, and focal lengths of 51.2 mm., mounted 2.5 cm. (1 inch) apart with the curved sides facing each other (*C*, Figure 3). The tubular housing, which was made of two sections, one 7.6 cm. (3 inches), the other 2.5 cm. (1 inch) in length, permits a total movement of the lenses of 1.9 cm. (0.75 inch). The shorter section tapers at the lower end so that it has an aperture of 0.48 cm. (0.19 inch). The lamp housing is adjusted so that the small end of the tube comes to rest directly above the slit. The film is placed between the end of the tube and the slit, and an image of the filament of the lamp is focused on the slit.

Figure 4 is a view looking down on the top of the instrument. The photo-exciter lamp is held in position by means of an arm, which fits on a post, *h*. The post is a steel rod about 2.2 cm. (0.875 inch) in diameter, mounted by means of a shoe on the side of the case. The arm connecting the lamp housing to the post is about 5 cm. (2 inches) wide at the narrowest point (Figure 2) and 0.6 cm. (0.25 inch) thick. The arm may be lengthened or shortened slightly by adjusting the nut on a bolt extending through the flanges of a loop, *j*, near its middle (Figure 4). The lamp housing can be raised or lowered on the post, since the sleeve of the arm rests on a clamp which is adjustable on the post. The lower edge of the sleeve adjacent to the clamp fits into a circular groove in the clamp which also contains a V-shaped groove into which the beveled edge of the projecting arm comes to rest. In this way, the lamp can be readily lifted out of position and swung around out of the way while exchanging films, but returns to the same position each time it is placed over the film.

The compound mechanical stage with which the patterns are centered and brought into position for the measurements is made of three units. The large square metal plate (1, Figure 4) is movable in the direction parallel with the long axis of the instrument. It rides on two tracks, one of which contains a groove to keep the stage in a fixed position perpendicular to the direction of movement. This stage moves by means of a rack and pinion and has a metric scale with a vernier reading to 0.1 mm.

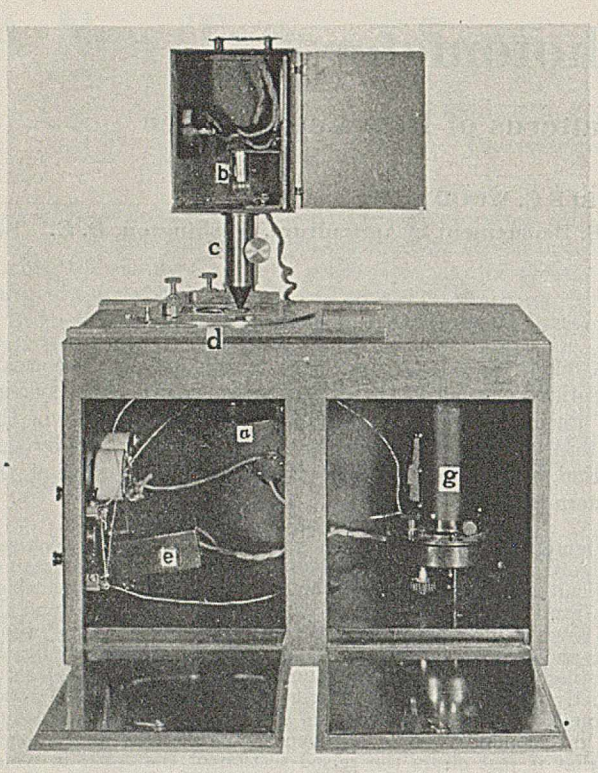


FIGURE 1. SIDE VIEW OF MICROPHOTOMETER

Showing various parts through opened doors

The second unit (2, Figure 4) consists of a rotary stage built into the large square stage. It is 19 cm. (7.5 inches) in diameter and is calibrated in degrees, with a vernier reading to 0.1 degree. The central portion of this stage is removable, so that a variable-sized central opening can be used, depending on the size of the x-ray pattern to be measured. Ability to match the size of the pattern with the central opening aids considerably in centering the films. The rotary stage is revolved by means of a gear which permits accurate adjustment.

The third unit (3, Figure 4) is similar to a microscope mechanical stage, but is adapted for x-ray films. One carriage of this stage contains two thin metal arms, 0.08 cm. (0.03 inch) thick and 0.6 cm. (0.25 inch) wide which ride flush with the surface of the rotary stage. A steel spring on each arm holds the film in position. Cross lines drawn on an unexposed film are slipped under the metal arms and adjusted over the central opening of the rotary stage to facilitate centering of the pattern. For final adjustment, the cross lines are removed, the exciter lamp is placed in position, and the film is accurately centered with the aid of the galvanometer.

A section of the top cover of the case beneath the mechanical stage, about 5 cm. (2 inches) in diameter at the base and 1.9 cm. (0.75 inch) at the top, is raised in the form of a section of a cone (*n*, Figure 5). The top portion of this cone is covered over except for a circular hole about 2.5 mm. in diameter, about 0.6 cm. (0.25 inch) from the center. This opening is located so that a line drawn through its diameter and the diameter of the raised portion of the cover is parallel with the movement of stage 1 (Figure 4) into which the rotary stage is built. Such a line would bisect the rotary stage and the pinhole cap when they are in position.

Two metal caps, each of which fits over the top of the conelike projection, were constructed. Each cap contains a series of pinholes or openings, one a series of five circular holes, the other a series of four rectangular slits, all located 0.6 cm. (0.25 inch) from the center of the cap, so that any one can be rotated into position. The circular pinholes are 0.5, 0.75, 1.0, 1.5, and 2.0 mm. in diameter, respectively. Three of the rectangular slits are arranged with their long axes in the direction of radii of the cap and the fourth is perpendicular to a radius. The three slits along the radii may be used to determine the intensity around an arc or ring, whereas the other may serve to determine the distance between rings or spots or the relative intensity of the individual rings. The openings along the radii are 0.5 × 1.0, 0.5 × 1.5, and 0.5 × 2.0 mm., respectively, and the one perpendicular to the radius is 0.5 × 1.0 mm. The smallest of the radial rectangular slits has been found most satisfactory.

For the particular purpose for which this instrument was designed, readings need not be made in standard units, since the technic depends solely upon ratios of a given set of measurements. However, means were provided for calibrating the apparatus so that, if desired, the readings can be expressed in the usual terms of density, transmission, or opacity. This is done by means of a set of three "neutral" glass filters with densities of 0.357, 0.603, and 1.19, respectively. The first was mounted permanently in the instrument, so that it can be swung into position above the photocell at any time.

The luminous transmissions of the filters for the conditions of operation have been calculated and the results may be used to convert the galvanometer readings to actual transmissions when so desired. (The spectral transmissions of the filters were measured with a General Electric recording spectrophotometer by the Bureau of Standards, U. S. Department of Commerce. Data on the spectral response of the Photox cell were also supplied by the Bureau of Standards.) The filters are sufficiently "neutral" so that small errors in color temperature of the lamp or in color response on the cell will not appreciably affect the values obtained for their luminous transmission.

Although exact linearity of response of the photocell to light intensity is not essential to this particular problem, such a response was found to exist over the entire range of the light intensity used. By using low light intensity, with a sensitive galvanometer, the galvanometer deflections were found to be proportional, within the limits of accuracy in

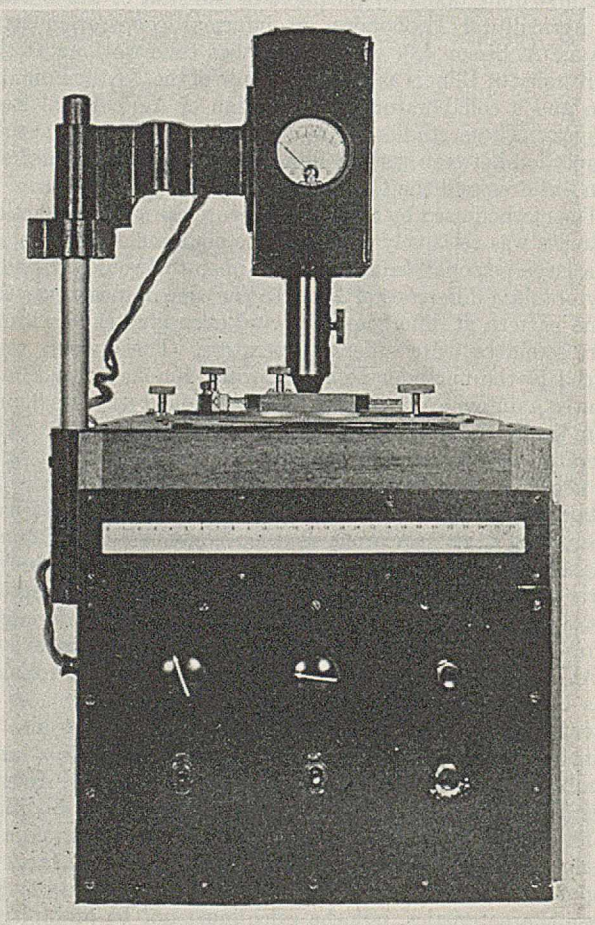


FIGURE 2. FRONT VIEW OF MICROPHOTOMETER

reading the scale, to the transmissions (0.440, 0.250, and 0.0646, respectively) of the filters.

The microphotometer has been found very stable and reliable. The 6-volt lamp used as a source of illumination on the film is operated at about 4.7 volts from a heavy-duty battery and has remained stable over long periods of time. When properly adjusted, the galvanometer readings, as checked by the filter, did not drift more than 1 mm. in an hour and often not enough in this time to be noticeable on the scale. Changes over long periods of time in the lamp or in the photocell will not affect the results. The only exception is the error which would be introduced if the shape of the response curve of the photocell should change. In over a year's use, such change as may have occurred was not sufficient to be observed in repeated measurements of the standard filters.

Method

The bundles of fiber were prepared in the same way as in the Chandler method (*1*) except for wrapping. After combing and adjusting to the correct size (0.3-cm., 0.125-inch, circumference) they were placed in the clamps and wrapped from one end to the opposite end, using a single thread with a 907-gram (2-pound) weight on it. Cellulose acetate or collodion was then placed on the wrapped thread and allowed to dry. A portion of the thread near the middle of the bundle was then cut away, exposing the parallel fibers—the collodion preventing the balance of the thread from coming off.

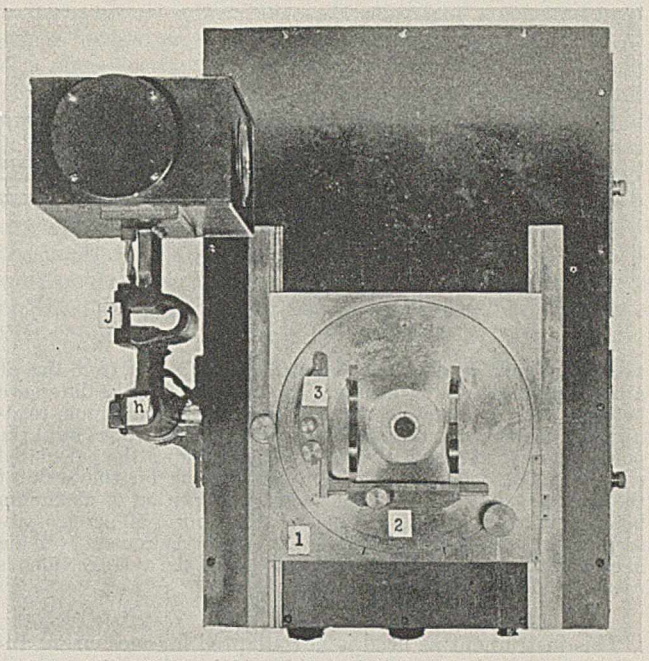


FIGURE 4. TOP VIEW OF INSTRUMENT
Showing compound mechanical stage

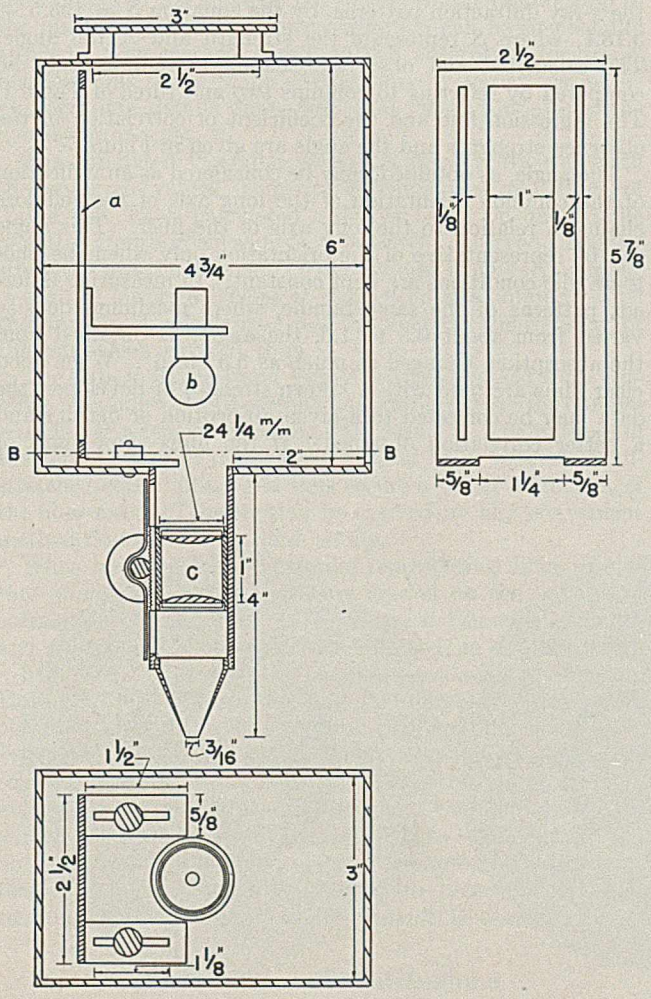


FIGURE 3. CROSS SECTION OF PHOTO-EXCITER LAMP HOUSING
b. Lamp *C.* Lens system

The x-ray diffraction patterns were obtained by passing a parallel beam of copper radiation through this bundle of paralleled cotton fibers held with the long axis perpendicular to the x-ray beam. The diffracted radiation was allowed to fall upon a flat photographic film placed 4 cm. from the center of the bundle. Because of their prominence and simple relation to the fiber axis, the arcs resulting from the 002 plane (Figure 6) were measured.

The method consists essentially in determining relative lengths, in degrees, of these arcs. Since the arcs taper off gradually, it is difficult to set an end point for practical use in determining their lengths. Sisson and Clark (*4*) tried several measures, including the angular distance from the point of maximum density to the points of the median, the mean, and the standard deviation of density.

In the present set of measurements, various criteria have been tried, based on transmission, opacity, and density. As a measure of orientation, the angular displacement from the point of maximum blackening to the point where 60 per cent of maximum transmission occurs is being used. This is equivalent to 40 per cent of the maximum absorption and, because of a slightly greater convenience in calculating, the scale of the microphotometer has been placed so as to show the absorption of the film. An important advantage over the method based on density is that a better relation is obtained with shorter exposures. Best results are obtained when the maximum density is about 0.5, whereas it is indicated that the maximum density should be around 1.0 if the measurements are based on density. This represents a saving of at least 50 per cent in exposure time—an important factor in a process of this type.

The details of measurement are as follows:

The film is centered accurately upon the stage, so that the arcs from the 002 plane will remain over the pinhole while the stage is being rotated through 360°. This is readily done by centering the film with the aid of the removable cross lines and then checking the centering by galvanometer readings at a few points around the circle. The positions on the stage corresponding to maximum blackening are then recorded. The galvanometer readings are

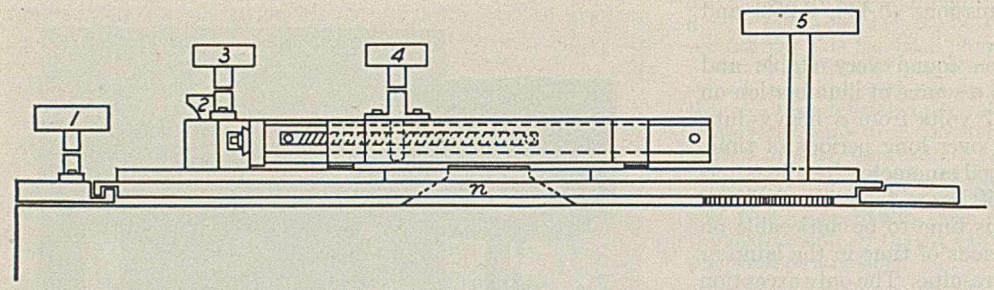


FIGURE 5. DIAGRAM OF MECHANICAL STAGE

Showing details of construction in relation to raised portion, *n*, of base plate

taken at a point of maximum blackening and at 90° from this at a point of minimum blackening. Forty per cent of the difference between these two readings is then added to the minimum reading, and the stage is rotated between these two positions until the point is found at which the galvanometer shows the calculated reading. The difference between the reading on the scale of the circular stage at this point and at the position of maximum blackening is the angular distance of the point of 40 per cent absorption (60 per cent transmission). This measurement is made in each of the four quadrants of the circle and the average value is used.

This instrument as used is not adapted for measuring absolute density or transmission with a high degree of accuracy. Furthermore, the "absorption" values contain a reflection factor which has not been taken into account; but for this particular purpose variations in the photographic method far outweigh any possible sources of error in the microphotometer. The difficulties in the photographic method, such as variation in developer strength or in film emulsions, have been studied at some length and further experiments along this line are planned. To date, however, the results show a sufficiently high reproducibility to justify the use of the method under well-controlled conditions.

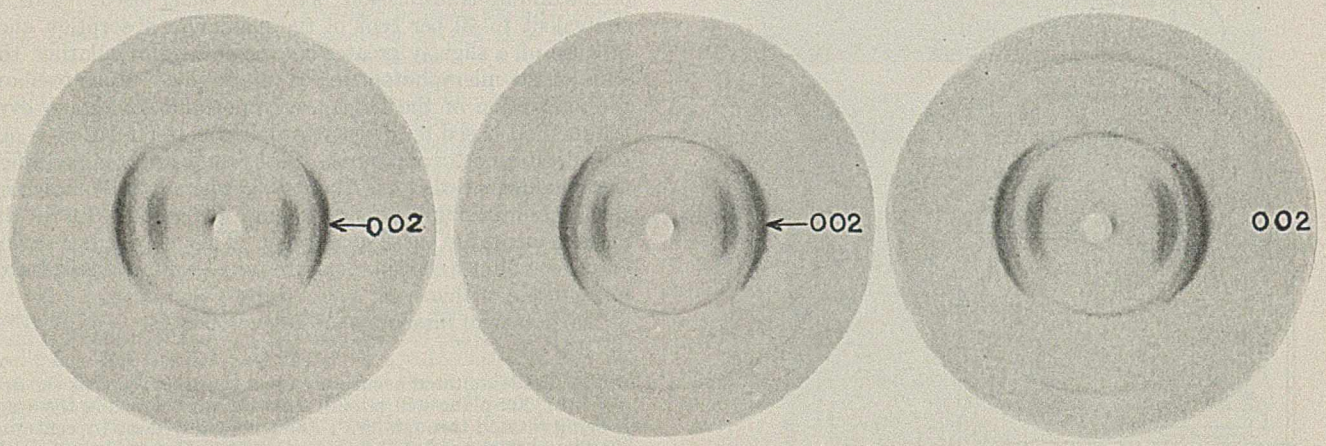
Results

The new microphotometer has a number of advantages over such previous instruments as the microdensitometer used by Sisson and Clark (*4*) and Sisson (*2*, *3*) for analyzing the x-ray diffraction patterns of raw cotton fibers. The compound mechanical stage allows for a more rapid adjustment and the high sensitivity to changes in the blackening of the film assures accurate centering of the pattern. A given operator can accurately measure from two to four times as many patterns in a day on the microphotometer as on the microdensitometer.

The error of measuring a film on the microphotometer is negligible in comparison with the errors in preparation of sample and the reproducibility of the photographic method. A standard error of 0.05° in the angle of 40 per cent absorption was obtained with the microphotometer when three different operators measured the same patterns repeatedly, whereas a standard error of 0.52° was obtained on the microdensitometer when only two different operators made repeated measurements on the same patterns. This indicates that in addition to being more rapid the microphotometer has a precision approximately ten times that of the microdensitometer.

The Chandler strength values for 30 different cottons were found to be related to the 40 per cent angle, as obtained from the x-ray diffraction patterns, by the equation $S = 193.5 - 3.18A$, where S represents the strength and A the angle. The calculated and observed Chandler strengths may be compared by referring to columns two and three of Table I. The regression line and the coefficient of correlation of the observed strengths and the angle are given in Figure 7.

The angle, as obtained, may be considered as an indication of the common orientation of the long axis of the cellulose chains in relation to the long axis of the fiber. This angle can be representative of the orientation only when the photographic conditions are kept constant. In measuring different patterns of the same bundle, where maximum density varied from about 0.5 to 1.0, the angle, as obtained from the absorption, changed as much as 5.5° to 6°. When good clear films are used with a known strength of developer, the data may be corrected to a given absorption or density and a better correlation obtained. If the films show signs of



112,000 pounds per sq. nch 80,300 pounds per sq. inch 61,000 pounds per sq. inch

FIGURE 6. TYPICAL X-RAY DIFFRACTION PATTERNS FOR BUNDLES OF COTTON FIBERS OF DIFFERENT CHANDLER STRENGTHS

TABLE I. THE 40 PER CENT ANGLE AND THE CALCULATED AND OBSERVED CHANDLER STRENGTHS OF 30 SAMPLES OF COTTON

40% Angle	Calculated Chandler Strengths	Observed Chandler Strengths
Degrees	*1000 lb./sq. inch*	*1000 lb./sq. inch*
43.6	54.9	60.0
43.4	55.5	62.6
38.2	72.0	65.2
38.5	71.1	67.2
36.5	77.4	71.5
38.1	72.3	73.4
36.0	79.0	75.3
34.0	85.4	77.9
33.6	86.7	80.0
34.9	82.5	83.2
33.5	87.0	85.8
33.7	86.3	88.6
32.6	89.8	90.0
31.4	93.7	90.2
31.0	94.9	91.8
31.8	92.4	92.0
32.7	89.5	93.0
30.7	95.9	93.1
31.5	93.3	95.5
29.6	99.4	97.9
29.3	100.3	98.5
28.6	102.6	101.0
32.3	90.8	101.0
30.1	97.8	102.2
29.4	100.0	102.2
29.7	99.1	102.6
29.1	101.0	103.2
29.5	99.7	104.0
28.4	103.2	105.0
27.6	105.7	105.2

deterioration or the developer varies too widely in strength, the changes in the angle do not have a simple relationship to the blackening and no known correction can be successfully applied. The method based on the absorption (or transmission) was found to be affected less by variations in the photographic details than was that based on either the density or opacity.

The estimated Chandler strengths agree fairly well with the observed values (compare columns two and three of Table I). Each calculated value is based on one bundle of cotton from a given sample, whereas the observed Chandler strengths are the averages of ten bundles from each sample. A slightly lower coefficient of correlation was obtained when the angle was calculated from the opacity, the density, or the equivalent exposure time.

The data in Table I and Figure 7 were obtained from patterns of bundles of raw cotton photographed while the bundles were under a tension of 6.8 kg. (15 pounds). The purpose of the tension was to remove the natural wave or curl of unstretched cotton fibers and thus secure a better alignment of the fiber axes. Tension may be applied by any convenient method that gives consistent results.

When bundles from 48 samples representing different cottons were photographed without tension on the bundles, a correlation coefficient of -0.82 between Chandler strength and the 40 per cent angle was obtained, as compared with -0.95 when 30 samples were photographed with tension. However, the patterns for the "no tension" series were obtained under a wide range of photographic conditions. When these conditions are not kept relatively constant, the correlation coefficient would not be expected to be as high, even after the results are adjusted to a common absorption value. Correlation coefficients as high as -0.88 have been obtained for small numbers of bundles photographed without tension. Further work is planned to determine whether tension is essential and, if so, the amount necessary.

Summary and Conclusions

A microphotometer for measuring x-ray diffraction patterns has been developed and successfully used to measure patterns of raw cotton fibers. It consists of a constant light source focused on the pattern, a measuring device composed of a photoelectric cell and galvanometer system, and a compound mechanical stage for holding and moving the patterns under the light beam while measuring them. The instrument is stable and shows a high degree of precision in repeated measurement of the same x-ray diffraction patterns of raw cotton fibers. A coefficient of correlation of 0.95 between single x-ray patterns from each of 30 different cottons and their Chandler strength was obtained when the samples were photographed under tension. Lower correlations were obtained when no tension was used. These results indicate that the x-ray method may be used with considerable precision to estimate the strengths of undegraded raw cotton.

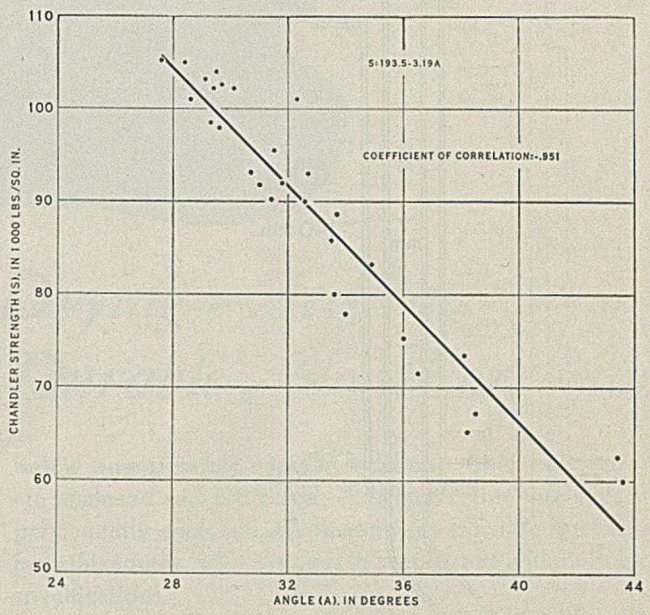


FIGURE 7. RELATION OF CHANDLER STRENGTH TO THE 40 PER CENT ANGLE AS MEASURED ON X-RAY DIFFRACTION PATTERNS

Further experiments are planned to determine the effects of preparation of sample, tension, fiber-wall development, and biological decay on the x-ray patterns and their relation to the tensile strength of raw cotton.

Acknowledgments

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The authors are indebted to the Tung-Sol Lamp Works, Inc., for the temperatures of the lamp at different voltages.

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A Constant-Temperature Bath for Stodola's Acetylation Microapparatus

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STODOLA'S method (*1*) for the microdetermination of hydroxyl and amino groups involves quantitative acetylation of these groups, which is carried out in a small flask fitted with a vertical condenser (shown with broken lines in the diagram). For complete reaction the contents of the flask must be held at 95° to 100° C. for one hour. This is ordinarily achieved by means of a glycerol bath and it requires intermittent attention on the part of the operator to maintain this temperature.

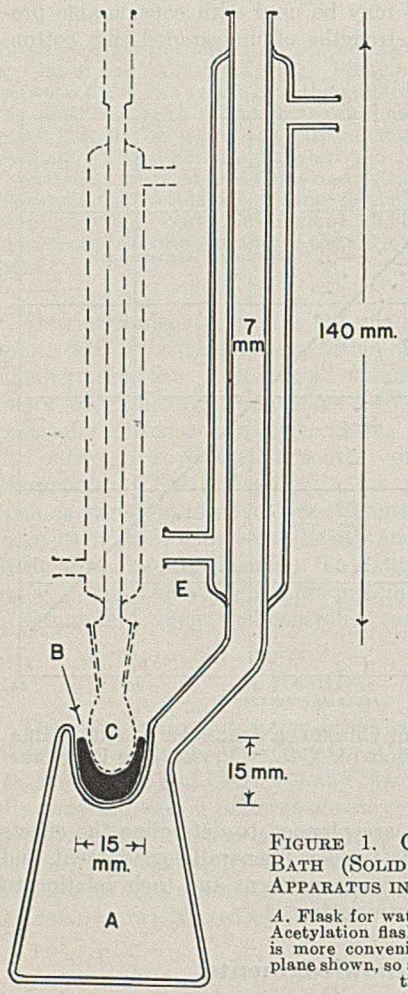

FIGURE 1. CONSTANT-TEMPERATURE BATH (SOLID LINES). ACETYLATION APPARATUS IN PLACE (BROKEN LINES)

A. Flask for water. *B*. Glycerol in well. *C*. Acetylation flask. Condenser jacket tube *E* is more conveniently placed at 90° to the plane shown, so as to be out of way of acetylation apparatus

The apparatus described maintains the required temperature indefinitely without attention. This frees the operator, and the use of two reaction flasks allows him, while one reaction is in progress, to titrate the previous reaction, clean the flask, and weigh out the sample for the next reaction without interruption. Over the course of four consecutive analyses as much as 2 hours may thus be saved. The apparatus employs the principle of the Abderhalden dryer, in that the desired temperature is maintained by the vapors of a suitable refluxing liquid (water). The reaction flask of Stodola's apparatus is partially immersed in glycerol in a well which is heated by the vapors from the boiling liquid in the large flask, *A*. The vapors are returned by a short water condenser which is connected in series with, and following, the condenser of Stodola's apparatus.

Glycerol is used in the well because it is very easily washed from the reaction flask before the titration is carried out. Mineral oil is inconvenient here, because it is hard to wipe it from the reaction flask. Mercury could be used if the laboratory were well ventilated, but it is not recommended on account of possible danger to the operator.

Flask *A* is conveniently fashioned from a 50-cc. Pyrex Erlenmeyer. The dimensions as shown in the figure are adjusted to suit the measurements of the acetylation flask.

To use the apparatus a small amount of water is placed in flask *A* and the condenser water is turned on. The well is filled about two-thirds full of glycerol to assure good thermal contact with the reaction flask, and the water is brought to a boil with a microburner. The apparatus needs no further attention. A little zinc dust in flask *A* will promote smooth boiling.

This type of bath is, of course, suitable for other reactions which require a constant temperature over a period of time.

Acknowledgments

The author wishes to acknowledge with thanks the aid given by James Cason and Walton Geiger in developing and testing this device.

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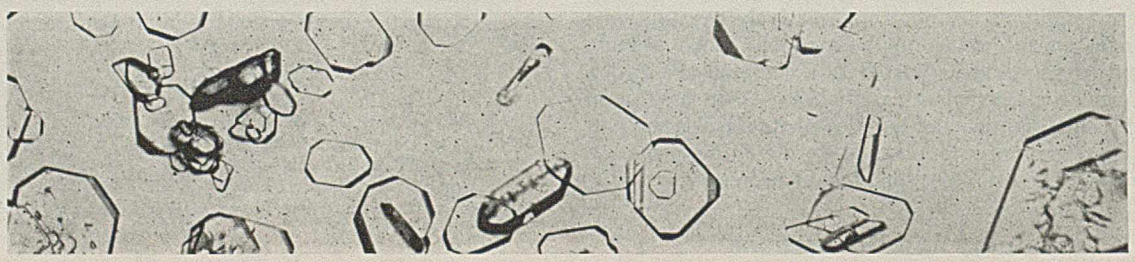

Courtesy, M. L. Willard

BARIUM CHLORIDE (PHOTOMICROGRAPH)

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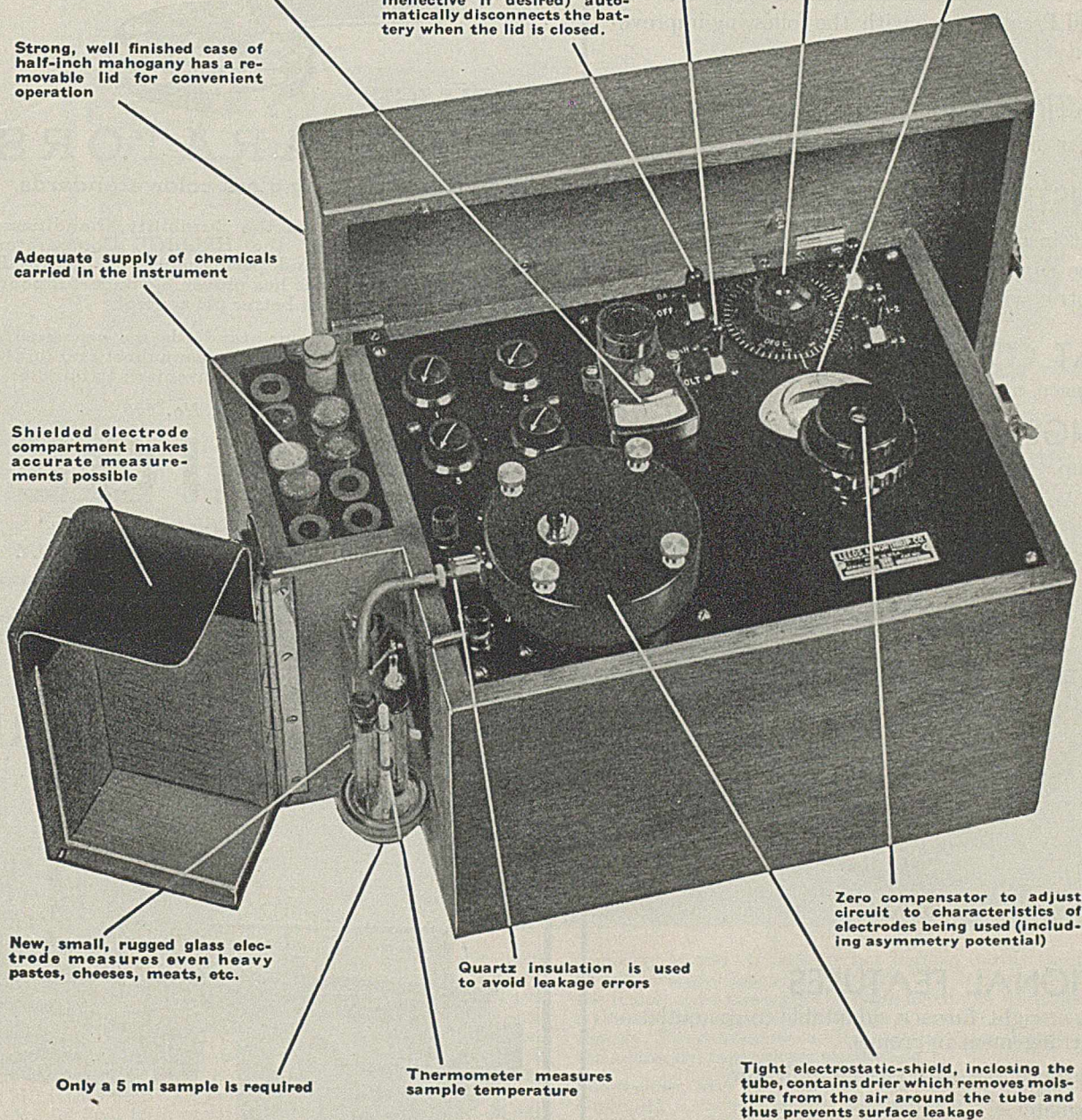

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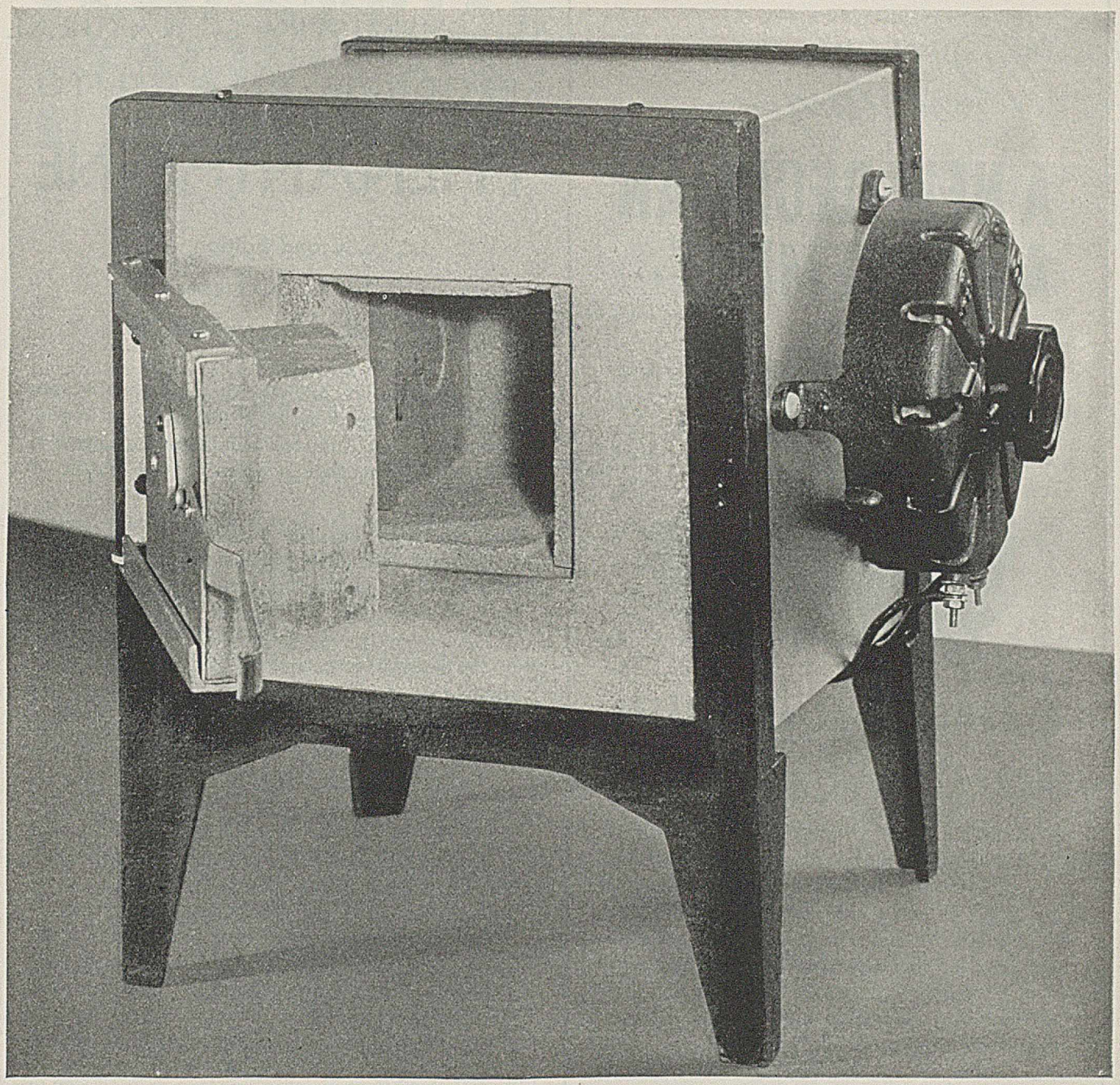

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