Anna SKORUPA*[1], Alicja PIASECKA-BELKHAYAT[1]

Chapter 5. APPLICATION OF FUZZY FINITE DIFFERENCE METHOD IN HEAT AND MASS TRANSFER DURING CRYOPRESERVATION PROCESS

5.1. Introduction

Cryopreservation is a process in which organs, cells, tissues or other biological constructs are preserved by cooling samples to a very low temperature (between –80ºC to –196ºC). During cryopreservation, the biological activity of the tissues is reduced or completely stopped and then the physiological temperature is restored again. Successful cryopreservation does not significantly affect the basic functions of cryopreserved biological tissues or cells, such as their mechanical properties [1,2]. Cryopreservation has many practical applications, including medicine. The process is used to preserve stem cells (tissue engineering research) or to cryobank transported organs (in transplantology) [3].

In recent years, the success of cryopreservation of cells and tissues has gradually increased, thanks to the use of cryoprotectants and temperature control equipment. It is important to choose, depending on the cell type, the right cooling rate, heating rate and the CPA, which is used to reduce the amount of ice formed at a given temperature. A cryoprotectant that is commonly used during an articular cartilage cryopreservation is dimethyl sulfoxide (DMSO). In order to avoid the formation of ice regardless of the cooling and heating rates, the liquidus tracking method is used. In the LT process, the temperature decreases/increases gradually during addition/removal of cryoprotectant, and the thermophysical state of the articular cartilage sample remains on or above the liquidus line so that no ice is formed, independently of the cooling/warming rate [4, 5].

* Corresponding author: anna.skorupa@polsl.pl, Konarskiego 18A, 44-100 Gliwice, PL.
[1] Department of Computational Mechanics and Engineering, Faculty of Mechanical Engineering, Silesian University of Technology.

In the present study, the numerical analysis of the articular cartilage sample cryopreservation process including heat and mass transfer proceeding is presented. In the model, the liquidus-tracking protocol for the cryopreserved articular cartilage sample is simulated. Additionally, in the mathematical model, thermophysical parameters such as thermal conductivity and volumetric specific heat are given as fuzzy numbers because they cannot be determined in a deterministic way and their values are approximated in an experimental way. The problem discussed has been solved using the fuzzy finite difference method algorithm using α-cuts and the rules of directed interval arithmetic [6, 7]. The application of α-cuts allows one to simplify mathematical operations in the fuzzy number set. In the research, the thermal proceeding is modelled using the fuzzy Fourier equation, whereas the cryoprotectant transport through extracellular matrix are described by fuzzy mass transfer equation.

5.2. Fuzzy governing equations

Thermal processes proceeding in the axially symmetrical heterogeneous articular cartilage sample can be described by the fuzzy energy equation [5,8]

$$\tilde{c}\frac{\partial \tilde{T}(r,\,z,\,t)}{\partial t}=\left[\frac{1}{r}\frac{\partial}{\partial r}\left(\tilde{\lambda}\,r\frac{\partial \tilde{T}(r,\,z,\,t)}{\partial r}\right)+\frac{\partial}{\partial z}\left(\tilde{\lambda}\,\frac{\partial \tilde{T}(r,\,z,\,t)}{\partial z}\right)\right] \tag{1}$$

where $\tilde{\lambda}$ is the fuzzy thermal conductivity, $\tilde{c}$ is the fuzzy volumetric specific heat, $\tilde{T}$ is the fuzzy temperature, t is the time, r and z denote the cylindrical coordinates.
The considered equation (1) is supplemented by the boundary conditions of the 2nd or 3rd type (see Fig. 5.1) and initial condition

$$\begin{cases} r=0,\;\; 0\le z\le \dfrac{H}{2}: & \tilde{\lambda}\dfrac{\partial \tilde{T}(r,\,z,\,t)}{\partial r}=\tilde{0} \\ 0\le r\le R,\;\; z=\dfrac{H}{2}: & -\tilde{\lambda}\dfrac{\partial \tilde{T}(r,\,z,\,t)}{\partial z}=\tilde{0} \\ r=R,\;\; 0\le z\le \dfrac{H}{2}: & -\tilde{\lambda}\dfrac{\partial \tilde{T}(r,\,z,\,t)}{\partial r}=\gamma(\tilde{T}-T_{\text{bulk}}) \\ 0\le r\le R,\;\; z=0: & \tilde{\lambda}\dfrac{\partial \tilde{T}(r,\,z,\,t)}{\partial z}=\gamma(\tilde{T}-T_{\text{bulk}}) \\ t=0: & \tilde{T}(r,\,z,\,0)=T_0 \end{cases} \tag{2}$$

where T_{bulk} is the temperature of the bathing solution, T_0 is the initial temperature and γ is the the natural convection heat transfer coefficient [5,8].

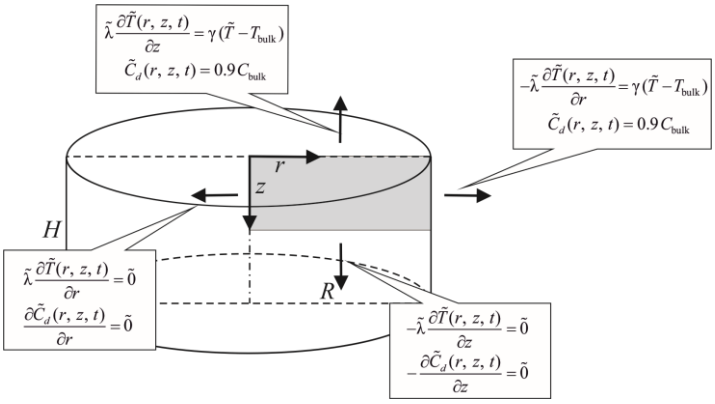


Fig. 5.1. Domain considered and boundary conditions
Rys. 5.1. Schemat rozpatrywanego obszaru oraz warunki brzegowe

The modelling of the cryoprotectant transport through the extracellular matrix can be written as follows

$$\frac{\partial \tilde{C}_d(r,z,t)}{\partial t} = \left[\frac{1}{r}\frac{\partial}{\partial r}\left(\tilde{D}_d\, r\,\frac{\partial \tilde{C}_d(r,z,t)}{\partial r}\right) + \frac{\partial}{\partial z}\left(\tilde{D}_d\,\frac{\partial \tilde{C}_d(r,z,t)}{\partial z}\right)\right] \tag{3}$$

where $\tilde{C}_d$ is the fuzzy cryoprotectant concentration in the extracellular matrix, while $\tilde{D}_d$ is the fuzzy diffusion coefficient of the cryoprotectant in the extracellular matrix estimated by the Einstein-Stokes equation [8, 9]

$$\tilde{D}_d = \frac{k_B \tilde{T}}{6\pi\eta\, r_s} \tag{4}$$

where k_B is the Boltzmann constant, r_s is the radius of the spherical particle molecule and η is the dynamic viscosity.

The mathematical model (see equation (3)) should be supplemented by the boundary-initial conditions (see Fig. 5.1)

$$\begin{cases} r = 0,\ \ 0 \le z \le \dfrac{H}{2}: & \dfrac{\partial \tilde{C}_d(r,z,t)}{\partial r} = \tilde{0} \\ 0 \le r \le R,\ \ z = \dfrac{H}{2}: & -\dfrac{\partial \tilde{C}_d(r,z,t)}{\partial z} = \tilde{0} \\ r = R,\ \ 0 \le z \le \dfrac{H}{2}: & \tilde{C}_d(r,z,t) = 0.9C_{\text{bulk}} \\ 0 \le r \le R,\ \ z = 0: & \tilde{C}_d(r,z,t) = 0.9C_{\text{bulk}} \\ t = 0: & \tilde{C}_d(r,z,0) = C_0 \end{cases} \tag{5}$$

where C_{bulk} is the cryoprotectant concentration in the bathing solution (the coefficient 0.9 relates to the real mass exchange between the sample and the bathing solution) and C_0 is the initial cryoprotectant concentration [5, 8].

The presented mathematical model does not include the phenomenon of phase changes. This is due to the fact that the LT method is used to model heat and mass transfer. The LT protocol regulates the temperature and concentration in such a way that the temperature of the sample is above or on the liquidus line, which eliminates the probability of ice crystallization in cells – see the calculated eutectic temperatures and the melting points of tissue in [4].

5.3. Fuzzy finite difference method

Numerical model of thermal processes and mass transfer proceeding in domain of tissue is based on the fuzzy finite difference method in the version presented in [6, 10]. At the beginning, a time grid with a constant step Δt and a geometrical mesh are introduced. The boundary nodes are located at the distance 0.5 h or 0.5 k with respect to the real boundary (h, k are the steps of regular mesh in directions r and z), respectively.

The approximate form of the interval energy equation (1) for the internal nodes $(i,\ j)$ and the transition $t^{f-1} \to t^{f}$ is the following

$$\tilde{c}_{i,j}^{f-1}\frac{\tilde{T}_{i,j}^{f}-\tilde{T}_{i,j}^{f-1}}{\Delta t}=\frac{\Phi_{i,j-1}}{\tilde{R}_{i,j-1}^{f-1}}\left(\tilde{T}_{i,j-1}^{f-1}-\tilde{T}_{i,j}^{f-1}\right)+\frac{\Phi_{i,j+1}}{\tilde{R}_{i,j+1}^{f-1}}\left(\tilde{T}_{i,j+1}^{f-1}-\tilde{T}_{i,j}^{f-1}\right)+ \\ \frac{\Phi_{i-1,j}}{\tilde{R}_{i-1,j}^{f-1}}\left(\tilde{T}_{i-1,j}^{f-1}-\tilde{T}_{i,j}^{f-1}\right)+\frac{\Phi_{i+1,j}}{\tilde{R}_{i+1,j}^{f-1}}\left(\tilde{T}_{i+1,j}^{f-1}-\tilde{T}_{i,j}^{f-1}\right) \tag{6}$$

where

$$\Phi_{i,j-1}=\frac{r_{i,j}-0.5h}{r_{i,j}\,h} \qquad \Phi_{i,j+1}=\frac{r_{i,j}+0.5h}{r_{i,j}\,h} \qquad \Phi_{i-1,j}=\Phi_{i+1,j}=\frac{1}{k} \tag{7}$$

are the shape functions of differential mesh, while the fuzzy thermal resistances are defined as follows:

$$\tilde{R}_{i,j+1}^{f-1}=\frac{0.5h}{\tilde{\lambda}_{i,j}^{f-1}}+\frac{0.5h}{\tilde{\lambda}_{i,j+1}^{f-1}} \qquad \tilde{R}_{i,j-1}^{f-1}=\frac{0.5h}{\tilde{\lambda}_{i,j}^{f-1}}+\frac{0.5h}{\tilde{\lambda}_{i,j-1}^{f-1}}$$
$$\tilde{R}_{i+1,j}^{f-1}=\frac{0.5k}{\tilde{\lambda}_{i,j}^{f-1}}+\frac{0.5k}{\tilde{\lambda}_{i+1,j}^{f-1}} \qquad \tilde{R}_{i-1,j}^{f-1}=\frac{0.5k}{\tilde{\lambda}_{i,j}^{f-1}}+\frac{0.5k}{\tilde{\lambda}_{i-1,j}^{f-1}} \tag{8}$$

The final approximate form of the interval mass equation (3) for the internal nodes $(i,\ j)$ and the transition $t^{f-1}\to t^{f}$ is the following

$$\frac{\left(\tilde{C}_d\right)_{i,j}^{f}-\left(\tilde{C}_d\right)_{i,j}^{f-1}}{\Delta t}=\frac{\Phi_{i,j-1}}{\tilde{W}_{i,j-1}^{f-1}}\left[\left(\tilde{C}_d\right)_{i,j-1}^{f-1}-\left(\tilde{C}_d\right)_{i,j}^{f-1}\right]+\frac{\Phi_{i,j+1}}{\tilde{W}_{i,j+1}^{f-1}}\left[\left(\tilde{C}_d\right)_{i,j+1}^{f-1}-\left(\tilde{C}_d\right)_{i,j}^{f-1}\right]$$
$$+\frac{\Phi_{i-1,j}}{\tilde{W}_{i-1,j}^{f-1}}\left[\left(\tilde{C}_d\right)_{i-1,j}^{f-1}-\left(\tilde{C}_d\right)_{i,j}^{f-1}\right]+\frac{\Phi_{i+1,j}}{\tilde{W}_{i+1,j}^{f-1}}\left[\left(\tilde{C}_d\right)_{i+1,j}^{f-1}-\left(\tilde{C}_d\right)_{i,j}^{f-1}\right] \tag{9}$$

where the fuzzy diffusion resistances between the central node and the adjoining ones are the following:

$$\tilde{W}_{i,j+1}^{f-1}=\frac{0.5h}{\left(\tilde{D}_d\right)_{i,j}^{f-1}}+\frac{0.5h}{\left(\tilde{D}_d\right)_{i,j+1}^{f-1}} \qquad \tilde{W}_{i,j-1}^{f-1}=\frac{0.5h}{\left(\tilde{D}_d\right)_{i,j}^{f-1}}+\frac{0.5h}{\left(\tilde{D}_d\right)_{i,j-1}^{f-1}}$$
$$\tilde{W}_{i+1,j}^{f-1}=\frac{0.5k}{\left(\tilde{D}_d\right)_{i,j}^{f-1}}+\frac{0.5k}{\left(\tilde{D}_d\right)_{i+1,j}^{f-1}} \qquad \tilde{W}_{i-1,j}^{f-1}=\frac{0.5k}{\left(\tilde{D}_d\right)_{i,j}^{f-1}}+\frac{0.5k}{\left(\tilde{D}_d\right)_{i-1,j}^{f-1}} \tag{10}$$

The system of equations (6) and (9) has been solved using the assumption of the stability condition for explicit differential scheme [10]. The method of attaching boundary conditions is discussed in detail in [8].

More information about the fuzzy finite difference method and fuzzy trapezoidal numbers used in the next part of the paper can be found in [6, 7].

5.4. Results of computations

As a numerical example, the heat and mass transport in the homogenous cylindrical articular cartilage sample of dimensions H = 1 mm and R = 3 mm has been anlysed. The following thermophysical parameters as fuzzy trapezoidal numbers have been introduced: thermal conductivity $\tilde{\lambda}=\left[\lambda-0.05\lambda,\lambda-0.025\lambda,\lambda+0.025\lambda,\lambda+0.05\lambda\right]$ and volumetric specific heat

$\tilde{c} = [c - 0.05c, c - 0.025c, c + 0.025c, c + 0.05c]$, where λ = 0.518 $W \cdot m^{-1} \cdot K^{-1}$ and $c = 3.924 \cdot 10^6$ $J \cdot m^{-3} \cdot K^{-1}$. The mathematical model has also been supplemented by: initial temperature in tissue domain T_0 = 22°C, initial cryoprotectant concentration C_0 = 0% (w/w) and heat transfer coefficient γ = 525 $W \cdot m^{-2} \cdot K^{-1}$. Additionally, to determine the diffusion coefficient, the following input data have been used: the Boltzmann constant $k_B = 1.38 \cdot 10^{-23}$ $J \cdot K^{-1}$, radius of the spherical particle molecule $r_s = 2.541 \cdot 10^{-10}$ m and the dynamic viscosity $\eta = 1.996 \cdot 10^{-3}$ Pa·s [5, 8].

In this work, the LT protocol proposed by Pegg et al. [4] and improved by Yu et al. [5] was applied. It consists of seven steps in cooling and addition phase and six steps in warming and removal phase. This approach prevents ice crystal formation without causing toxicity, because temperature and concentration of bathing solution for each step are properly regulated. The values of temperature (T_{bulk}) and concentration (C_{bulk}) of bathing solution in respective steps, which refer to boundary conditions in numerical model, can be found in Tab. 5.1.

Table 5.1

The assumption of the LT protocol [5]

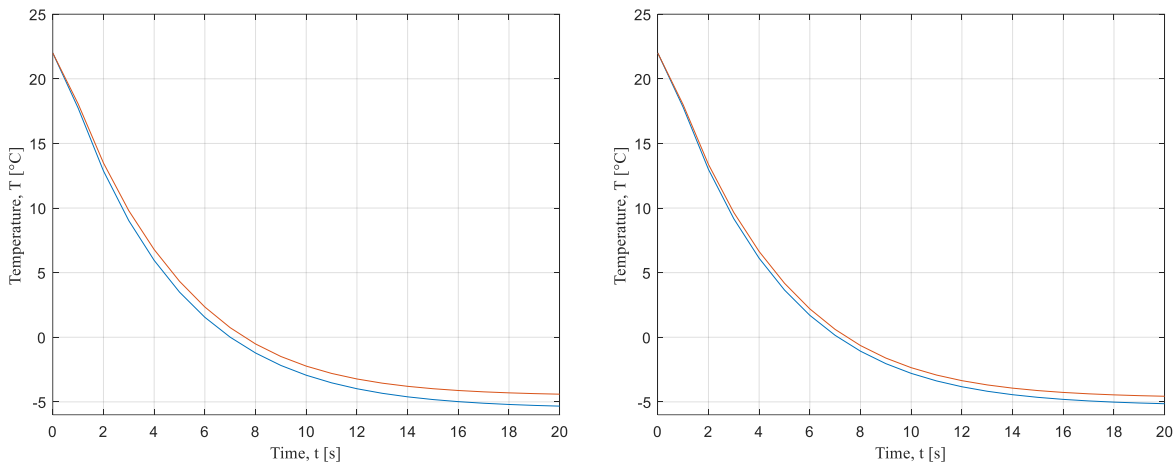
Phase	Step	Time	Temperature of bathing solution	Concentration of bathing solution
		t [min]	T_{bulk} [ºC]	C_{bulk} [%(w/w)]
Cooling and addition	1	10	22	10
	2	9.8	22	20
	3	18.2	-5	29
	4	25	-8.5	38
	5	19.8	-16	47
	6	26.4	-23	56
	7	23.8	-35	63
Warming and removal	1	23.8	-35	56
	2	26.4	-23	47
	3	19.8	-16	38
	4	25	-8.5	29
	5	18.2	-5	20
	6	29.85	22	0

The simulaton has been performed using the finite difference method (FDM) with the rules based on fuzzy analysis for trapezoidal numbers with application of α-cuts and the

directed interval arithmetic [7, 8]. The following assumption in numerical model was introduced: time step $\Delta t = 0.001$ s, mesh steps $h = 0.0001$ m and $k = 0.00005$ m, numbers of nodes: 10 x 30.

Fig. 5.2 illustrates the history of the temperature over time for transition from 22°C to –5°C from step 2 to step 3 in the cooling and addition phase for α equal to 0 and 0.75, where two curves represent the beginning and the end of α-cuts. The obtained results refer to the point with the coordinates $r = 0.1$ mm and $z = 0.45$ mm.

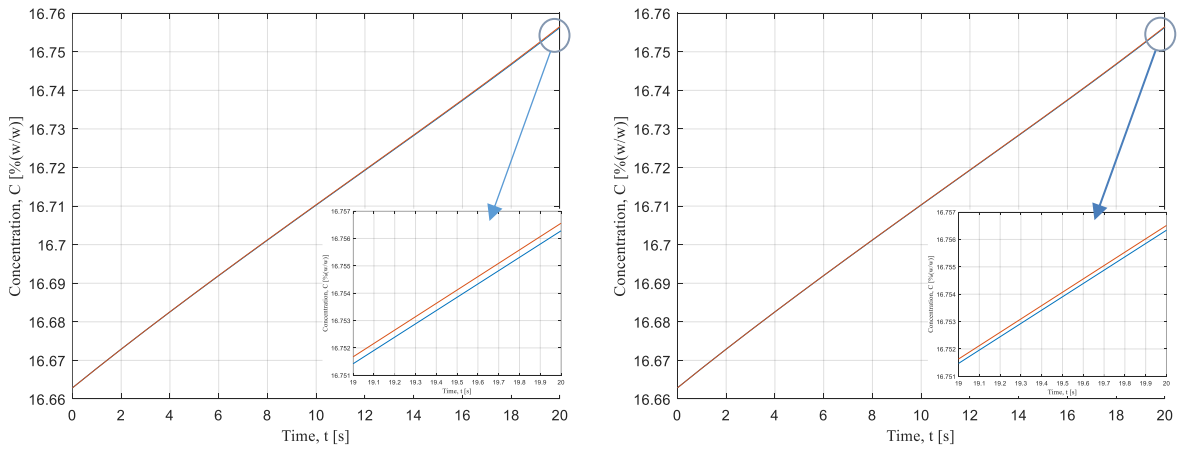
Fig. 5.3 shows the history of the concentration over time for transition from 22°C to –5°C from step 2 to step 3 in the cooling and addition phase for the same values of parameter α and in the same point. The gaps between the lines on the graphs are narrow, therefore the zoom for 19-20 s was made.



(a) (b)

Fig. 5.2. History of temperature over time from 22ºC to –5ºC for: α = 0; (b) α = 0.75

Rys. 5.2. Wykres zależności temperatury od czasu przy przejściu z 22ºC do –5ºC dla: (a) α = 0; (b) α = 0.75



(a) (b)

Fig. 5.3. History of concentration over time from 22ºC to –5ºC for: α = 0; (b) α = 0.75

Rys. 5.3. Wykres zależności koncentracji od czasu przy przejściu z 22ºC do –5ºC dla: (a) α = 0; (b) α = 0.75

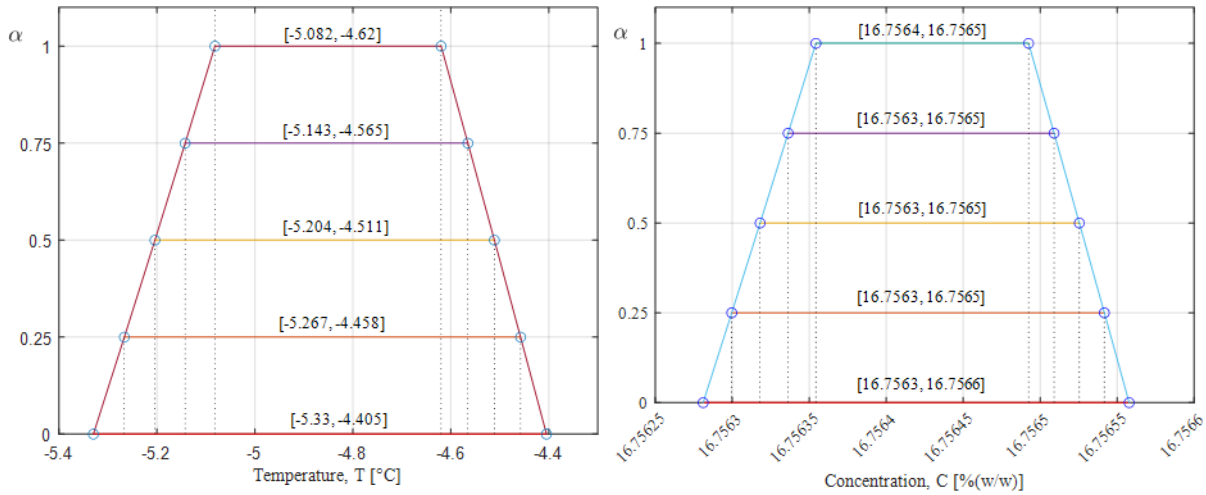
Table 5.2 contains a comparison of obtained temperatures and concentration with the values given by Yu et al. [5]. The computed interval temperatures coincide with the values proposed in the experiment. On the other hand, the concentrations determined in the simulation differ from the values both suggested in the experiment and studied by Yu et al. [5]. The discrepancies are visible mainly for those steps, in which higher concentration of bathing solution is assumed (e.g. step 7 in the cooling and addition phase or step 1 in the heating and removal phase).

Table 5.2

Interval values of temeprature and concetration at the end of each step

Phase	**Step**	**Temperature [°C]**			**Concentration [%(w/w)]**			
		proposed in [5]	obtained in simulation		proposed in [5]	calculated in [5]	obtained in simulation	
			α = 0	α = 0.75			α = 0	α = 0.75
Cooling and addition	1.	22	[22.00, 22.00]	[22.00, 22.00]	10	6.80	[7.839, 7.839]	[7.839, 7.839]
	2.	22	[22.00, 22.00]	[22.00, 22.00]	20	15.73	[16.663, 16.663]	[16.663, 16.663]
	3.	-5	[-5.51, -4.54]	[-5.31, -4.70]	29	23.23	[25.837, 25.840]	[25.838, 25.840]
	4.	-8.5	[-9.37, -7.71]	[-9.03, -7.00]	38	32.22	[34.138, 34.140]	[34.139, 34.140]
	5.	-16	[-17.64, -14.51]	[-17.00, -15.05]	47	37.32	[42.096, 42.106]	[42.099, 42.104]
	6.	-23	[-25.36, -20.86]	[-24.44, -21.64]	56	44.30	[50.336, 50.342]	[50.337, 50.341]
	7.	-35	[-38.58, -31.75]	[-37.20, -32.93]	63	47.50	[56.595, 56.608]	[56.598, 56.606]
Warming and removal	1.	-35	[-38.58, -31.75]	[-37.20, -32.93]	56	-	[50.503, 50.491]	[50.501, 50.493]
	2.	-23	[-25.36, -20.86]	[-24.44, -21.64]	47	-	[42.364, 42.358]	[42.362, 42.359]
	3.	-16	[-17.64, -14.51]	[-17.00, -15.05]	38	-	[34.404, 34.394]	[34.402, 34.396]
	4.	-8.5	[-9.37, -7.71]	[-9.03, -7.00]	29	-	[26.161, 26.159]	[26.162, 26.160]
	5.	-5	[-5.51, -4.54]	[-5.31, -4.70]	20	-	[18.236, 18.233]	[18.236, 18.233]
	6.	22	[22.00, 22.00]	[22.00, 22.00]	0	-	[0.023, 0.023]	[0.023, 0.023]

Figure 5.4 presents results of calculation at the given point after 20 s of step 3 in cooling and addition phase for particular α-cuts. It can be seen that the intervals between respective temperatures or concentrations are wider for lower values of parameter α.



(a) (b)

Fig. 5.4. Interval temperature (a) and interval concentration (b) at the given node after 20 s of step 3 in cooling and addition phase for chosen values of parameter α.

Rys. 5.4. Przedziały temperatur (a) oraz przedziały koncentracji (b) w danym węźle po 20 s kroku 3 w fazie chłodzenia i dodawania dla wybranych wartości parametru α.

5.5. Conclusions

In the paper, the numerical analysis of heat and mass transfer phenomena during crypreservation is presented. The process is considered in the 2D axialy symmetrical articular cartilage sample. The discussed problem has been solved using fuzzy Fourier equation and fuzzy mass diffusion equation. In the numerical simulation, the fuzzy FDM has been applied, assuming that the parameters, such as volumetric specific heat and thermal conductivity, are defined as trapezoidal fuzzy numbers.

The application of FDM gives the solutions as intervals. This type of analysis allows for a better interpretation of real phenomena, because imprecise parameters determined experimentally are entered into the model as fuzzy numbers.

Furthermore, using α-cuts during numerical modelling allows to avoid complicated arithmetic because it lets one consider fuzzy numbers as directed interval values. It can be also concluded that the intervals are narrower for the higher values of parameter α.

The obtained results have been compared with the results of the experiment. While the fuzzy temepratures include suggested values, the concentrations differ from the calculations presented in [5]. It can be caused by estimation of the diffusion coefficient using the Einstein–Stokes model (compared with the diffusion coefficient in [5]).

It should be noted that obtianed data are not fully comparable. In the work of Yu et al [5], in addition to using a different way to calculate the diffusion coefficient, a model of mass transport across the cell membrane is also included.

In the future, it is planned to extend the presented model with the mass (cryoprotectant) transport across cell membrane.

Bibliography

1. G. Zhao, J. Fu, Microfluidics for cryopreservation, *Biotechnol. Adv.* (2017) **35**: 323-336.
2. F. Xu, S. Moon, X. Zhang, L. Shao, Y.S. Song, U. Demirci, Multi-scale heat and mass transfer modelling of cell and tissue cryopreservation, *Philos. Trans. R. Soc. A Math. Phys. Eng. Sci.* (2010) **368**: 561-583.
3. T.H. Jang, S.C. Park, J.H. Yang, J.Y. Kim, J.H. Seok, U.S. Park, C.W. Choi, S.R. Lee, J. Han, Cryopreservation and its clinical applications, *Integr. Med. Res.* (2017) **6**: 12-18.
4. D.E. Pegg, L. Wang, D. Vaughan, Cryopreservation of articular cartilage. Part 3: The liquidus-tracking method, *Cryobiology* (2006) **52**: 360-368.
5. X. Yu, S. Zhang, G. Chen, Modeling the addition/removal of dimethyl sulfoxide into/from articular cartilage treated with the liquidus-tracking method, *Int. J. Heat Mass Transf.* (2019) **141**: 719-730.
6. A. Piasecka-Belkhayat, *Interval boundary element method for imprecisely defined unsteady heat transfer problem* (in Polish), Wyd. Politechniki Śląskiej, Gliwice, 2011.
7. A. Piasecka-Belkhayat, A. Korczak, Analysis of ultrashort laser pulse irradiation with 2d thin metal films using the fuzzy lattice boltzmann method, *J. Theor. Appl. Mech.* (2021) **58**: 209-219.
8. A. Skorupa, A. Piasecka-Belkhayat, Numerical Modeling of Heat and Mass Transfer during Cryopreservation Using Interval Analysis, *Appl. Sci.* (2021) **11**: 302.

9. W. Liu, G. Zhao, Z. Shu, T. Wang, K. Zhu, D. Gao, High-precision approach based on microfluidic perfusion chamber for quantitative analysis of biophysical properties of cell membrane, *Int. J. Heat Mass Transf.* (2015) **86**: 869-879.
10. B. Mochnacki, J. Suchy, *Numerical methods in computations of foundry processes* (in Polish), Wydawnictwo Naukowe PWN, Kraków, 1993.

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Abstract

In the paper, the numerical analysis of heat and mass transfer proceeding in the articular cartilage sample subjected to a cryopreservation process is presented. The two-dimensional, axially symmetrical model with fuzzy thermophysical parameters is considered. The base of the heat transfer model is the fuzzy Fourier equation, while the phenomenon of cryoprotectant transport through the extracellular matrix is described by the fuzzy mass transfer equation. The liquidus tracking (LT) approach was used to control the temperature and the concentration of cryoprotectant (CPA) to prevent the formation of ice regardless of the cooling or heating rate. The problem under discussion was solved using the fuzzy finite difference method using α-cuts with the application of the rules of directed interval arithmetic. Additionally, the trapezoidal approximation of fuzzy articular cartilage thermophysical parameters is applied. In the final part of the paper, the results of numerical simulations are compared with the results of experiments carried out for deterministically defined thermophysical parameters.

Keywords: cryopreservation, heat and mass transfer, fuzzy finite difference method, fuzzy numbers, directed interval arithmetic.