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Considering uncertain quantities in the model of cryopreservation process of biological samples

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Abstract

Purpose: This paper presents numerical modelling of the heat and mass transfer process in a cryopreserved biological sample. The simulation of the cooling process was carried out according to the liquidus-tracking (LT) protocol developed by Pegg et al., including eight stages in which both the bath solution concentration and temperature are controlled to prevent the formation of ice crystals.

Methods: To determine the temperature distribution during cryopreservation processes, one uses the Fourier equation, while mass transfer was taken into account using an equation based on the Fick's laws. This paper considers a model assuming fuzzy thermophysical parameters described by a triangular and a Gaussian membership function. The numerical problem was solved using the finite difference method including fuzzy set theory.

Results: The diagrams of temperature and mass distributions as a function on time and the distribution of the fuzzy variable at a given moment in time were prepared. Moreover, the fuzzy temperatures and concentrations were compared with experimental results from the literature in table.

Conclusions: In the conclusions, two different types of membership functions were compared with each other, with which the fuzzy variables were described. It can be said that the Gaussian membership function works well for experimental data where the mean and standard deviation are known. In addition, the obtained results were confronted with experimental data. The calculated fuzzy temperatures are consistent with the temperature values occurring in the LT protocol. Larger differences between the experimental data and the calculated values are observed for the fuzzy dimethyl sulfoxide (DMSO) concentration.

Keywords: cryopreservation; heat transfer, mass transfer; fuzzy numbers; Gaussian membership function; α-cuts concept

1. Introduction

It is quite common to model biological and engineering processes as deterministic phenomena. However, simulations of physical problems that occur in nature are associated with some uncertainties. They are caused, for example, by the parameters adopted in the model, which are determined experimentally and that the measurements depend on the condition, sex, and quality of the acquired samples 0.

Two approaches can be distinguished for considering uncertain variables in the model: probabilistic and non-probabilistic techniques. The first is based on modelling the characteristics of uncertainty through the use of probability distributions that describe how a given random variable might behave. The aim of probabilistic techniques is to predict outcomes under uncertainty. However, their effectiveness is related to access to relevant empirical data obtained for a given parameter, which can be a limitation to their use [23],[29].On the other hand, non-probabilistic methods include fuzzy set theory and interval set theory. In fuzzy set theory, imprecise variables that are elements of the set are assigned a membership function that determines the degree of membership in the set. The membership function can be described by a linear function, such as a triangular or trapezoidal function, or by more complex relationships, for example, a Gaussian function or a bell function [2],[16],[23]. Fuzzy set theory was first proposed by Zadeh in 1965 [34].

Slightly different definitions are given to inaccurate parameters in interval set theory. The interval number is represented by an interval with a given specified lower and upper limit [16],[23]. This concept was invented by Moore in 1966 [19].

Let us introduce some information on cryopreservation. This is a process in which the biological activity of biological material is reduced by lowering the temperature. The purpose is to preserve samples in such a way that when they are rewarmed, their physiological activities are restored [31],[35].

During cryopreservation, there is a possibility of cell or tissue damage. This is caused, for example, by ice crystallisation or osmotic stress. To eliminate this risk, the cooling (heating) rate is properly regulated and cryoprotective agents (CPAs) are introduced. The most common CPAs are glycerol, dimethyl sulfoxide (DMSO), ethylene glycol, propylene glycol, etc. [11],[12].

Depending on the cooling rate and the CPA concentration used, cryopreservation can be performed by different methods. Conventional slow freezing, for example, is characterised by a low cooling rate (approximately 1 °C/min according to Mazur [17]) and a low CPA concentration. Vitrification, on the other hand, involves rapidly cooling the sample to achieve amorphous ice instead of ice crystallisation. This process continues at high CPA concentration [11],[26].

Other cryopreservation techniques are worth mentioning. The liquidus-tracking (LT) method, for example, involves controlling the cooling rate and CPA concentration to maintain the temperature in the sample above the melting point, which is altered by the presence of CPA [13],[26].

Cryopreservation is a complex multi-physical problem with coupled transport phenomena. The mathematical model includes a description of heat flow and mass transfer associated with molecular diffusion, as well as osmotic transport (microscale process) [15],[26],[31],[33].

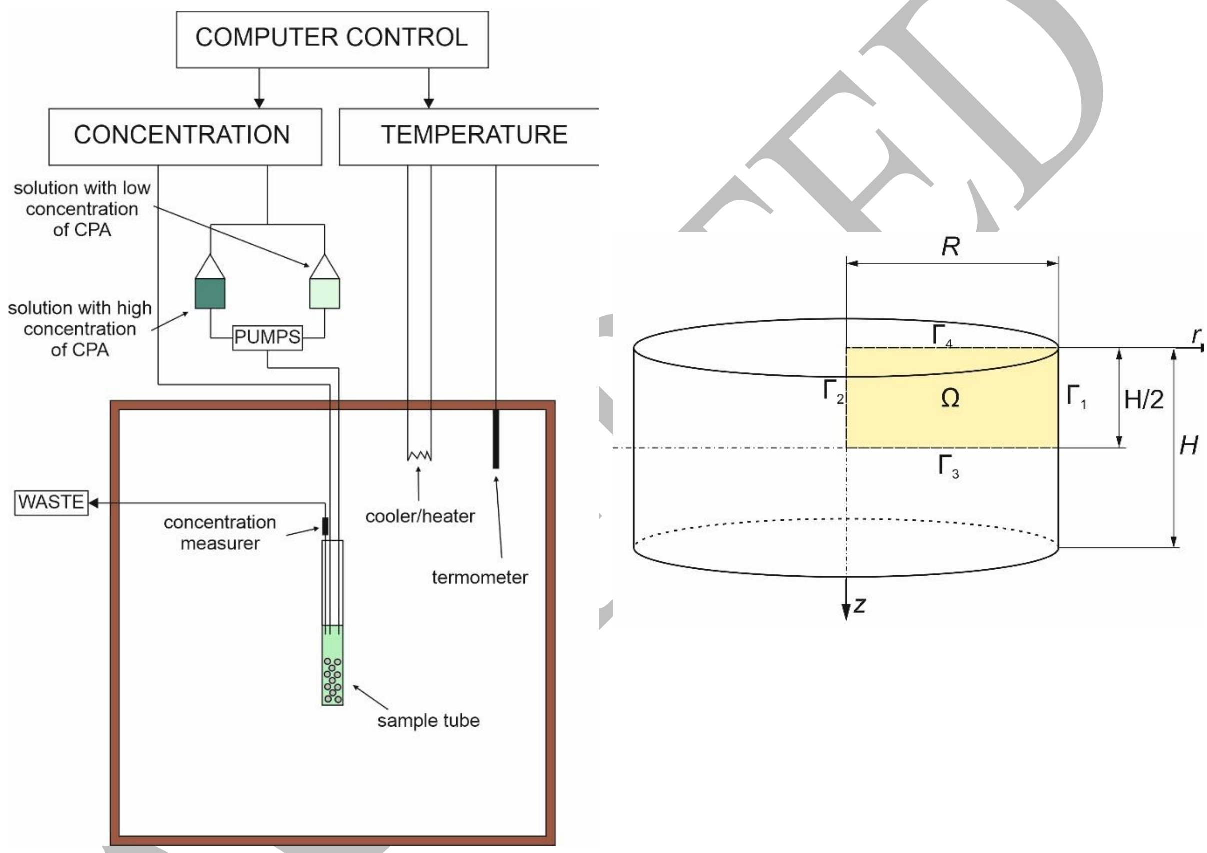
The paper contains a numerical simulation of the cryopreservation process for a sample made of articular cartilage. The thermal processes occurring during the cryopreservation were examined using the Fourier equation. Furthermore, mass transfer (molecular diffusion) was also analysed applying an equation based on Fick's laws. The study does not consider the phenomenon of osmotic transport. Similar analyses using a deterministic model can be found in the literature [15],[33]. However, there are also uncertainties in the cryopreservation model. Our previous work used interval set theory [22],[24],[26],[27] and fuzzy set theory [23],[26], where a triangular or trapezoidal membership function was introduced. In this study, simulation was performed for fuzzy thermophysical parameters described by a Gaussian membership function, which is a novel approach. The obtained fuzzy results were compared with those for a triangular membership function. For the preparation of the numerical model, the finite difference method (FDM) was implemented.

This paper is divided into four chapters. The first chapter provides an introduction, while the second chapter describes the materials selected for the analysis and the methods, which include a heat and mass transfer model and a numerical model. The next chapter presents computational examples. The final chapter contains the conclusions. The study is completed with an Appendix containing the basics of fuzzy numbers and a description of the α-cuts.

2. Methods

The study analysed the heat and mass transfer macroscopically in a biological sample during the cryopreservation process. It simulated the cooling process performed according to the LT protocol developed by Pegg et al. [20]. The LT protocol involves eight steps, during which the temperature and concentration of the bath solution are adjusted to prevent the solidification process in the sample by changing its melting point in a controlled manner. The melting point is influenced by CPA, which enters the extracellular matrix of the sample from the bath solution. Taylor and Hunt [28] and Pegg et al. [20] propose a CPTes2 solution that consisting mainly of water, DMSO, and also KCl (a potassium-rich mixture). Our research only investigated changes in the concentration of DMSO.

Figure 1a shows a schematic of an example cryopreservation device using the LT protocol invented by Wang et al. [30]. The study considered the computational domain (Ω) of an axisymmetric sample (cf. Figure 1b).



(a) (b)

Figure 1. Simplified scheme of device to cryopreservation by LT protocol (a) and scheme of sample computation domain (b)

2.1. Heat and mass transfer model

Changes in temperature distribution in the computational domain were calculated using the Fourier equation [3],[8]:

$$\tilde{c}_V \frac{\partial \tilde{T}(X,t)}{\partial t} = \nabla\left(\tilde{k}\nabla\tilde{T}(X,t)\right) + Q(X,t), \quad (1)$$

where $\tilde{T}$ is the fuzzy temperature, X refers to the coordinate system, t is the time, Q is the heat source $\tilde{c}_V$ and $\tilde{k}$ represent the fuzzy thermophysical parameters such as the fuzzy volumetric specific heat capacity and fuzzy the thermal conductivity, respectively.

For the axisymmetric problem considered in our study, Equation (1) can be expressed:

$$\tilde{c}_V \frac{\partial \tilde{T}(r,z,t)}{\partial t} = \frac{1}{r}\frac{\partial}{\partial r}\left(\tilde{k} r \frac{\partial \tilde{T}(r,z,t)}{\partial r}\right) + \frac{\partial}{\partial z}\left(\tilde{k}\frac{\partial \tilde{T}(r,z,t)}{\partial z}\right), \tag{2}$$

where r and z are the cylindrical coordinates. The heat source Q is neglected in further considerations because articular cartilages do not have blood or lymphatic vessels and therefore.

The mathematical model of heat transfer was completed for initial-boundary conditions [27],[33]:

$$\begin{cases} \Gamma_1 \text{ and } \Gamma_4: & -\tilde{k}\boldsymbol{n} \cdot \nabla \tilde{T}(r,z,t) = \alpha_\Gamma[\tilde{T}(r,z,t) - T_{bath}], \\ \Gamma_2 \text{ and } \Gamma_3: & -\tilde{k}\boldsymbol{n} \cdot \nabla \tilde{T}(r,z,t) = 0, \\ t = 0 & \tilde{T}(r,z,0) = T^0, \end{cases} \tag{3}$$

where **n** is the normal vector to the boundary, α_Γ is the natural convection heat transfer coefficient, T_{bath} is the temperature of the surrounding medium (a bathing solution), T_0 is the initial temperature.

The relationship describing the mass transfer between external medium and extracellular solutions of the cell, which is named as the molecular diffusion, is the diffusion equation based on Fick's law:

$$\frac{\partial \tilde{c}_d(X,t)}{\partial t} = \nabla\left[\tilde{D}\left(\tilde{T}\right)\nabla \tilde{c}_d(X,t)\right], \tag{4}$$

where $\tilde{c}_d$ is the fuzzy molar concentration, $\tilde{D}$ is the fuzzy molecular diffusion coefficient. The subscript d represents the DMSO as CPA.

After conversion of Equation (4) for the axisymmetric problem [3],[6],[7]:

$$\frac{\partial \tilde{c}_d(r,z,t)}{\partial t} = \frac{1}{r}\frac{\partial}{\partial r}\left(\tilde{D}\left(\tilde{T}\right) r \frac{\partial \tilde{c}_d(r,z,t)}{\partial r}\right) + \frac{\partial}{\partial z}\left(\tilde{D}\left(\tilde{T}\right)\frac{\partial \tilde{c}_d(r,z,t)}{\partial z}\right). \tag{5}$$

Please note that the fuzzy diffusion coefficient depends on temperature, which confirms that the mathematical model of cryopreservation represents a multi-physics coupled problem . The diffusion coefficient can be calculated from the Einstein-Stokes equation [4],[18]:

$$\tilde{D}(\tilde{T}) = \frac{k_B \tilde{T}(r,z,t)}{6\pi r_s \mu}, \tag{6}$$

where k_B is the Boltzmann constant (k_B = 1.38×10^{-23} J·K^{-1}), r_s is the radius of the spherical particle, μ is the dynamic viscosity.

The mass transport model also includes initial-boundary conditions [27]:

$$\begin{cases} \Gamma_1 \text{ and } \Gamma_4: & \tilde{c}_d(r,z,t) = 0.9c_{bath}, \\ \Gamma_2 \text{ and } \Gamma_3: & -\boldsymbol{n} \cdot \tilde{D}(\tilde{T})\nabla\tilde{c}_d(r,z,t) = 0, \\ t = 0: & \tilde{c}_d(r,z,0) = c^0, \end{cases} \tag{7}$$

where c^0 is the initial concentration, c_{bath} is the concentration of the surrounding medium (a bathing solution). The 0.9 factor reflects the mass transfer between the domain Ω and the surrounding medium.

2.2. Numerical model

The numerical model was prepared applying the finite difference method (FDM) considering fuzzy numbers theory (see Appendix). An explicit scheme was used to analyse transport phenomena for unsteady state [18].

A time mesh was established with a constant step, defined by $\Delta t = t^{f-1} - t^{f}$. The grid for computational domain (Ω) was created based on the five-point star illustrated schematically in Figure 2, where h_1 and h_2 represent the mesh step in the r- and z-direction, respectively, node (i, j) is the central node. This concept assumes that boundary nodes are located at a distance of $0.5h_1$ and $0.5h_2$ from the edge. [18].

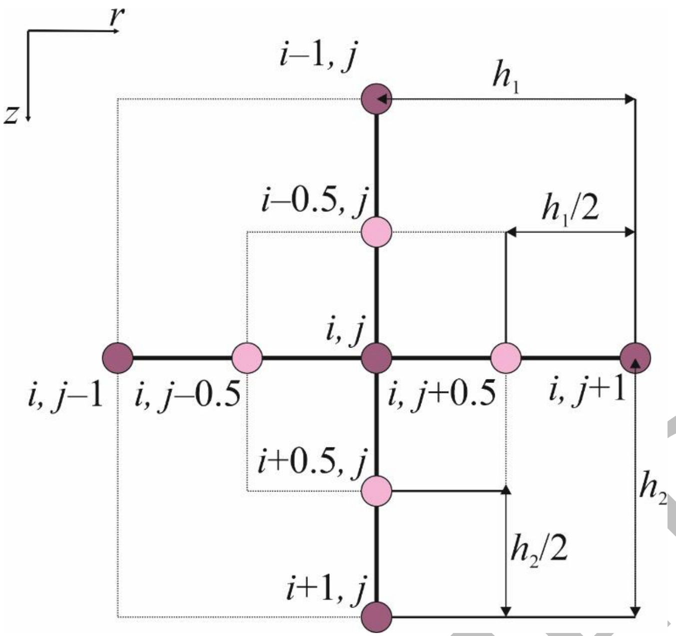


Figure 2. Five-points star

The idea of FDM is to convert differential equations into algebraic equations by replacing the appropriate differential quotients. Different types of differential quotients can be consulted in the literature [26].

By substituting the relevant relations into Equation (2), and after transformation, the following formula for internal nodes was obtained [26]:

$$\tilde{T}_{i,j}^{f} = \tilde{T}_{i,j}^{f-1} + \frac{\Delta t}{\tilde{c}_V}\left[\sum_{a=1}^{4} \frac{\Phi_e}{\tilde{R}_e^{f-1}}\left(\tilde{T}_e^{f-1} - \tilde{T}_{i,j}^{f-1}\right)\right], \tag{8}$$

where $i = 2, 3, \ldots, n-1$ and $j = 2, 3, \ldots, m-1$, n and m are the number of nodes, a corresponds to e = {$(i, j+1)$; $(i, j-1)$; $(i+1, j)$; $(i-1, j)$}, $\tilde{R}_e$ and Φ_e is the fuzzy thermal resistance and the shape function, respectively, where:

$$\begin{aligned} \tilde{R}_{i,j-1}^{f-1} &= \frac{0.5h_1}{\tilde{k}_{i,j}^{f-1}} + \frac{0.5h_1}{\tilde{k}_{i,j-1}^{f-1}}, \quad \tilde{R}_{i,j+1}^{f-1} = \frac{0.5h_1}{\tilde{k}_{i,j}^{f-1}} + \frac{0.5h_1}{\tilde{k}_{i,j+1}^{f-1}}, \\ \tilde{R}_{i-1,j}^{f-1} &= \frac{0.5h_2}{\tilde{k}_{i,j}^{f-1}} + \frac{0.5h_2}{\tilde{k}_{i-1,j}^{f-1}}, \quad \tilde{R}_{i+1,j}^{f-1} = \frac{0.5h_2}{\tilde{k}_{i,j}^{f-1}} + \frac{0.5h_2}{\tilde{k}_{i+1,j}^{f-1}}, \end{aligned} \tag{9}$$

and

$$\Phi_{i,j-1} = \frac{r_{i,j} - 0.5h_1}{r_{i,j}h_1}, \quad \Phi_{i,j+1} = \frac{r_{i,j} + 0.5h_1}{r_{i,j}h}, \quad \Phi_{i-1,j} = \Phi_{i+1,j} = \frac{1}{h_2}, \tag{10}$$

where $r_{i,j}$ is the radial coordinate of the node (i, j).

In a similar procedure, a numerical model was created for the mass transfer, hence Equation (5) for internal nodes has the form [26]:

$$(\tilde{c}_d)_{i,j}^{f} = (\tilde{c}_d)_{i,j}^{f-1} + \Delta t \sum_{a=1}^{4} \frac{\Phi_e}{\widetilde{W}_e^{f-1}} \left[(\tilde{c}_d)_e^{f-1} - (\tilde{c}_d)_{i,j}^{f-1} \right], \tag{11}$$

where $i = 2, 3, \ldots, n - 1$ and $j = 2, 3, \ldots, m - 1$, $\widetilde{W}_e$ is the fuzzy mass diffusion resistance:

$$\begin{aligned} \widetilde{W}_{i,j-1}^{f-1} &= \frac{0.5h_1}{\widetilde{D}_{i,j}^{f-1}} + \frac{0.5h_1}{\widetilde{D}_{i,j-1}^{f-1}}, \quad \widetilde{W}_{i,j+1}^{f-1} = \frac{0.5h_1}{\widetilde{D}_{i,j}^{f-1}} + \frac{0.5h_1}{\widetilde{D}_{i,j+1}^{f-1}}, \\ \widetilde{W}_{i-1,j}^{f-1} &= \frac{0.5h_2}{\widetilde{D}_{i,j}^{f-1}} + \frac{0.5h_2}{\widetilde{D}_{i-1,j}^{f-1}}, \quad \widetilde{W}_{i+1,j}^{f-1} = \frac{0.5h_2}{\widetilde{D}_{i,j}^{f-1}} + \frac{0.5h_2}{\widetilde{D}_{i+1,j}^{f-1}}. \end{aligned} \tag{12}$$

The implementation of differential quotients for boundary nodes was reported in the literature [26], therefore this element of the numerical model will not be presented here.

A stability condition was also specified for the given model [26]:

$$\Delta t \le \sum_{a=1}^{4} \frac{\tilde{R}_e^{f-1}}{\Phi_e} \quad \text{and} \quad \Delta t \le \sum_{a=1}^{4} \frac{\widetilde{W}_e^{f-1}}{\Phi_e}. \tag{13}$$

3. Results

Our study modelled the cryopreservation process for a homogeneous biological sample made of articular cartilage with dimensions $H = 1\times10^{-3}$ m and $R = 3\times10^{-3}$ m (see Figure 1b). The thermophysical parameters were introduced as fuzzy numbers described by a triangular function and a Gaussian function. For the analysis for triangular fuzzy numbers, the following parameter values were introduced: $\tilde{c}_V = (3.728\times10^6; 3.924\times10^6; 4.120\times10^6)$ J·K^{-1}·m^{-3} and $\tilde{k} = (0.492; 0.518; 0.544)$ W·m^{-1}·K^{-1}. For Gaussian fuzzy number, it was assumed that: the mean values are $m_{cv} = 3.924\times10^6$ J·K^{-1}·m^{-3}, $m_k = 0.518$ W·m^{-1}·K^{-1} and standard deviations are $\sigma_{cv} = 1.962\times10^4$ J·K^{-1}·m^{-3}, $\sigma_k = 0.026$ W·m^{-1}·K^{-1} for the volumetric specific heat capacity and the thermal conductivity, respectively [1],[32],[33]. Convection heat transfer coefficient is equal to $\alpha_\Gamma = 525$ W·m^{-2}·K^{-1} [33]. Other parameters used in the simulation were input data characterizing the chemical properties of CPA (DMSO) in the context of the diffusion phenomenon, which are $r_s = 2.541\cdot10^{-10}$ m and $\mu = 1.996\cdot10^{-3}$ Pa·s [25],[33].

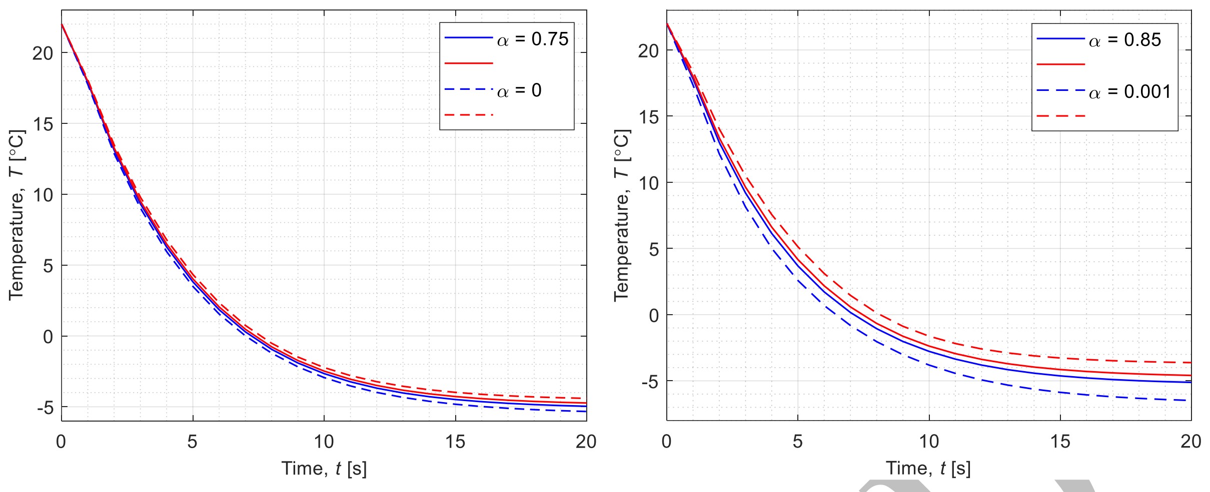
The model was completed with initial conditions, where $T^0 = 22$ °C, $c^0 = 0$ %(w/w) [27],[33]. However, the values of temperature and DMSO concentration of the bath solution used to calculate the boundary variables are determined based on Pegg's protocol for cooling, as shown in Table 1 [20].

For the fuzzy numbers described by the triangular membership function, the simulations were performed for α = {0; 0.25; 0.5; 0.75; 1}, while for the fuzzy numbers described by the Gauss membership function, for α = {0.001; 0.15; 0.25; 0.35; 0.45; 0.5; 0.65; 0.75; 0.85; 0.95; 1}. It is also assumed that time step $\Delta t = 0.005$ s and mesh steps $h_1 = 0.0001$ m and $h_2 = 0.00005$ m.

Table 1. Temperature and DMSO concentration of the bath solution

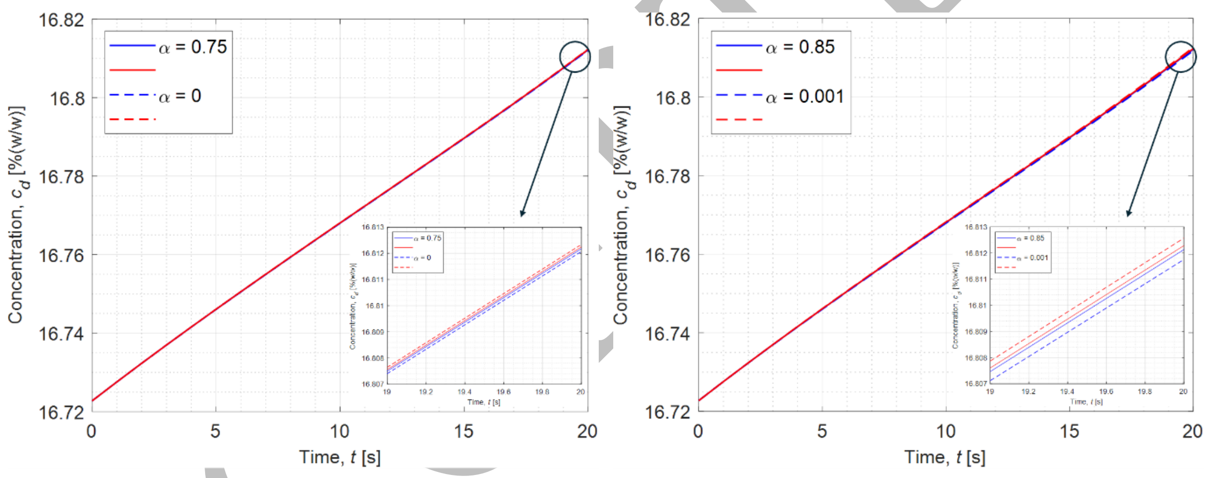
Step	Time duration	Temperature of Bath Solution	Concentration of Bath Solution
	t [min]	T_{bath} [°C]	c_{bath} [%(w/w)]
1.	10	22	10
2.	10	22	20
3.	30	−5	29
4.	30	−8.5	38
5.	30	−16	47
6.	30	−23	56
7.	30	−35	63
8.	30	−48.5	72

Figures 3-6 show the results of the simulation, which were collected at point $r = 0.00005$ m, $z = 0.000475$ m. Figure 3 illustrates the fuzzy temperature curves in the selected period of time (for 20 s of step 3) for different parameters α using triangular (a) and Gaussian (b) membership function. Figure 4, in analogy to Figure 3, presents the dependence of the fuzzy concentration of DMSO over a selected period of time (for 20 s of step 3). for different parameters α using triangular (a) and Gaussian (b) membership function.



(a) (b)

Figure 3. Fuzzy temperature in time (for 20 s of step 3, point $r = 5\times10^{-5}$ m, $z = 4.75\times10^{-4}$ m) for triangular (a) and Gaussian (b) membership functions

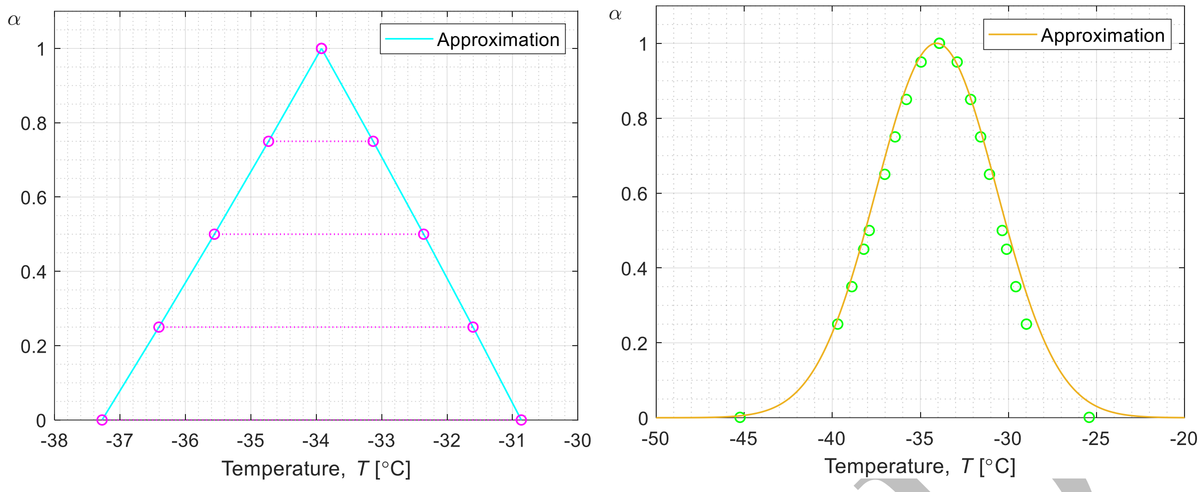


(a) (b)

Figure 4. Fuzzy concentration in time (for 20 s of step 3, point $r = 5\times10^{-5}$ m, $z = 4.75\times10^{-4}$ m) for triangular (a) and Gaussian (b) membership functions

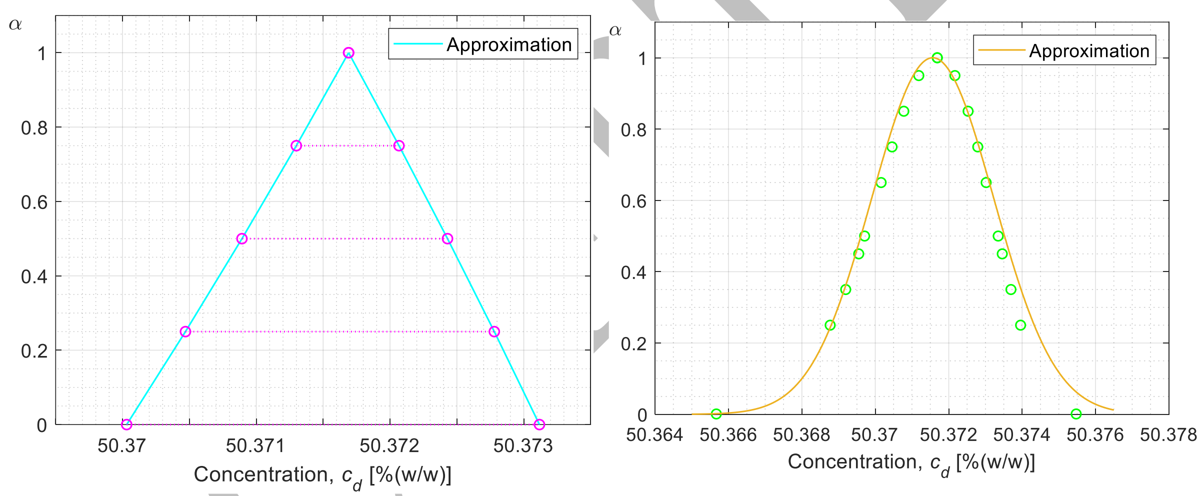
1.

Figure 5 depicts the fuzzy temperature at the selected moment of simulation time (10 s at step 7) obtained for the triangular (a) and Gaussian (b) membership functions. Please note that the distribution of the variable was approximated from the results for the Gaussian membership function. Similarly, Figure 6 shows the fuzzy DMSO concentration at a selected moment of simulation time (10 s at step 7) received for the triangular (a) and Gaussian (b) membership functions.



(a) (b)

Figure 5. Fuzzy temperature at the selected moment of simulation time (10 s at step 7, point $r = 5\times10^{-5}$ m, $z = 4.75\times10^{-4}$ m) for the triangular (a) and Gaussian (b) membership functions



(a) (b)

Figure 6. Fuzzy concentration at the selected moment of simulation time (10 s at step 7, point $r = 5\times10^{-5}$ m, $z = 4.75\times10^{-4}$ m) for the triangular (a) and Gaussian (b) membership functions

Table 2 compares the obtained concentration for the triangular and Gaussian membership functions with the experimental data from the literature [20]. The first two columns show the obtained fuzzy temperature results for the triangular and Gaussian membership functions. It can be seen that the given fuzzy temperatures coincide with the bath solution temperatures (compare with Table 1).The next sections of the table show a comparison of the fuzzy DMSO concentration in the cellular matrix described by the triangular and Gaussian membership functions with the experimental results. For the DMSO concentration, there are differences between the simulation results and the experimental data, as shown by the calculated relative error, the highest value of which is 15.82% (step 8) and the lowest value of which is 0.06% (step 4).

Table 2. Comparison of results with experimental data

Step	Fuzzy temperature for α = 0 (triangular m. f.), $\tilde{T}$ [°C]	Fuzzy temperature (Gaussian m. f.), $\tilde{T}$ [°C]	Fuzzy concentration for α = 0 (triangular m. f.), $\tilde{c}_d$ [%(w/w)]	Fuzzy concentration (Gaussian m. f.), $\tilde{c}_d$ [%(w/w)]	Experimental data, c_d [%(w/w)]	Relative error, δ [%]
1	[22.0000; 22.0000]	m = 22.0000 σ = 0.0000	[7.8386; 7.8386]	m = 7.8386; σ = 0.0000	–	–
2	[22.0000; 22.0000]	m = 22.0000 σ = 0.0000	[16.7228; 16.7228]	m = 16.7228 σ = 0.0000	16.3 ± 1.3	2.59
3	[−5.5120; −4.5355]	m = −5.0454 σ = 0.6996	[26.0787; 26.0792]	m = 26.0790 σ = 3.55×10^{-4}	24.5 ± 1.1	6.44
4	[−9.3704; −7.7104]	m = −8.5773 σ = 1.1893	[34.1789; 34.1798]	m = 34.1793 σ = 5.95×10^{-4}	34.2 ± 0.9	0.06
5	[−17.6384; −14.5136]	m = −16.1454 σ = 2.2386	[42.2743; 42.2762]	m = 42.2752 σ = 0.0013	41.7 ± 3.3	1.38
6	[−25.3552; −20.8633]	m = −23.2090 σ = 3.2180	[50.3691; 50.3722]	m = 50.3705 σ = 0.0023	47.8 ± 2.8	5.38
7	[−38.5840; −31.7485]	m = −35.3181 σ = 4.8969	[56.6669; 56.6719]	m = 56.6692 σ = 0.0037	52.2 ± 1.3	8.56
8	[-53.4664; −43.9944]	m = −48.9408 σ = 6.7857	[64.7393; 64.7516]	m = 64.7449 σ = 0.0093	55.9 ± 2.9	15.82

m. f. – membership function

4. Discussion

To begin with, it is worth examining the results in Figures 3-6 and the Table 2. It can be seen that the temperature distribution in the sample stabilises relatively quickly and reaches the value of the bath solution (cf. Figure 3). In the case of a change in DMSO concentration, a continuous increase is observed without any apparent stabilisation as in the case of the temperature curve (cf. Figure 4). In addition, it is noticeable in the graphs in Figures 3 and 4 that the smaller the value of the parameter α, the narrower the width of the interval. From Figures 5 and 6 it can also be observed that the value of parameter α affects the width of the interval. Similar conclusions about the effect of the parameter α on the distribution of a given quantity described as a fuzzy number are provided, for example, in the dissertation [26]. This thesis considers different computational problems for the cryopreservation process applying fuzzy numbers described by triangular and trapezoidal membership functions.

In this study, numerical simulations were performed for fuzzy thermophysical parameters described by a Gaussian membership function, which is a novel approach (in [21],[23],[26] only the triangular and trapezoidal membership function are presented). The results obtained were compared with those for the triangular membership function (see Table 2 and Figures 5-6). Triangular fuzzy numbers have sharp and linear membership boundaries, which makes them easier to implement. The Gaussian membership function, on the other hand, has smooth boundaries and tends asymptotically to zero. Gaussian fuzzy numbers are more complex to calculate due to the exponential nature of the membership function. It can be assumed that it is worth using them to model probabilistic phenomena. The use of Gaussian fuzzy numbers is certainly an interesting extension of the research topic dealt with by the authors of this paper.

On the other hand, analysing Table 2, it is noticeable discrepancies between numerical results and experimental data. Referring to previous articles, it can be suggested that it is worthwhile to analyse, for example, the mathematical model, the calculated values of the diffusion coefficient, as well as the introduced thermophysical parameters. A similar study of cryopreservation using the LT protocol and the deterministic thermophysical parameters presents Yu et al. [33]. However, Yu et al. in their assumptions determined that the extracellular matrix of articular cartilage is a porous and isotropic material. As a consequence, the diffusion coefficient depends on the properties of the porous media, such as the tortuosity. This assumption can consequently lead to more accurate numerical simulation results. Articular cartilage as a porous material is also described in the work of Behrou et al. [1], who distinguish the liquid and solid phases in the tissue, and explore the effect of temperature on its properties.

5. Conclusion

This paper presents the results of a simulated cryopreservation of a biological sample. The cryopreservation of an articular cartilage sample was modelled using the LT protocol. This approach allows the temperature and concentration to be controlled in order to avoid the formation of ice crystals which would lead to the destruction of the biological sample. Due to the imprecise nature of the thermophysical parameters, they were introduced as fuzzy numbers described by a triangular and a Gaussian membership function. It should be noted that Gaussian fuzzy numbers do not have the sharp interval boundaries that characterise triangular numbers. Therefore, the Gaussian membership function works well for experimental data where the mean and standard deviation are known. Triangular and Gaussian fuzzy numbers also share common characteristics. Using the α-cut concept, the width of the interval is widest for $\alpha = 0$ and narrowest for $\alpha = 1$ (is equal to 0).

The obtained fuzzy concentrations and temperatures in eight stages of the LT protocol for triangular and Gaussian membership functions were compared with experimental data taken from the literature. The calculated fuzzy temperatures are consistent with the temperature values occurring in the LT protocol. Larger differences between the experimental data and the calculated values are observed for the fuzzy DMSO concentration, where the maximum relative error is 15.82%. It is suggested that this is due to an inappropriate selection of thermophysical parameters or a model describing the diffusion coefficient.

Acknowledgment

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Appendix

Sets $\widetilde{A}$ of fuzzy numbers are sets in which each element x is assigned a relevant membership function [5],[9],[21]:

$$\widetilde{A} = \{(x, \mu_{\widetilde{A}}(x)); x \in \mathbb{X}\}, \tag{A.1}$$

where $\mu_{\widetilde{A}}$ is the membership function, which takes the value from 0 to 1. Fuzzy numbers which belong to a set can be described by different membership functions. In our study, the triangle membership function described as a straight line and the Gaussian membership function were implemented.

The membership function for the triangular fuzzy number $\tilde{a} = (a^-, a_0, a^+)$ is expressed by the relation [10],[21]:

$$\mu_{\tilde{a}}(x) = \begin{cases} 0, & x < a^-, \\ \frac{x-a^-}{a_0-a^-}, & a^- \le x \le a_0, \\ \frac{a^+-x}{a^+-a_0}, & a_0 \le x \le a^+, \\ 0, & x > a^+, \end{cases} \tag{A.2}$$

where a_0, a^-, a^+ are the core of the number and the left and right ends of the fuzzy number, respectively.

On the other hand, the Gaussian membership function for a fuzzy number $\tilde{a} = (m_a, \sigma_a)$ has the form [14]:

$$\mu_{\tilde{a}}(x) = exp\left[\frac{-(x-m_a)^2}{2\sigma_a^2}\right], \tag{A.3}$$

where m_a, σ_a denote the mean value and standard deviation of data set a, respectively.

The α-cut for a given fuzzy set $\widetilde{A}_\alpha$ is defined as the set of all elements $\widetilde{A}$ whose membership function is greater than α [5],[10]:

$$\widetilde{A}_\alpha = \{x \in \mathbb{X}: \quad \mu_{\widetilde{A}}(x) \geq \alpha\}. \tag{A.4}$$

As a consequence, a fuzzy number is calculated as the sum of all α-cuts:

$$\widetilde{A} = \sum_{\alpha \in [0,1]} \alpha \widetilde{A}_\alpha. \tag{A.5}$$

Then the fuzzy numbers are expressed as closed intervals, where for triangular fuzzy numbers it is given as [21]:

$$\tilde{a}_\alpha = [(a_0 - a^-)\alpha + a^-, (a_0 - a^+)\alpha + a^+], \tag{A.6}$$

and for fuzzy numbers described by Gaussian membership function [14]:

$$\tilde{a}_\alpha = \left[m_a - \sigma_a\sqrt{-2\ln\alpha}, m_a + \sigma_a\sqrt{-2\ln\alpha}\,\right]. \tag{A.7}$$

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