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Numerical Simulation of Freeze-Thaw Biopharmaceutical Processes

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Freezing and thawing of protein in aqueous solutions are two essential tasks in the area of biopharmaceuticals manufacturing. Freezing allows to store and transport proteins without chemical degradation or microbial growth. Thawing, complicated and intricate as freezing, recovers the protein solution for further processing. Conducting experiments using biological materials have proven to be costly, and accurate results using less expensive tools can be obtained by means of numerical simulations based on Computational Fluid Dynamics. The objective of this study was to develop freeze-thaw models for biological substances. The final goal was to predict the mechanical behavior of the proteins and the variation of certain chemical parameters in the liquid in which they are contained. Our methods were validated on a simple freezing case and then applied to a real industrial case. The outlook of the work is the development of physical models that take into account the local concentration of solutes (salts) and the pH, as well as their impact on protein stability.

Keywords: freezing, proteins, phase change, numerical simulation, CFD.

1. Introduction

Freezing and thawing of protein in aqueous solutions are two essential process steps in the area of biopharmaceuticals manufacturing. As reported for example by Kohle et al. (2010) freezing allows store and transport proteins without chemical degradation or microbial growth. However, the freeze/thaw process offers numerous challenges due to the complexity of the physics and the poor understanding of how the local solute concentrations are affected by various process and system parameters. Furthermore, the impact of salt and solute concentrations on protein stability is an unknown. Because of the general lack of testable knowledge, trial and error methods are often used to solve industrial problems related to freeze/thaw processing.

There are a number of studies that have published with regards to proteins freezing, for example Liu et al. (2005), but often they are limited to small or microscopic volumes which are not representative of the entire bulk drug system. Hence, rational scale-up, i.e., the control of effects occurring and dominating at different scales, is a critical step in the design of freeze/thaw processes.

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Experiments conducted in this field can be very expensive, especially if performed on full-scale geometries, as they require relatively large amounts of proteins that are lost at the end of the experimental campaign. Moreover, the available experimental tools do not easily allow characterization of the mechanical and thermal behavior of proteins and the solution properties during freezing.

While analytical procedures are available allowing the analysis of one-dimensional cases involving an infinite or semi-infinite region with simple initial and boundary conditions and constant thermal properties (Hu and Argyropoulos., 1996), modern Computational Fluid Dynamics (CFD) tools enable an accurate analysis of freeze/thaw processes. In order to solve the moving boundary problems, many authors have presented numerical methods based on finite-difference (Vlasichev, 2001), finite-element (Chakraborty and Dutta, 2003) or finite-volume procedures (Kim et al., 2002). Depending on the issue under investigation, simplified approaches can be adopted to freeze/thaw problems in order to match the industrial requirements in terms of computational costs. This study investigates and compares also a simplified model that solves only the thermal behavior of a fluid and can be used for problems where buoyancy is not an issue. This model saves a significant amount of computational time. For problem including buoyancy effects a more detailed model has been validated and applied to an industrial case. The outlook of the current work is the development of a physical model that also takes into account the movement of proteins.

AVL FIRE was employed to solve the model equations. The simulations ran on 8 CPUs Intel Xeon E5540 @ 2.53GHz machines (4 dual core) with 24GB RAM.

2. Theoretical Model

Two theoretical models are presented here: a simple model for tracking the ice front (single phase model) and a more complex model that involves the presence of buoyancy effects (multiphase model). Both models were solved using a finite-volume approach.

2.1 Single Phase Model

A simple model used only for tracking the ice front would contribute greatly to the understanding of the behavior of ice evolution and characteristic freezing time for various configurations. Indeed for problems involving phase-change phenomena, the position of the moving boundary cannot be assumed in advance. On the contrary, the position must be determined by the solution procedure (Mechigel and Kadja, 2007). The theoretical model involves the presence of a single liquid, i.e., pure water, which changes its characteristics abruptly depending on the temperature in each cell. The energy equation (Eq.1) rules such a behavior. The core of the model is the change of density (ρ) thermal conductivity (k) and viscosity (μ) as function of the temperature. When the temperature in each cell reaches the critical freezing temperature (CFT), the system properties change in a step-wise function, i.e.:

$$\rho\frac{\partial H}{\partial t} = k\nabla^2 T + S_H \qquad \begin{cases} T_c \geq CFT \Rightarrow (\rho,k,\mu) = (\rho,k,\mu)_{liquid\_phase} \\ T_c < CFT \Rightarrow (\rho,k,\mu) = (\rho,k,\mu)_{solid\_phase} \end{cases} \tag{1}$$

2.2 Multiphase Model

In this approach the presence of buoyancy effects are taken into account. The solid phase is described as a porous media, and the methodology employs a multi–fluid modeling in conjunction with an enthalpy-porosity technique to track the melting/solidification front effectively on a fixed grid. The presence of a phase change requires addition of equations to consider the latent heat exchange. This has been mathematically realized by adding an enthalpy source terms in the thermal equation (Swaminathan and Voller, 1992), within an Eulerian multiphase modeling framework (AVL, 2010). The continuity equation for each phase, namely water and ice, has been written in the form indicated by Drew and Passman (1998),

$$\frac{\partial \alpha_k \rho_k}{\partial t} + \nabla \cdot \alpha_k \rho_k \mathbf{v}_k = \sum_{l=1, l \neq k}^{N} \Gamma_{kl} \qquad \left(\sum_{k=1}^{N} \alpha_k = 1; \qquad \Gamma_{kl} = \rho_k \cdot f(\Delta H) \right) \tag{2}$$

where **v** is the velocity vector, α is the volume fraction and ρ is the density of k-th phase, while the right hand side of Eq. 2 describes the sources due to phase change. Momentum and continuity equations are also calculated as suggested by Drew and Passman (1998).

3. Validation Cases

3.1 Single Phase Model Setup and Results

The theoretical model that involves only the adoption of the energy equation was validated using the data provided by Buyruk et al. (2009) (Figure 1 a). The initial temperature in the field was 4°C and the semi-pipe of diameter d was kept at the constant temperature of -10°C. The walls intersecting the pipe were defined as symmetry surfaces; the other surfaces were adiabatic. The material employed for this validation was water. As suggested by theory (Fachinotti, 2006), (Rabello et al., 2005), for ice-front tracking problems the time step is a critical issue that can affect results. The condition $\Delta t < \alpha(\rho c_P / k)\Delta x^2$ determines the time-step as a function of physical properties (the media) and cell dimension, avoiding numerical instabilities. The ice front was tracked for almost 7000 s and a sensitivity analysis of the mesh size was performed. Figure 1 b shows numerical and experimental data obtained by Buyruk et al. (2009) and comparison with present numerical results. The use of a very fine mesh would allow our case to match perfectly the numerical solution reported in literature. In this case, according with Eq.3, the shorter time-step makes the problem computationally significantly expensive. However, an intermediate refinement of the mesh yielded sound results that agreed well with the experimental data, even with less computational efforts.

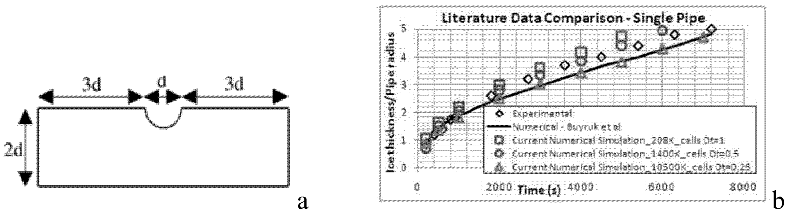

Figure 1: Single phase model: validation and mesh sensitivity

3.2 Multi-Phase Model Setup and Results

The multiphase model was validated using the experimental and numerical data provided by Giangi et al. (1999), where the geometry is a cube. Two opposite faces are kept at constant T of +10°C and -10°C, whereas the lateral walls are adiabatic. Figure 2 shows that buoyancy effects are present and that they affect the ice-front evolution. The change of density in the water induced the densest water (close to the coldest wall) to travel towards the bottom of the box, thus producing a clockwise flow.

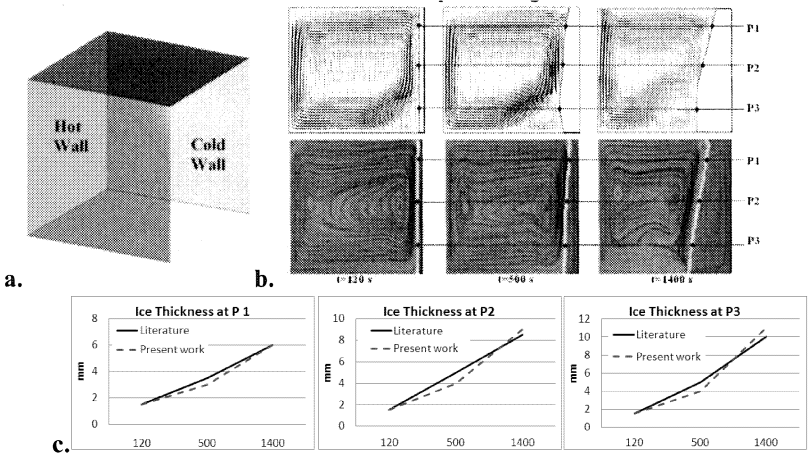

Figure 2: (a) validation test case; (b) simulation results; (c) comparison of numerical results with literature data on ice thickness at different locations.

Due to this flow the heat flux from/to the wall is not uniform, leading to a non-symmetric ice front. The evolution of ice thickness along different lines (P1, P2, P3) for our simulations shows a very good agreement with the literature data (Figure 2, c).
A mesh sensitivity study was performed according to the methodology proposed by Celik et al. (2008). Monitoring the ice thickness along P1, P2 and P3 for three different meshes, the Fine-Grid Convergence Index (GCI) was less than 5.9% for the worst case.

4. Application of the Models to an Industrial Case

The two presented models for single and multiphase freezing have been applied to an industrial case, i.e., a freeze/thaw vessel unit of 350l from ZETA Biopharma (Figure 3 a). The pipes, the bottom and the lateral walls were all elements of the cooling system, whereas the top was set as adiabatic wall. The computational mesh consists of around 1.1 millions cells. The initial temperature of the liquid, i.e., pure water, was set to 18°C, while the temperature of the cooling system was -40°C for the base case. The temperature profile in a probe inside the tank was also available for comparisons with the numerical data. The probe coordinates (x, y, z) are (0.1, 0.1,-0.06)m, according to a coordinate system located along the symmetry axis of the vessel at a distance of 0.194 m from the bottom (see Figure 3). The first simulations were performed with the faster single-phase model, allowing us to compare numerical results and measurements for a wall temperature T_w of -40°C (see Figure 3).

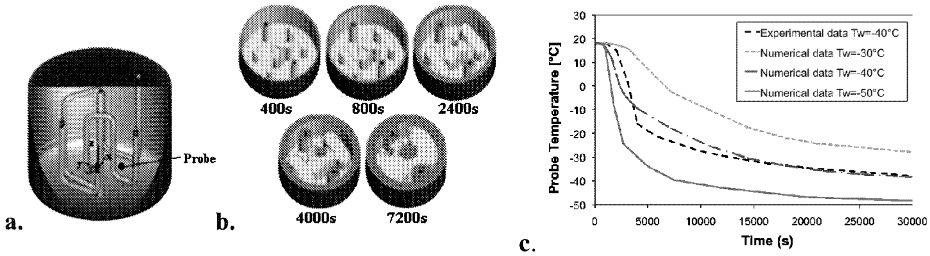

Figure 3: Geometry of the freeze/thaw unit (a); ice front evolution for the single phase model (b); temperature evolution at the probe location (c).

Here, the evolution of the ice front is also presented as contour plots for different times during freezing process. These results indicate that the ice growth occurred mainly in the first 2 hours. The rest of the time is then used to reduce the temperature of the solid phase. Even if the simulated temperatures qualitatively agree with the experimental data, different gradients at the beginning and during the cooling phase are still detectable in the diagrams due to the approximation adopted in the model. Nevertheless, this method may be used as simple engineering tool to predict the temperature evolution inside the tank for different wall conditions, for example -30°C and -50°C (see diagram in Figure 3, c).

As in real freeze-thaw units buoyancy effects are always present, the multiphase model has been also used to characterize the freezing process. Due to the high computational costs of this approach, only the first 2000 s have been simulated and are presented in this work. The application of the multiphase model to the industrial case produces results that are strongly affected by the presence of a velocity field. The streamlines of the velocity field clearly show recirculation zones close to the walls (see Figure 4, a).

The profiles of the mean temperature at the probe location for both presented approaches are shown in Figure 4 (b). The quantitative results of the multiphase method, presented as average temperature of both phases at the probe location, weighted by volume fraction, appear to match the experimental data quite well. This underlines the importance of convective effects in freezing simulations. In fact, the flowing medium induces a non-homogeneous thermal distribution inside the tank. Furthermore, the buoyancy behaviour due to the local temperature (and thus density) leads to a migration of warmer water to the top of the freezing unit (see Figure 4, c). As a consequence, the ice front starts to grow from the bottom zone.

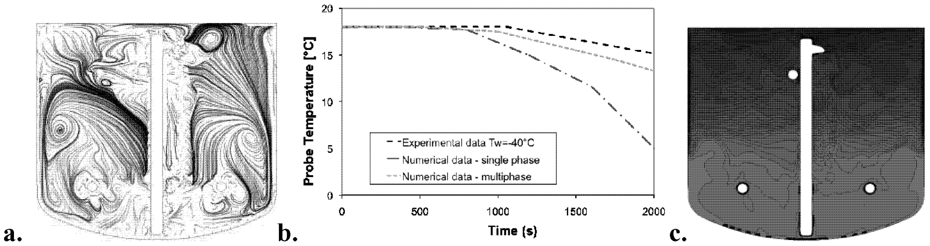

Figure 4: Flow streamlines at t=1000s (a); temperature profiles at the probe location (b); temperature field for water phase at t=1000s (c).

5. Conclusions and Outlooks

Two numerical models have been developed and validated for freezing problems involving a phase change. The presented methods aim at providing a deep understanding of the process, thus facilitating development and design of a freeze/thaw unit for biological products. The next steps will include the integration of models for the transport of biological compounds and proteins, and simulation of concentration effects.

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