

A numerical model of protein crystallization with counter diffusion method.

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Abstract. A theoretical model is presented to simulate the growth and nucleation of protein lysozyme crystals by counter diffusion method. The comparison of experimental and simulation results shows that the numerical solution accurately describes the initial stages of crystallization. The developed model can be used to describe crystallization processes with various counter diffusion experiment parameters.

1. Introduction

The solution of many biomedical problems requires detailed information about chemical structure of biological molecules. This information can be obtained from X-ray analysis of protein crystal. However, for greater resolution of the protein space structure this crystal should be of sufficient size and quality. One of the widely used methods of protein crystallization is counter diffusion method [1]. In this method, protein crystallization occurs due to diffusion of protein and precipitant solution towards each other through the agar gel layer. This process allows to obtain gradient of concentration from one edge of capillary to another. Diffusion rate of protein solution is much slower than that of precipitant. Thus we get the opposed concentration gradients for protein solution and precipitant, which is an initial condition for protein nucleation process. The advantage of this method is that one experiment with counter diffusion technique is equivalent to a huge amount of experiments with “hanging” or “sitting drop” method. Its main characteristic is nonlinear relation between different physical processes: diffusion of protein and precipitant; nucleation and growth of protein crystal. Vacuum silicon grease and wax film were used to seal the crystallization cell.

2. Experiment

2.1. Experiment preparation

In the experiment solubility of lysozyme is controlled by the sodium chloride. Figure 1 illustrates a simplified scheme of counter diffusion experiment.



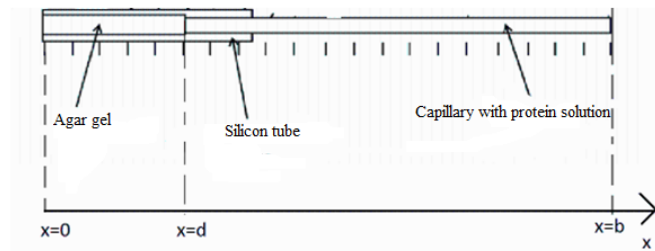


Figure 1. Configuration of the simplified counter-diffusion method

2.2. Samples preparation

In the experiment we used 1%(w/v) agar gel with 0.04% NaN_3 , obtained in a standard way [2]. Capillary was 200 μm in diameter. We prepared 30, 65, 100 mg/ml hen-egg lysozyme solution, in 50mM acetate buffer with $\text{pH}=4.5$. Also we prepared 0.5, 0.8, 0.1 M of sodium chloride solution, in 50mM acetate buffer. For one experiment 8 ml of sodium chloride solution is needed. Protein and precipitant solution were degassed. After filling all capillaries, one edge of capillary was sealed with wax with another edge placed in silicon tube filled by agar gel. Finally, samples were placed in precipitant solution.

2.3. Snapshots of the protein crystal

The experiment was filmed using Carl Zeiss Axio Observer microscope with 1 hour intervals. To get more accurate results, for every picture along the capillary length (X-axis) different cross section of the capillary were taken (Z-axis) with 1mm step along the X-axis and 50 microns step along the Z-axis. Thus, for a capillary with internal diameter of 200 μm five cross-sections were taken for each point on the X-axis.

3. Theoretical model

3.1. Model assumptions

We divide the capillary by N cells. Each i cell ($i=1..N$) has volume ΔV . Precipitant and protein solution concentrations are considered homogenous, thus dependant only of time.

3.2. Main physical processes

Diffusion of Sodium chloride ions through the agar gel from reservoir to capillary. Diffusion of protein molecules (with C_i concentration in i cell) from capillary to reservoir through agar gel. Nucleation of crystals with critical radius when supersaturation $S \geq 1$ occurs

$$S(x,t) = \frac{C(x,t)}{C_0(x,t)} \quad (1)$$

Nucleation rate for each cell of capillary at time t is defined by supersaturation function $S(x,t)$.

According to Volmer-Becker-Doring theory, nucleation rate can be described with Arrhenius equation [3]:

$$W(x,t) = k_0 C(x,t) \exp\left(-\frac{\Delta G_{crit}}{(kT)}\right) \quad (2)$$

$$\Delta G_{crit} = \frac{16\pi\Omega^2\gamma^3}{3(kT)^2 \ln^2 \left(\frac{C(x,t)}{C_0(x,t)} \right)} \quad (3)$$

where k_0 – empirical constant. $C(x,t)$, $C_0(x,t)$ – concentrations of protein molecules in solution and solubility respectively, Ω – volume of one molecule in crystal, γ – surface free energy of the critical cluster, T – temperature k – Boltzmann constant.

To change solubility of lysozyme sodium chloride was used. When using a simple electrolyte as the precipitant, the protein solubility can be expressed as function of salt concentration [4]

$$C_0(x,t) = C_1(x,t) + C_2(x,t) \quad (4)$$

$$\ln(C_1(x,t)) = \ln(C_{10}) - k_{o1}C_s(x,t) \quad (5)$$

$$\ln(C_2(x,t)) = \ln(C_{20}) + k_i(C_s(x,t))^{1/2} - k_{o2}C_s(x,t) \quad (6)$$

where $C_{10}, C_{20}, k_{o1}, k_{o2}, k_i$ empirical constants

Numerical value of function C_s can be obtained from diffusion equation

$$\frac{\partial C_s(x,t)}{\partial t} = \frac{\partial}{\partial x} D_s(x) \frac{\partial C_s(x,t)}{\partial x} \quad (7)$$

Diffusion equation for protein molecules is nonlinear partial differential equation

$$\frac{\partial C_i(x,t)}{\partial t} = \frac{\partial}{\partial x} D(x) \frac{\partial C_i(x,t)}{\partial x} - \sum_j \sum_i \alpha_q D(x) C_i(x,t) R_{q_j}(t) C_{q_j}(t) \quad (8)$$

where C_i – protein molecules concentration in i cell; α_q – empirical constant which depends on interaction between protein molecules and protein crystal; R_{q_j} – radius of j crystal in i cell; C_{q_j} concentration of crystal in j cell

Growth rate of protein crystal in j cell [5]:

$$\frac{dR_{q_j}(t)}{dt} = \frac{\Omega \alpha_q D(x) C_i(x,t)}{4\pi R_{q_j}(t)} \quad (9)$$

$D_s(x)$, $D(x)$ – are piecewise functions, because diffusion coefficient of protein molecules and ions of salt in agar gel and water are different [1,4,6].

Nucleation rate W is number of crystals nucleated per unit volume per unit time:

$$W(x,t) = \frac{\partial^2 N}{\partial V \partial t} \quad (10)$$

The waiting time until the crystal nucleates we can express with τ_0 . To get nucleation time for one crystal in i cell we integrate W , with $N=1$, and set the upper limit of integration to τ_0 :

$$\frac{1}{\Delta V} = \int_0^{\tau_0} W(x,t) dt \quad (11)$$

Equations (3,8,9,11) combined give us the final system:

$$\frac{\partial C_i(x,t)}{\partial t} = D(x) \frac{\partial C_i(x,t)}{\partial x} - \sum_j \sum_i \alpha_q D(x) C_i(x,t) \left(R_{crit}^2(x,t) + \frac{\Omega \alpha_q D(x)}{2\pi} \int_0^{\tau_0} C_i(x,t) dt \right)^{\frac{1}{2}} C_{q_j}(t) \quad (12)$$

$$\frac{1}{\Delta V} = \int_0^{\tau_0} k_0 C_i(x,t) \exp \left(- \frac{16\pi\Omega^2\gamma^3}{3(kT)^3 \ln^2 \left(\frac{C_i(x,t)}{C_{0i}(x,t)} \right)} \right) dt \quad (13)$$

$$R_{crit}(x,t) = \frac{2\Omega\gamma}{kT \ln \left(\frac{C_i(x,t)}{C_{0i}(x,t)} \right)} \quad (14)$$

The numerical model was made using MATLAB software package. Coordinate Δx and time steps Δt of differencing scheme were selected so that (in capillary and in agar gel)

$$\frac{D\Delta t}{\Delta x^2} = 0.2 \quad (15)$$

4. Results

The experiment was conducted for 24 hours. During this time the crystals appeared at a distance of 10 mm from the beginning of the capillary. Capillary snapshots were taken for analysis of experimental data; the whole capillary was divided into segments of 200 mkm length. To compare the experimental data with numerical simulation, we set step - $\Delta x = 200$ mkm in simulation.

Figure 2 shows the theoretical and experimental dependencies of the size and number of crystals. Crystal size: $L = 2 * R$.

The drop near $x=10$ mm is due to the features of the experiment, as the outside boundary of the silicone tube is in this area Figure 3.

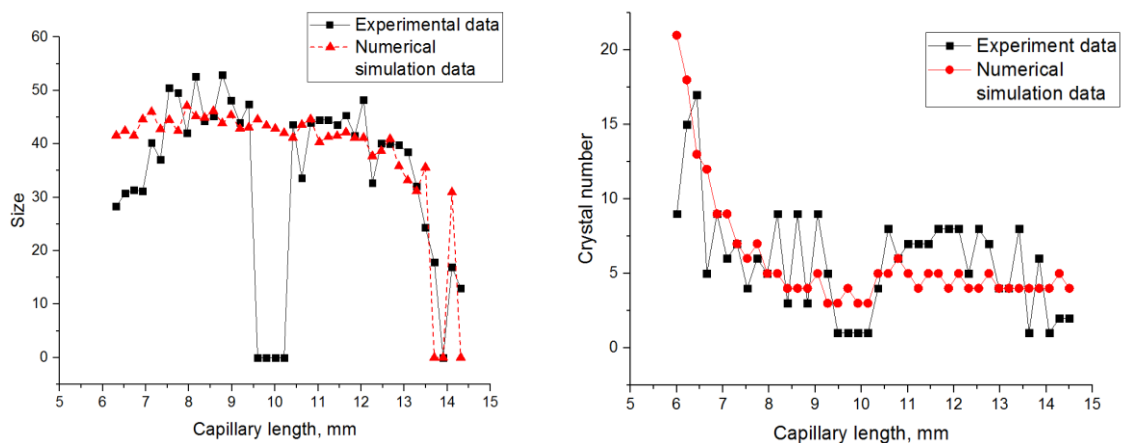


Figure 2. Size and number distribution along the length of capillary.



Figure 3. The piece of the capillary with a shaded area.

5. Conclusion

During this work the series of experiments with a model object - lysozyme were conducted and a numerical model for nucleation process was created. The numerical model predicts with high accuracy coordinates of the appearance and size of the crystal, which is confirmed by experiment. This numerical model can be used to determine the optimal crystallization conditions for other macromolecules in the non-ionic composition buffers.

References

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