Analysis of the influence of coupled diffusion on transport in protein crystal growth for different gravity levels

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Diffusion has a central role in protein crystal growth both in microgravity conditions and on ground. Recently several reports have been focused on the importance to use the generalized Fick's equations in *n*-component systems where crystals grow. In these equations the total flux of each component is produced by the own concentration gradient (main flow) and by the concentration gradient of the other components (cross-flow) present in the system. However in literature the latter effect is often neglected, and the so-called pseudo-binary approximation is used. Lin *et al.* (1995) proposed a mathematical model to evaluate the concentration profile of the species present around a growing protein crystal. Although the model is reliable, it suffers of the pseudo-binary approximation (neglecting cross term diffusion coefficients and using binary diffusion coefficients), probably because of the lack of multicomponent diffusion data. The present model is based on the experimental set-up proposed by Lin *et al*. (1995). Nevertheless we have included the coupled diffusion effects, according to the correct description of the matter transport through the generalized Fick's equations. The crystal growth rate is calculated for different gravity levels. The model has been applied to the ternary lysozyme-NaCl-water and quaternary lysozyme-poly(ethylene glycol) (PEG)-NaCl-water systems using recent diffusion data.

Keywords: numerical simulation, coupled diffusion, microgravity

1. Introduction

Protein crystal growth has been studied extensively both from experimental and numerical point of view. Whatever the complexity of the crystallization process, protein crystal growth can be divided in three steps: nucleation, transport of protein from the bulk to the solution-crystal interface and growth kinetics at the crystal surface.

Usually the nucleation probability is considered to be directly proportional to the supersaturation degree according to different functional laws. The kinetics at the interface is determined by growth rate law at the first order of the supersaturation (eventually including dissolution, segregation (Lin *et al.*, 2001), defects formation (Vekilov & Alexander, 2000)). Transport properties are usually described through a single diffusion coefficient for each solute (pseudobinary diffusion coefficients), even if frequently the necessity of including the cross diffusion coefficients are discussed (Rosenberger, 1996; Lin *et al.* 1995, Castagnolo *et al.* 2001).

From a thermodynamic point of view the mutual diffusion process is due to a chemical potential gradients of solutes. In the case of solutions, where a protein crystal grows, the pseudobinary approach means that the chemical potential of protein is considered as not being affected by the crystallizing agent concentration, which is obviously in contrast to the precipitating action: protein is driven out from the solution right because of the presence of a precipitating agent.

Accordingly to these considerations, recently some of the authors determined experimentally diffusion properties of solutions where protein crystals grow (Albright *et al.*, 1999; Annunziata *et al.*, 2000; Paduano *et al*,. 2001; Vergara *et al.*, 2002). The relevance of these studies is the multicomponent use of the diffusion description through the generalized Fick's equations (Vitagliano, 1991) for n component system:

$$J_i = -\sum_{j=1}^{n-1} D_{ij} \nabla C_j \qquad i = 1,...,n-1 \qquad (1)$$

Eq. 1 contains the main-terms D_{ii}, which account for the flux of each component on its own concentration gradient, and the off-diagonal terms D_{ij}, which account for the flux of each component on the gradient of the other component. It can be useful for the reader to give an intuitive way to explain the cross-flux: the ratio D_{21}/D_{11} is equal to the grams of molecules 2 dragged by one gram of molecule 1, if the concentration gradients are expressed in mg/ml.

Our approach explicitly takes into account the fact that the crystallizing agent must affect the chemical potential gradient of protein and *vice-versa*. Actually diffusion data have been used to extract chemical potential derivative of protein (crystallizing agent) with respect to crystallizing agent (protein) concentration (Annunziata *et al.* 2000, Vergara *et al.*, 2002).

From the experimental diffusion studies, a strong concentration dependence of diffusion coefficients were observed (Albright *et al.*, 1999; Annunziata *et al.*, 2000; Paduano *et al.* 2001; Vergara *et al.* 2002). Interestingly the cross-term diffusion coefficients linking the crystallizing agent flow to the protein gradients are huge. Considering that the protein concentration gradient is quite large at the interface, the presence of cross flow can markedly modify the matter transport. Therefore a multicomponent description can be more suitable to describe the diffusion process occurring during crystal growth.

The zone around crystals is obviously the most interested to the growth rate, and the concentration depletion occurring in this zone (CDZ) was suggested to have an enormous importance in the interpretation of the crystal order achieved in gel and in microgravity (Otalora *et al.*, 2001). In fact in these two environments the transport is essentially diffusive and the CDZ is almost undisturbed. Alternatively the absence of convection was suggested as the cause of a lower incorporation of impurity inside the growing crystal. Both these hypotheses were confirmed by experimental and numerical studies, therefore both must be considered as two reasons of the gravity level effect. Some of the authors are involved in a study of the effect of the crystallization environment on the crystal quality: the full comparison of four different environments was presented elsewhere (Vergara *et al.*): solutions on ground, solutions in microgravity, gel on ground and gel in microgravity. The present study provides the numerical model to investigate quantitatively the crystal growth rate in the four environments.

We have already presented a preliminary analysis of the effect of the cross-term diffusion on the transport description in free interface diffusion (FID) hydrodynamics, without the inclusion of any crystal (Castagnolo *et al.*, 2001). In the present paper a batch crystal growth experiment is performed through a numerical simulation to emphasize the role of cross-term diffusion coefficients both in normal and reduced gravity conditions. The possibility to translate the crystallization conditions from batch methods to FID, vapor diffusion and dialysis was already reviewed in this journal (Chayen, 1998).

Furthermore we propose here a useful way to predict the occurrence of convection, starting from density and diffusion data, that should always be available in order to have an idea of the transport behavior in protein crystallization. Unfortunately these data

are not available, even for protein models in crystallogenesis. Only lysozyme starts being characterized from this point of view (Albright *et al.*, 1999; Annunziata *et al.*, 2000; Paduano *et al.* 2001; Vergara *et al*. 2002).

Studies of the concentration profiles data around growing crystals were developed for some systems by several interferometric techniques (Mach-Zehnder, Michelson and holography), by using facilities on ground and in microgravity. APCF (FID and dialysis techniques) and the future ProMISS (by using gel acupuncture techniques) missions are examples of this kind of efforts. Many of these studies were presented in this journal (Vekilov *et al*. 1995; Snell *et al*. 1996; Otalora *et al*., 2001). The interpretation of the interferometric fringes demands (beside data about refractive properties) a correct transport description, as the one presented herein.

Authors are conscious that there are too many proteins and too little time to measure diffusion coefficients (D_{ij} from Eq. 1) by interferometry. Gouy and Rayleigh interferometry are the most accurate techniques to determine D_{ij}, but they are not sufficiently widespread. Therefore predictive equations to evaluate cross-term diffusion coefficients (Vergara *et al.*, 2000; Vergara *et al.*, 2001*a*), based on molecular exclusion concepts, were already presented to the crystal grower community (Castagnolo *et al.*, 2001). These equations supply the tool to perform the present analysis for any protein- crystallizing agent system that any bio-crystallographer can handle in his crystallization trials.

The numerical simulation considers the presence of two precipitating agents (salt and polymer). The combined use of both salt and PEG is analyzed in order to understand some recent results about the use of PEG as nucleation promoter in systems containing already a salts (Galkin & Vekilov, 2000; Kulkarni & Zukoski, 2001). The present study uses experimental diffusion coefficients collected both in undersaturated and saturated conditions (Albright *et al*., 1999; Annunziata *et al*. 2000). The diffusion data used here, will be discussed elsewhere in larger details (Albright *et al*., in preparation; Annunziata, 2001 and Vergara, 2001*b*).

2. The modeling

The non-steady incompressible balance equations of mass, momentum and species have been solved under the assumption of the Boussinesque approximation. The non-dimensional form of these equations can be found in Lin *et al.* (1995) as well as the adopted reference quantities and the resulting definition of the non-dimensional numbers. The geometry of the protein chamber, crystal height and width are the same, i.e. the length is 0.3 cm and the height 0.1 cm, the crystal' s length is 0.06cm, its height is 0.04cm. Both chamber boundary conditions (insulating walls) and initial values for species concentrations (C_1^0 = 50 mg/cm^3 for protein and C_3^0 = 25 mg/cm^3 for salt) are also the same. A symmetry axis is imposed at mid of the crystal, which is attached on the bottom of the protein chamber.

A difference between the present paper and Lin *et al.* (1995), consists in the definition of the surface crystal boundary conditions. The crystal surface is assumed to promote protein precipitation to the solubility value for each time. Neglecting the kinetic barrier amplifies the protein gradient at the interface, but allows a parametric investigation for the transport in the bulk for different systems. Accordingly the protein concentration is fixed at 50 mg/ml at initial time and 10 mg/ml at crystal surface, the salt is 25 mg/ml at initial time while an "*ad hoc*" barrier has been imposed at the crystal surface. This is the case where, for each mole of protein precipitating into the crystal, 5 moles of salt do as well. This hypothesis is imposed, as the salt concentration jump ΔC_3 at interface is related to the protein concentration jump ΔC_1 as:

$$\Delta C_3 = 5\, M_3/M_1\, \Delta C_1 \qquad (2)$$

M_3 and M_1 being salt and protein molecular weights respectively. Since proteins have typically higher molecular weight than salt crystallizing agents, this condition means that a salt depletion is a few percents of the corresponding protein one.

Another fundamental difference with respect to Lin's model for the ternary system lysozyme-NaCl-water is the presence of cross diffusion coefficients in the present model, namely four diffusion coefficients (two main terms and two cross terms). This implies adding cross term diffusion terms in the mass balance equations and physically allows the motions of the solutes to be correlated.

The model is also applied to a quaternary system (Lysozyme, NaCl, PEG 2000) where nine diffusion coefficients (three main terms and six cross terms) describe the matter transport. According to experimental evidence carried out by Knoll and Hermans (1983), we assume there is no segregation of PEG in the crystal. In order to perform a comparison between quaternary and ternary system, concentration values are the same at the initial time, while PEG is assumed to be 100 mg/ml.

3. The numerical scheme

A finite volume approach has been adopted for the solution of the discrete form of the balance equations. Single step time marching procedure has been chosen together with quick method (Leonard, 1979) for the evaluation of the convective flux, and central differencing scheme for diffusion terms.
The velocity pressure method was used as numerical technique, the pressure equation has been solved by using a SOR (Successive Overrelaxation Method), since this method remains the best compromise between convergence velocity and computational load.
Since we are interested in modeling boundary layers occurring at crystal surface, an accurate grid refinement has been used there. The total number of grid points is 45x21 whatever the simulation carried out.

4. Considered systems

4.1 Ternary system : lysozyme (1) - NaCl (3) – water (0)

According to Lin *et al.* (1995), the solution density (ρ_s) and kinematic viscosity (ν) are 1.02 (g/cm^3) and 0.0153 (cm^2 s^{-1}). The derivatives of solution density with respect to lysozyme ($\partial\rho/\partial C_1$) and NaCl ($\partial\rho/\partial C_3$) are 0.28 and 0.66 respectively. The diffusion coefficients (Albright *et al.*, 1999) are :

$D_{11} = 1.20\ 10^{-6}$ (cm^2 s^{-1}) $\quad D_{13} = 2.49\ 10^{-7}$ (cm^2 s^{-1})
$D_{31} = 6.80\ 10^{-7}$ (cm^2 s^{-1}) $\quad D_{33} = 1.46\ 10^{-5}$ (cm^2 s^{-1})

The Rayleigh and Schmidt numbers for protein and salt are Ra_1 = 20101, Ra_3 = 1924 and Sc_1 = 12750, Sc_3 = 1048 respectively. Here the Rayleigh number for the i-th species is $Ra_i = g\, L^3\, (\partial\rho/\partial C_i)\, C_i^0\, /\rho_s\, \nu$, where g is the gravity level, L is half of the crystal length. The Schmidt number is $Sc_i = \nu/D_{ii}$ and the cross value of Schmidt number for cross terms in the mass balance equation can be determined as $Sc_{ij} = D_{ij}\, Sc_i / D_{ii}$. Since Ra_1 is one order of magnitude larger than Ra_3, the protein concentration gradient is the main driving force to convection in cell. As pointed out by Lin *et al.* (1995), the large value of these numbers is influenced by the choice of large crystal (size about 1 mm), the Rayleigh number being proportional to the cube of the crystal width. Nevertheless it is worth noting that a crystal smaller by one order of magnitude (say 30 μm) than the present one still produces a Rayleigh number which is larger than unity. Typical crystals size ranges from 10 to 1000 μm.

4.2 Quaternary system : lysozyme (1) - PEG 2000(2) - NaCl (3) - water (0)

Diffusion coefficients, viscosity and solution density for this system have been measured by Albright *et al*, (1999) and by Annunziata (2001) and Vergara (2001*a*). The concentration of PEG 2000 is 100 mg/ml, whereas lysozyme and NaCl compositions are as above. The diffusion coefficients corresponding to the initial concentrations used for the present simulations are reported in Table 1.

Table 1 Diffusion coefficients (cm^2 s^{-1}) corresponding to the quaternary system lysozyme (1)-PEG 2000(2)-NaCl(3)-water at the composition reported in the text.

$D_{11}= 4.80\ 10^{-7}$	$D_{12} = 7.00\ 10^{-8}$	$D_{13} = 2.25\ 10^{-7}$
$D_{21}= 2.90\ 10^{-7}$	$D_{22} = 1.95\ 10^{-6}$	$D_{23} = 2.67\ 10^{-6}$
$D_{31}= 6.84\ 10^{-7}$	$D_{32} = 1.02\ 10^{-6}$	$D_{33} = 1.08\ 10^{-5}$

The density of the solution amounts to 1.097 g/cm^3, kinematic viscosity to 0.0259 cm^2/s, and the derivative of density with respect to lysozyme, PEG2000 and NaCl are 0.28, 0.66 and 0.155 respectively. Using the reference geometrical quantities of Lin *et al.* (1995), the relevant non-dimensional numbers are Ra_1 = 27511, Ra_2 = 7401, Ra_3 =1422 and the Sc_1 = 53912, Sc_2 = 13298, Sc_3 = 2392. Carotenuto *et al*. (2002) have pointed out that the larger value of the Schmidt numbers for protein and salt (Sc_1, Sc_2) suggests the presence of boundary layer structure nearby the crystal to be larger for the quaternary system than that corresponding to ternary system.

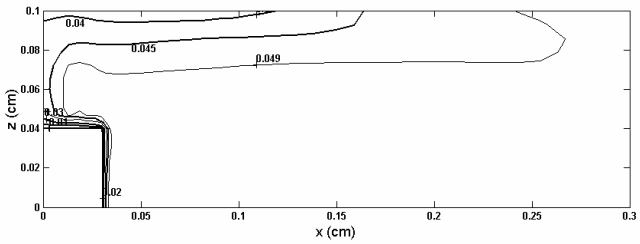

Figure 1 Protein contours [g/cm^3] for the ternary (lysozyme-NaCl) system after 180 seconds for g=1.

5. Results and discussion

5.1 On ground results

5.1.1 Comparison between quaternary and ternary systems. As observed by Lin *et al.* (1995), gradients induced by the protein depletion create solutal buoyancy convection on earth. The plume forming on the topside of the crystal is evident in Fig. 1 and Fig. 2 for both ternary and quaternary systems. As well known, this plume arises due to the gradient of protein concentration at interface, which forms since protein depletion. Even if the ordinary diffusion coefficients are almost the same as in Lin *et al.* (1995), comparing Fig. 1 of present paper and Fig. 6b in Lin *et al.* (1995), the transport of this plume in the bulk is faster since the protein precipitates directly to the saturation value.

Furthermore Fig. 2 shows that the plume diffuses faster in the quaternary than in the ternary system, as expected by the comparison of the corresponding Ra_1 values.

Protein and salt concentration profiles at height z = 0.025 cm are shown in Fig. 3 along the x-distance from the crystal lateral interface for both systems at the same time. The quaternary system shows a protein gradient at the crystal boundary layer higher than for a ternary system, as expected, because of the larger value of the Schmidt numbers for the former system. Nevertheless the protein depletion in the region out of the boundary layer for the quaternary system is higher than for the ternary system because of the larger

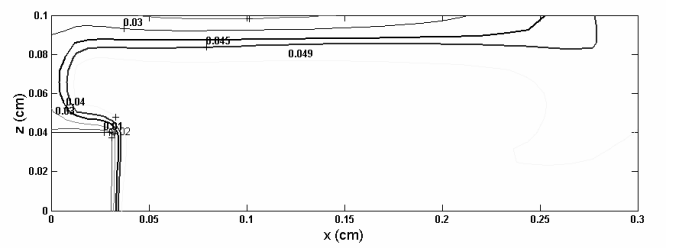

Figure 2 Protein contours [g/cm^3] for the quaternary (lysozyme-NaCl-PEG2000) system after 180 seconds for g=1.

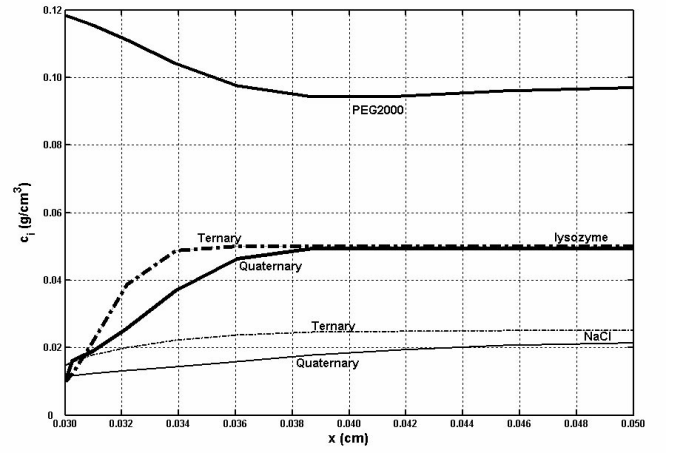

Figure 3 Protein, salt and PEG2000 concentration profiles in the solution at crystal height z = 0.025 cm for ternary (dash-dot lines) and quaternary system (solid lines) at the same time (250 s) for g = 1.

Rayleigh number. According to Eq. 2, the salt depletion is also larger for a quaternary system (see Fig. 3). It has to be pointed out that not only the larger protein depletion but also the larger salt depletion, which modifies the interface local solubility, indicate that quaternary systems shall produce better protein crystal on earth since the probability for parasitic crystals is strongly reduced.

Finally in the quaternary system, a PEG accumulation nearby the crystal can be observed in Fig. 3, while it depletes in the bulk phase. As mentioned above, the PEG does participate to the crystal growth even if it does not segregate within the crystal (Knoll & Hermans, 1983).

5.1.2 Influence of cross-term diffusion coefficients in the quaternary system. In order to determine the influence of cross diffusion coefficients on the present systems, simulations with and without cross diffusion coefficients have been carried out for the quaternary system. Differently from what observed by Lin *et al.* (1995), the simulations with cross diffusion presence indicate a large transport of crystallizing agents.

The salt concentration distributions remain practically uniform throughout the solution only in the case of absence of cross diffusion terms, while a "salt" depletion occurs if cross diffusion terms are taken into account in the model. Salt contours (Fig. 4 and Fig. 5) and profiles (Fig. 6) show how the presence of cross-coupled diffusion coefficient promote this depletion, even if protein profiles (Fig. 6) are not affected by the presence of cross-coupled coefficients. This effect can be explained by the counter contributions produced by the accumulation of PEG at the interface (Fig. 3) that depresses the salt transport, but does not influence the protein transport since the cross

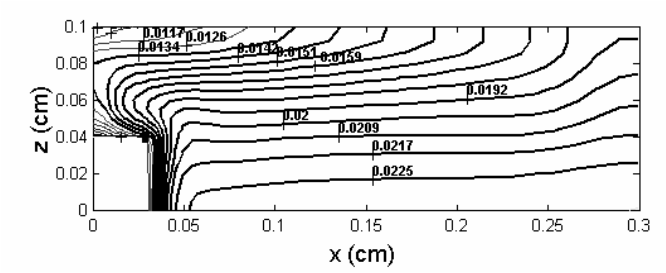

Figure 4 Salt (NaCl) surface contours [g/cm^3] with cross difussion coefficients for quaternary system at time = 256s for g=1.

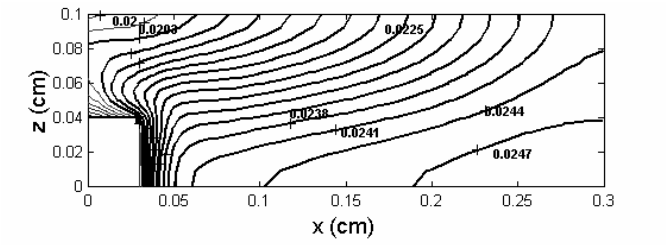

Figure 5 Salt (NaCl) surface contours [g/cm^3] without cross difussion coefficients for quaternary system at time = 256s for g=1

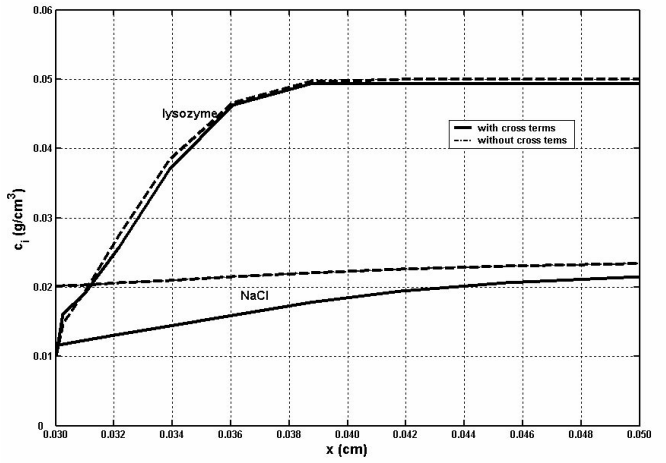

Figure 6 Protein and salt concentration profiles at z = 0.025cm along distance from interface (solid line with cross diffusion coefficients; dashed line without cross diffusion coefficients) for time = 256 seconds for g = 1.

diffusion coefficient D_{12} is two order of magnitude smaller than D_{32}. This correlation between the motion of salt and PEG can justify the action of PEG as a nucleation modulator (Kulkarni & Zukoski, 2001; Galkin & Vekilov, 2000). In fact even though PEG 2000 is a very poor precipitating agent for lysozyme, it affects sensitively the salt concentration in the presence of chemical potential gradients of the protein, as during the nucleation process, characterized by large fluctuations.

Lin *et al.* (1995) speculated that salt accumulation could occur if the crystallizing agent possesses a much lower diffusivity than the protein, which is especially "contrary to actual measured values". The authors carried out a simulation for purely diffusive transport with same diffusion coefficients for protein and salt, and concluded that the interfacial protein solubility through accumulation of crystallizing agent at the interface was insignificant.

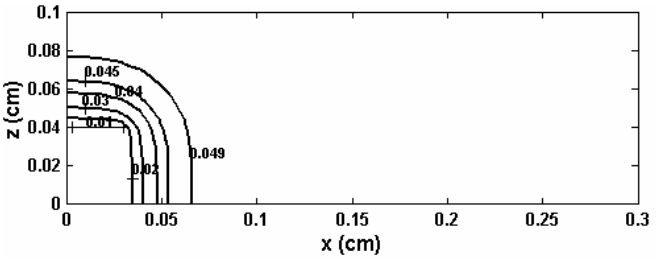

Figure 7 Protein contours [g/cm^3] for the quaternary system at time = 256 seconds for g=0.

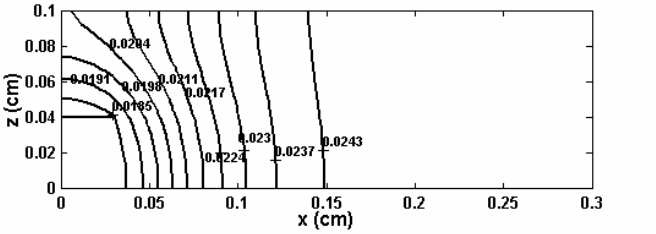

Figure 8 Salt (NaCl) contours [g/cm^3] for the quaternary system at time = 256 seconds for g = 0.

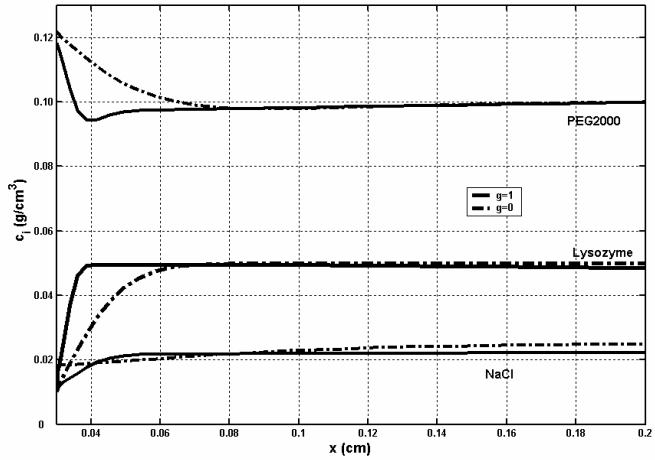

Figure 9 Protein, salt and PEG2000 profiles (g/cc) along distance from interface for the quaternary system for g=1 (solid line) and g=0 (dash-dot line) after 256 seconds.

The cross coupling enhances the accumulation, since the PEG cross diffusion coefficients D_{21} and D_{23} are comparable and higher than D_{11} respectively.

These results indicate that reliable modeling for protein crystallization has to include presence of cross term diffusion coefficients in order to determine correctly the crystallizing agent concentration profiles, and the suitable conditions for carrying out efficient experiments.

5.2 Microgravity conditions

Low gravity conditions provide the protein crystal growth mechanism to be under pure diffusion phenomena and the crystals are expected to have high quality. As well known for g=10^{-6} the depletion zone is larger than for g=1 as shown by the protein contour lines shown in Fig. 7 and the concentration profile in Fig. 9. Contrary to what was obtained by Lin *et al.* (1995), the salt contour line distribution, which is shown in the Fig. 8, and the corresponding

profile along distance from lateral crystal interface (Fig. 9) shows a large NaCl diffusion in the bulk, clearly transported by the protein depletion.

Fig. 9 also shows a comparison between the PEG2000 profiles along distance from the interface at t = 256 seconds for g = 1 and g = 0. The absence of gravity promotes a larger crystallizing agent accumulation than on earth, thus lowering the solubility conditions. The minimum of the profile for g = 1 is more narrow than that for g = 0, which is actually a broad minimum. A further paper is in progress in order to determine by measurement the presence of local solubility as function of salt and PEG2000.

6. Conclusions

Numerical simulations have been performed on protein crystal growth in the presence and in the absence of gravity for quaternary systems. The parameters used in the simulations are typical of the lysozyme diffusivity and growth kinetics. The introduction of cross-term diffusion coefficients sensitively affects the concentration profile and velocity of protein and crystallizing agent around the growing crystal. The effect is more evident for the case of low gravity. Even if the PEG2000 is not segregated in the crystal in our model, its accumulation occurs at the interface since the presence of cross term diffusion coefficients. Although PEG efficiency to precipitate lysozyme is low, for other proteins (Atha & Ingham, 1981) where PEG acts as precipitating agent, this accumulation would have more pronounced effects.

The addition of PEG to a salt-protein aqueous solution cause a strong coupling between protein and salt fluxes, possibly justifying the effect of PEG as a modulator of nucleation (Kulkarni & Zukoski, 2001; Galkin & Vekilov, 2000).

Once again the pseudobinary approximation appears misleading to describe protein crystal growth, and a multicomponent approach is needed. The larger protein and salt depletion in the quaternary indicate that on earth quaternary systems (at higher viscosity) should produce protein crystal better than ones grown from ternary systems, since the probability for parasitic crystals is strongly reduced.

Ultimately the multicomponent analysis was already considered, without any doubt, a more realistic description of the diffusion process, but it has been proved here that it provides different results from the pseudobinary approach, which should be abandoned in describing the transport for crystal growth.

Acknowledgments

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References

Albright, J. G., Annunziata, O., Miller, D. G., Paduano, L. & Pearlstein, J. (1999). *J. Am. Chem. Soc.* **121**, 3256-3266.
Albright, J. G., Annunziata, O., Miller, D. G., Paduano, L., Pearlstein, J., Satorio R. & Vergara A. In preparation
Annunziata, O., Padano, L., Pealrstein, A.J., Miller D.G. & Albright J.G. (2000). *J. Am. Chem. Soc*. **122**, 5916-5928.
Annunziata, O. (2001). Ph.D. Thesis, Texas Christian University, Texas, USA
Atha, D. H. & Ingham, K. C. (1981). *J. Biol. Chem.* **256**, 12108-12117.
Carotenuto, L., Cartwright, J. H. E., Castagnolo, D., Garcia Ruitz, J. M. & Otalora, F. (2002). *Microgravity Science and Technology* **XIII/3**. In the press.
Castagnolo, D., Carotenuto, L., Paduano, L., Vergara, A. & Sartorio, R. (2001). *J. Cryst. Growth*, **232**, 138-148.
Chayen, N. E. (1998). *Acta Cryst.* D**54**, 8-15.
Fletcher, C. A. J. (1991). *Computational Techniques for Fluid Dynamics*, Springer-Verlag, Berlin.
Galkin, A. & Vekilov, P. (2000). *Proc. Natl Acad. Sci*. **97**, 6277-6281.
Knoll, D. & Hermans, J. (1983). *J. Biol. Chem.* **258**, 5710-5715
Kulkarni, A. & Zukoski, C. (2001). *J. Cryst. Growth*, **232**, 156-164.
Leonard, B. P. (1979). *Comp. Methods Appl. Mech. Eng.* **19**, 59-98.
Lin, H., Rosenberger, F., Alexandera, J. I. D. & Nadarajah, A. (1995). *J. Cryst. Growth*, **151**, 153-162.
Lin, H., Petsev, S.-T., Yau, Thomas, B. R. & Vekilov, P. G. (2001). *Cryst. Growth Des.* **1**, 73-79.
Otalora, F., Novella, M. L., Gavira, J. A., Thomas, B. R. & García-Ruiz, G. M (2001). *Acta Cryst.* D**57**, 412-417.
Paduano, L., Annunziata, O., Pearlstein, A. J., Miller, D. G. & Albright, J. G. (2001). *J. Cryst. Growth*, **232**, 149-156.
Rosenberger, F. (1996). *J. Cryst. Growth*, **158**, 552-559.
Snell, E. H., Helliwell, J. R., Boggon, T. J., Lautenschlager, P. & Potthast, L. (1996). *Acta Cryst.* D**52**, 529-533.
Vekilov, P. G., Ataka, M. & Katsura, T. (1995). *Acta Cryst.* D**51**, 207-219.
Vekilov, P. & Alexandera, J. I. D (2000). *Chem. Rev.* **100,** 2061-2090.
Vergara, A., Paduano, L., Vitagliano, V. & Sartorio, R. (2000). *J. Phys. Chem. B*, **104**, 8086-8074.
Vergara, A., Ph.D. Thesis (2001*a*). University of Napoli "Federico II", Italy
Vergara, A., Paduano, L., Vitagliano, V. & Sartorio, R. (2001*b*) *Macromolecules*, **34**, 991-1000.
Vergara, A., Paduano, L. & Sartorio, R. (2002). *Macromolecules*, **35**, 1389-1398.
Vergara, A., Corvino, E., Sorrentino, G., Piccolo, C., Tortora, A., Carotenuto, L., Mazzarella, L. & Zagari, A. (2002). *Acta Cryst.* D**58**, 1687-1691.
Vitagliano, V. (1991). *Pure & Appl. Chem.* **63**, 1441-1448.

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