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Sensitivity of the non-linear dynamics of lysozyme "Liesegang Rings" to small asymmetries

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Abstract

This paper deals with the analysis of the sensitivity of the non-linear dynamics of the crystallization process of lysozyme and related "Liesegang Rings" phenomena to small asymmetries that may characterize the geometry and/or the boundary conditions of the system under investigation. Mathematical models and appropriate numerical methods are introduced to handle the complex phenomena related to protein nucleation and further precipitation (or resolution) according to the concentration distribution. The configuration under investigation consists of a protein chamber and a salt chamber separated by an "interface". The interface is strictly related to the presence of agarose gel in the protein chamber. Different models of the interface are considered. For the first group of simulations the deformation of the interface due to surface tension effects is neglected. For the second group of simulations this deformation is taken into account. The distribution of salt at the initial time is supposed to follow the shape of the gel meniscus whose interface cannot be horizontal due to surface tension effects. The shape is modeled using a sin function in order to have a minimum protruding in the protein chamber at the mean point along the horizontal length of the chamber. For the last group of numerical computations the gel meniscus is supposed to be not symmetrical with respect to this point in order to simulate small experimental imperfections. The numerical simulations show that neglecting the interface deformation leads to 1D results. The phenomenon is characterized by a certain degree of periodicity in time and along the vertical dimension (Liesegang patterns). The bands of Liesegang patterns are not spatially uniform. New solid particles are created on the lower boundary of depleted bands if the local concentration of salt reaches a value to let the local protein concentration overcome the "supersaturation limit". The numerical simulations show that the space distance between two consecutive layers tends to decrease towards the bottom of the protein chamber. If the shape of the meniscus is taken into account the numerical simulations show that lysozyme reacts to produce crystals distributed along both the horizontal and vertical directions. The effect of a non-planar boundary seems to be very important even if the deviation from the planar condition is very small. The third group of simulations finally shows that even if the asymmetry introduced in the shape of the meniscus is very small, the effect on the crystal pattern may be appreciable.

Keywords: crystallization process, periodic precipitation, non-linear dynamics

1. Introduction

Periodic precipitation is a generic term for material deposition processes which occur intermittently in terms of time or space or (generally) both. These processes represent a special case of fashionable topic: oscillatory reactions, with practical implications in crystal growth and material preparation, and a theoretical kinship with the complex of problems that come under the heading “order out of chaos”.

In the present paper the “periodic precipitation” of lysozyme is considered. In the last years great interest has been directed towards crystals of biological macromolecules and in particular towards the crystallization process of protein substances [1-3].

Typically these crystals are obtained by precipitation from super-saturated solutions with a number of techniques. In case precipitation is induced by diffusion of a precipitant agent (salt) into the protein solution, the related phenomenon is characterized by a certain degree of periodicity in time and/or in space. The term “periodic”, however, should be interpreted with caution and tolerance. Strictly speaking, it demands a period (i.e. a constant time or space interval), something that the phenomena here under discussion fail to show. These phenomena are nevertheless “periodic”. The periods are not constant, but they are not random either. Periodic precipitations are often referred to as “Liesegang Rings”, because they were first studied by Liesegang. Liesegang rings can manifest themselves in a great variety of media and ways.

In the present paper the crystallization process of lysozyme and the related “Liesegang Rings” phenomena will be presented and analyzed under different boundary conditions. Aim of the present study is in fact the analysis of the sensitivity of the non-linear dynamics of crystallization process of lysozyme and related “Liesegang Rings” phenomena to small asymmetries that may characterize the geometry and/or the boundary conditions of the system under investigation. The study requires the introduction of a mathematical model to handle

the complex phenomena related to protein nucleation and further precipitation or resolution according to the concentration distribution.

2. Study configuration

The configuration under investigation consists of a protein chamber and a salt chamber separated by an "interface". The interface is strictly related to the presence of agarose gel in the protein chamber. The salt is NaCl. For the case under investigation the protein chamber is filled with agarose gel in order to avoid crystal sedimentation and convection. The salt chamber is located on the top of the protein chamber (see Fig.1).

At the initial time, both chambers are filled with salt and protein solution at constant concentration respectively. For the first group of calculations the shape of the gel interface is supposed to be a planar surface. Then, in order to assess the effect of a non-planar interface, the distribution of salt at the initial time is supposed to follow the shape of the gel meniscus whose interface cannot be horizontal due to surface tension effects. The shape is modeled using a sin function (i.e. $\bar{\xi}(x)/h=1.1-0.1\sin(\pi x/L)$) in order to have $h(x=0)=h(x=L)$ and the minimum at $x=L/2$. The shape of the meniscus is not taken into account however for the protein chamber. Then the effect of asymmetries with respect to the point $x=L/2$ is taken into account defining appropriate parameters to characterize the deviation of the boundary condition and of the crystals patterns from the symmetric condition (shape symmetric with respect to $x=L/2$).

The data used as input for the numerical simulation are shown in table I.

3. Mathematical model and numerical method:

3.1 Governing field equations:

the diffusion of lysozyme and NaCl are governed respectively by the equations

$$\frac{\partial \overline{C_{Ly}}}{\partial \bar{t}} = D_{lys}\,\overline{\nabla}^2 \overline{C_{lys}} + \overline{\dot{J}} \tag{1}$$

$$\frac{\partial \overline{C_{NaCl}}}{\partial \bar{t}} = D_{NaCl}\;\overline{\nabla}^2 \overline{C_{NaCl}} \tag{2}$$

where and $\overline{\dot{J}}$ represents the local mass exchange flux, due to the formation, growth or dissolution of lysozyme solid phase (details showing how this flux is taken into account are described in the following sections) and the overbar denote dimensional quantities. Mutual diffusion is neglected.

The non-dimensional form of the equations results from scaling the lengths by the horizontal distance between the walls (L), the time by L^2/D_{lys} and the concentrations of protein and salt by their initial values (C_{lyso} and C_{NaClo}).

3.2 Periodic precipitation in monomer systems

Hereafter [Ly] represents the lysozyme which can precipitate without undergoing chemical change, [NaCl] represents the incoming solubility modulator, [M] represents the solid lysozyme phase (i.e. crystals).

Whenever [Ly], [NaCl] and [M] co-exist in equilibrium, we must have

$$C_{lys}=Ks \tag{3}$$

where Ks is called the "solubility" (or "*saturation level*") . As long as $C_{lys}<Ks$, more [M] will dissolve. If, on the other hand, $C_{lys}>Ks$, solute will condense on any [M] material *already*

existing and augment its size. When there is no pre-existing [M] deposit, it is generally found that the concentration of protein has to be greater than Ks to create one spontaneously, say

$$C_{lys} \geq Ksp \quad (4)$$

where Ksp is called the "*supersaturation limit*" (it is obvious that Ksp>Ks). The dependence of Ks of lysozyme on C_{NaCl} has been determined experimentally. In this work the expression reported in [3] is used and it is assumed that Ksp=3 Ks.

3.3 Grain Size

According to Ref. [4], once the solid particles [M] are formed it is assumed that they are in equilibrium with [Ly] and [NaCl]. Thus the concentration of [Ly] has to satisfy (3), i.e. the solute content of the solution will have to be decremented from the original C_{lys} value to a value which must satisfy (3); the decrement is: $\delta = C_{lys} - Ks$, the concentrations of [Ly] has to be adjusted as $C_{lys} = C_{lys} - \delta$. We assume that the newly formed solid phase is constituted by only one crystal with mass

$$M_{grain(o)} = \delta \cdot C_{lyso} \cdot dV \quad (5)$$

where dV is the volume of the grid cell whose average concentration is represented by C_{lys} (it is assumed that the concentrations are expressed e.g. in [g/cm^3]).

Of course this assumption is not verified for very large values of supersaturation when an intense precipitation takes place, leading to a large concentration of nuclei. Typically, this occurs when supersaturation is increased very rapidly, with respect to the time needed for nuclei formation (induction time). The counter-diffusion crystallization technique that is the subject of this analysis, ensures slow increase of supersaturation.

The phenomena here under investigation are driven by the diffusion of salt through the gel interface. The nucleation process and further growth, in fact, are strictly associated to the

modulation of the supersaturation limit and of the solubility due to salt diffusion. Therefore the characteristic time of the phenomena under investigation is the diffusion time of salt in the protein chamber ($t=h^2/D_{NaCl} \cong 2\ 10^5$). The induction time according to Ref. [5] is of the order of $2\ 10^3$ [s]. The relative importance of the two effects (characteristic induction time and characteristic diffusion time) is measured by the non-dimensional parameter $\tau_{r.i} = t_{induction}/t_{NaCl} \cong 10^{-2}$. Since τ is $O(10^{-2})$, the first effect can be neglected with respect to the latter. This explains why the increase of the supersaturation can be considered slow for the phenomena under investigation and at the same time allows the present mathematical-physical model to assume that when the supersaturation limit is exceeded, the amount of protein in liquid phase exceeding the saturation level is converted in solid phase without any time delay.

3.4 Rates of mass transfer

In the presence of a deposit, it is highly unlikely that the concentration of protein will ever reach Ksp again, but if that value came to be higher than Ks, the deposit would grow; if it were to fall below Ks, it would begin to re-dissolve.

In the presence of a deposit, if $C_{lys} > Ks$ then $\delta_g = C_{lys} - Ks$, $C_{lys} = C_{lys} - \delta_g$, $M_{grain} = M_{grain} + \delta_g \cdot C_{lyso} \cdot dV$.

Similarly in the case of dissolution ($C_{lys} < Ks$), the increment for [Ly] can be computed as $\delta_i = Ks - C_{lys}$ then [Ly] has to be increased as $C_{lys} = C_{lys} + \delta_i$ and the mass of each grain depleted as $M_{grain} = M_{grain} - \delta_i \cdot C_{lyso} \cdot dV$.

4. Results and discussion

The numerical simulations show, as expected, that the phenomenon under investigation is characterized by a certain degree of periodicity in time and in space.

4.1 Planar interface shape

In this case the phenomena under investigation do not exhibit dependence upon the x coordinate and the bands of Liesegang patterns are not spatially uniform along y. Nucleating particles deplete their surroundings of protein which causes a drop in the local level of supersaturation such that the nucleation rate falls in the neighborhood, leading naturally to a spacing among regions of nucleation and giving rise to the alternate presence of depleted bands and layers of crystals. Figs.2 show the protein and crystals distribution at $t=15.6\ 10^4$ s. New solid particles are created on the lower boundary of depleted bands when the local concentration of salt has reached a value to let the local protein concentration overcome the "supersaturation limit".

The present analysis points out that the width of the so-called depleted bands (this width corresponds to the average distance between two "layers" of crystals) is not constant. The band width and spacing change according to the distance from the origin of the imposed concentration gradient (for the present case the gel interface between the salt and protein chambers). The space distance between consecutive layers, in fact, tends to decrease uniformly towards the bottom of the protein chamber.

4.2 Non planar interface

If the shape of the gel interface is taken into account, numerical simulations show that lysozyme reacts to produce deposits spaced in both the x and y directions (see Figs. 3-5). The effect of a non-planar boundary seems to be very important even if the deviation from the planar condition is very small (10 % of L).

The figures show a 2D distribution of the crystals, and clearly the phenomenon related to the further growth of particles after their nucleation, i.e. the increase of the solid particles size. This behavior is due to the delicate balance between two counteracting effects:

1) protein condenses on any solid crystal already existing augmenting its size; this depletes the protein concentration and leads to lower values of C_{lys}

2) salt continues to diffuse through the interface so that Ks is reduced due to solubility modulation.

If the second effect prevails over the first one, i.e. the protein concentration is larger than the solubility then further protein precipitation in the considered region is possible.

It should be pointed out that, as highlighted for the case described in paragraph 4.1, the width of the depleted bands (this width corresponds to the average distance between two "layers" of crystals) is not constant. This is one of the reasons for which the term "periodic" should be interpreted with caution and tolerance for the problem under investigation. Strictly speaking, this term demands a period (i.e. a constant time or space interval), something that the phenomena here under discussion fail to show [4].

These phenomena are nevertheless "periodic". The periods are not constant, but they are not random either. The space distance between two consecutive layers, in fact, does not change in random way, but decreases towards the bottom of the protein chamber. The amplitude is maximum for the first "depleted band", then it is almost constant for two intermediate bands and then it reaches a minimum for the last band.

Moreover for a fixed layer a certain degree of space periodicity can be highlighted for the x-distribution of the solid particles along the layer. The average distance between two consecutive particles along x is not random. It is the same for the layers 2,3 and 4 whereas the layer 5 is characterized by a very small average x distance with respect to previous layers (see Fig. 5b).

4.3 Effect of asymmetric boundary conditions

The parameter (ε) used here to quantify the degree of asymmetry introduced in the problem is based on the ratio of the height of the meniscus at x=0 and at x=L:

$$\varepsilon = \bar{\xi}(x=0)/\bar{\xi}(x=L)-1 \qquad (6)$$

The corresponding "distortion" of the crystal distribution is defined as

$$\gamma = \frac{\int_{y=0}^{h}\int_{x=0}^{L/2} M_{grain}(x,y)\ dxdy}{\int_{y=0}^{h}\int_{x=L/2}^{L} M_{grain}(x,y)\ dxdy} - 1 \qquad (7)$$

Even if the asymmetry introduced in the boundary conditions is very small, the effect on the crystal pattern may be relatively appreciable as displayed in Figs.6 and reported in Table II where also the number of solid particles is shown to be a function of the degree of asymmetry. Table II shows that even if ε is $O(10^{-4})$, γ may be $O(10^{-2})$.

5. Acknowledgements

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6. References

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L [cm]	1
H [cm]	2.8
Width [cm]	0.1
Height of the protein chamber h [cm]	1.4
$D_{lysozyme}$ [cm^2/s]	10^{-6}
D_{naCl} [cm^2/s]	10^{-5}
C_{Lyo} [g/cm^3]	$4\ 10^{-2}$
C_{NaClo} [g/cm^3]	$7\ 10^{-2}$
Computational points along x	60
Computational points along y	90

Table I: data used as input for the numerical computations

ε	γ	Number of solid particles after $2\ 10^5$ s
0	0	128
$1\ 10^{-4}$	$1.1\ 10^{-2}$	128
$1\ 10^{-2}$	$4\ 10^{-2}$	117

Table II: Effect of the parameter ε

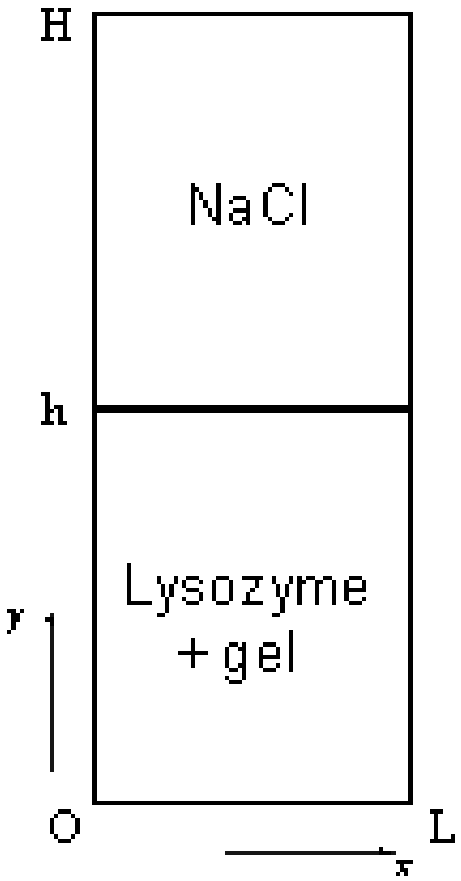
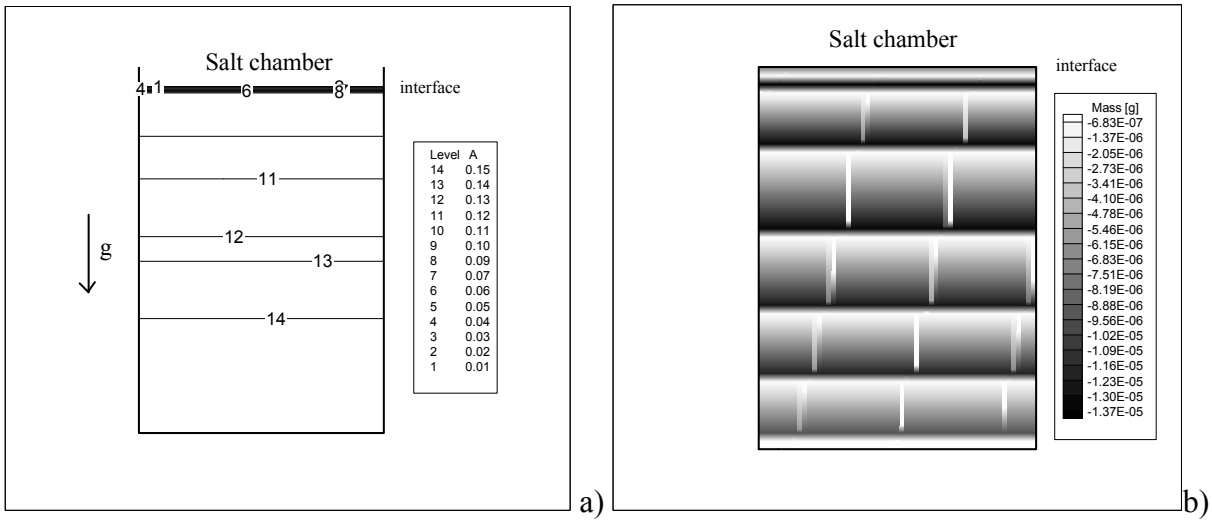
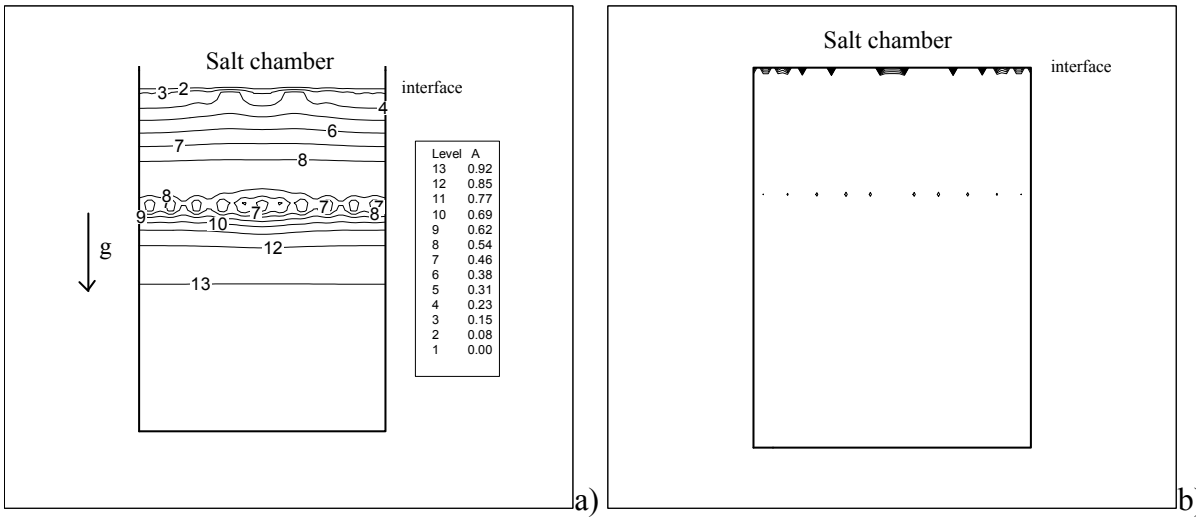


Fig.1: sketch of the configuration



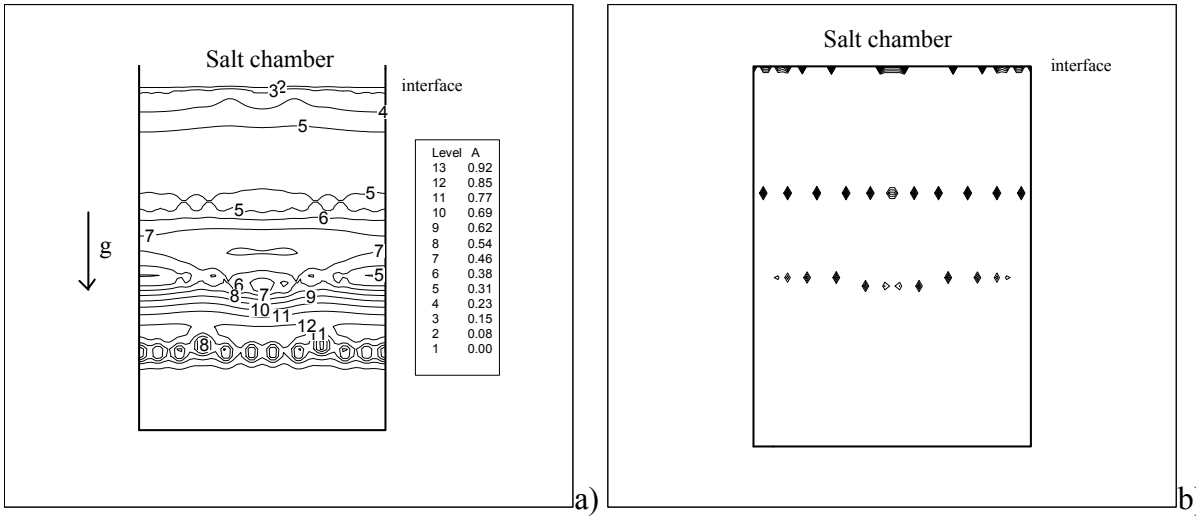
a) b)

Figs.2: 1D non-dimensional protein concentration contour lines (a) and 1D crystals distribution (each dark line corresponds to a layer of crystals) (b) – Time=15.6×10^4 s



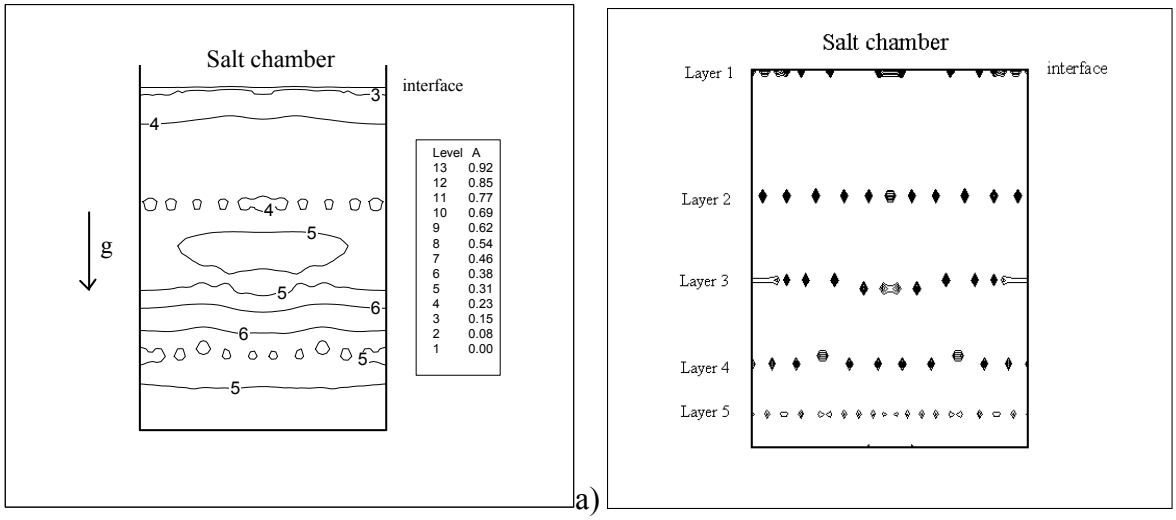
a) b)

Figs.3: non-dimensional protein concentration contour lines (a) and crystals distribution (b) – Time=12x10^4 s



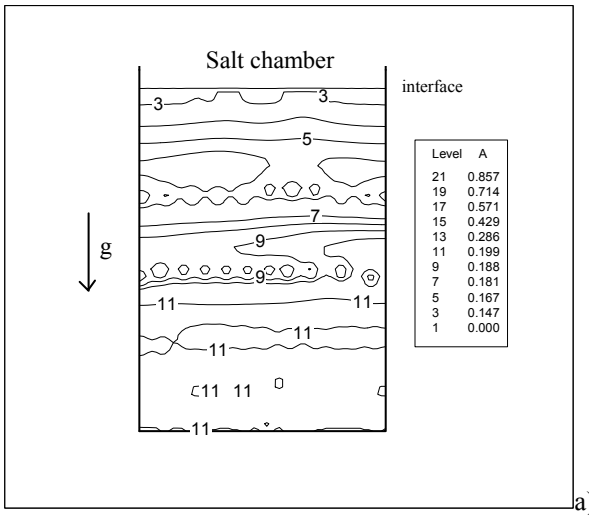
a) b)

Figs.4: non-dimensional protein concentration contour lines (a) and crystals distribution (b) – Time=14x10^4 s

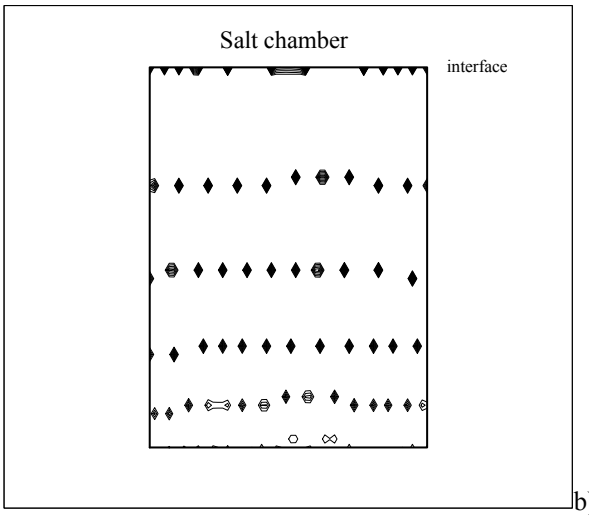


a) b)

Figs.5: non-dimensional protein concentration contour lines (a) and crystals distribution (b) – Time=15.6x10^4 s



a)



b)

Figs.6: non-dimensional protein concentration contour lines (a) and crystals distribution (b) – $\varepsilon=10^{-2}$, Time=$2x10^5$ s