An “attachment kinetics-based” Level-Set Method for macromolecular crystallization under buoyancy-driven convective effects

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Abstract

A level-set method, specifically conceived for the case of organic crystal growth from supersaturated solutions, is introduced and described in detail. The model can simulate the growth due to the slow addition of solute molecules to the surface of a lattice and can handle the shape of macromolecular growing crystals under the influence of natural convection. It is carefully developed according to the complex properties and mechanisms of protein crystal growth taking into account the possibility of anisotropic growth due to either "faceted" surface-orientation-dependent behaviors or the influence of external convection occurring in the protein reactor. The analogies and differences between this technique and a previous volume of fraction method are discussed in terms of theoretical aspects and fundamental equations. The advantages and limitations of both formulations are pointed out.

Categories:

65C20: Models, numerical methods
74N05: Crystals
76Z99: Biological Fluid Mechanics
76D05: Navier-Stokes equations
92C45: Kinetics in Biochemical Problems
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74N20: Dynamics of phase boundaries
1. Introduction

Proteins are the elementary building units of all living creatures and essential components for information and energy processing within the living systems. Protein crystallization provides the basis for determining protein structure, thereby opening the way to a host of applications in molecular biology and pharmacology. For this reason the crystallization process of these substances has been the focus of intense investigation for many decades and recently a tremendous effort has been devoted to the study of these topics. Progress in various biochemical and biomedical research and production tasks, in fact, is still impeded by lack of insight into the growth mechanisms of these complex molecules that are very labile and sensitive to the conditions under which are operated (Vekilov and Chernov, 2002; McPherson, 1990). Macromolecular solutions and crystals are multi-component systems whose properties can be influenced by a variety of physical and chemical parameters: e.g. temperature, supersaturation, defect formation, shear forces, buoyancy convection, the crystallographic structure (‘faceted’ or anisotropic growth; see, e.g. Coriell, Chernov, Murray and McFadden, 1998), etc.

Indirect proof for the effect of convection on crystallization was found in the observation that crystals grown in gels can be of better quality than crystals grown in ordinary solutions. Convection is suppressed by the gel network, which is believed to be one of the advantages of gels. Nevertheless, gel, in turn, can lead to a number of undesired effects. On the other hand, convective effects in protein precipitation processes without gel are very poorly understood. It is known that they impact protein structures in precipitates, particle size and morphology (Broom, Witherow, Snyder and Carter, 1988), but the mechanisms are still unknown. The problem is very complex and far to be well understood. An exhaustive review of fundamental protein crystal growth and morphology experimental studies has been given in Monaco and Rosenberger (1993) and in Vekilov and Chernov (2002). These reviews show in particular that previous experimental investigations have offered conflicting results on the ‘controlling forces’ in protein crystal growth.
Superimposed on the conflicting nature of the available experimental information is the lack of adequate numerical methods for the investigation of these aspects. While efficient numerical methods have been developed and made available for the scientific community in the case of growth of inorganic substances (in particular for the case of crystals grown by thermal solidification of melts under pure diffusive regime or under buoyancy effects), the modeling of the growth of protein crystals and of the mutual interaction between the growth and the presence of convective fields can still be regarded as an open task.

From both physical and numerical points of view, strictly speaking, the growing crystal gives rise to a moving boundary problem. Only a few of numerical approaches, however, have been undertaken over the years to get insights into the physics of this problem. An excellent effort to the understanding of the interaction between organic crystal growth and convection was initially given by Lin, Rosenberger, Alexander and Nadarajah (1995) who simulated buoyancy-induced flow around a crystal for a fixed crystal size using a kinetic-coefficient-based surface condition. The onset of morphological instabilities was treated by Coriell et al. (1998) within the framework of linear stability theories. Numerically generated orthogonal curvilinear co-ordinate systems, automatically adjusted to fit the boundary shape at any instant, were introduced by Noh, Koh and Kang (1998).

Despite these excellent contributions, an outstanding need is for additional scientific approaches capable of modeling the complex interplay between solutal flow and the crystal moving interface without resorting to stability theories and/or mathematical manipulations and transformations (i.e. moving grid boundary-fitted methodology).

The first method conceived on the basis of this philosophy was the Marker and Cell (MAC) of Harlow and Welch (1965). Instead of MAC, however, Volume tracking methods (e.g., volume of fluid, SLIC simple line interface calculation, and PLIC piecewise linear interface calculation), have became popular in the last years (for a very comprehensive discussion dealing with the genesis and the evolution of these methods see the excellent works of Rider and Kothe, 1998, Hirt and Nichols, 1981; Brackbill, Kothe and Zemach, 1992; Gueyffier, Li, Nadim, Scardovelli, Zaleski 1999 and references
therein). In particular, they have been used for the simulation of typical problems associated with
gas/liquid or liquid/liquid systems where the surface tension effects play a ‘critical role’ in
determining the shape of the fluid/fluid interface and/or its motion.

On the other hand, ‘enthalpy methods’ and similar techniques taking into account the release or
absorption of latent heat have been successfully applied to the case of thermal phase change problems
characterized by the presence of moving solid/melt interfaces due to the heating or the cooling of the
system under investigation. These phase-field models avoid the computational difficulties associated
with front tracking by introducing an auxiliary order parameter, or phase-field that couples to the
evolution of the thermal field. Because the interface is never explicitly tracked, complicated topology
changes are handled easily. These techniques were pioneered by Voller and Prakash (1987), Bennon
and Incropera (1987), Brent et al. (1988) and have been strongly improved over the years (see, e.g.,
the papers of Udaykumur, Mittal, Shyy, 1999; Beckermann, Diepers, Steinbach, Karma, Tong, 1999;
Knoll, Kothe, Lally, 1999; Kothe, Ferrel, Turner, Mosso, 1997; Reddy, Kothe, Beckermann, Ferrell,
Lam, 1997 and many others).

Recently these approaches have been extended to the case of macromolecular organic growth from
supersaturated solutions (addition/incorporation of solute molecules (building blocks or growth units)
to the crystal lattice) and to the growth of "living" biological tissues in bioreactors (Lappa, 2003a). For
instance, they were used by Lappa (2003b, 2003c) to investigate the relative importance of surface
kinetics and mass transport as "limiting steps" for the growth rate of lysozyme crystals under
microgravity conditions and by Lappa (2003d) to "track" the evolution of the solid/liquid interface of a
single sample of living tissue surrounded by the nutrient solution focusing on the surface metabolism
and its sensitivity to many "local" environmental factors.

Though the above-cited simulation models have proven to be an adequate choice to get insights into a
number of aspects, however, Level-set methods are recently enjoying a widespread use and are
progressively replacing numerical techniques based on the aforementioned volume tracking (volume
of fraction - phase field) philosophy. Level-set methods are becoming the most popular techniques for
the simulation of moving boundary problems, see, e.g., the landmark papers of Osher and Sethian (1988), Sussman, Smereka and Osher (1994), Osher and Fedkiw (2002), Sethian (1999). The great success of level set methods can be attributed to their versatility and simplicity as well as to significant advantages offered in taking into account some aspects (Osher and Fedkiw, 2002; Sethian, 1999). This is the reason why, for instance, the methodology and the formalism underlying modern level set methods has been extended also to the aforementioned techniques dealing with the solidification of melts (see, e.g., the excellent works of Chen, Merriman, Osher and Smereka, 1997 and Kim, Goldenfeld and Dantzig, 2000).

Along these lines and owing to the aforementioned need for new scientific/numerical approaches, the objective of the present analysis is to re-formulate the (VOF) kinetic model proposed by Lappa (2003b, 2003c) (that simulates the growth due to the slow addition of solute molecules to a lattice and can handle the shape of organic growing crystals under the influence of natural convection) within the framework of a Level-Set technique (in order to benefit from the advantages offered by this technique).

2. Organic crystal growth: models and basic assumptions

Crystallization of macromolecular substances shares many common properties with that of small solute molecules (e.g., growth by two-dimensional nucleation or by screw-dislocation mechanisms), but their crystals exhibit several peculiarities: most of them have a high solvent content [e.g. 30±80% in terms of volume], few intermolecular contacts and a high density of defects.

Many other distinguishing marks exist with respect to the crystallization of inorganic substances (see, e.g., Rosenberger, 1986). In many protein crystallization systems, interfacial kinetics are slow and are considered a major source of difficulties in crystal growth. In many inorganic crystal growth systems, however, the growth rate is limited by the transport of species to the interface so that the fluid-dynamics of the nutrient phase is at the root of growth problems. The inorganic crystal growth in general is not plagued by problems related to interfacial kinetics.
On a molecular level protein crystal growth consists of the successive addition of ‘growth units’ or ‘building blocks’ (nutrients) to a lattice. Growth units can consist of single molecules or possibly of clusters of molecules. In solution, growth units are typically solvated i.e. are surrounded by highly regularly arranged shells of solvent molecules that interact with the growth unit in a bond-like fashion. Protein crystallization does not fit any of the traditional (inorganic) models without major modification.

Pusey et al. (1986) expressed the growth rate of a protein substance as a function of supersaturation introducing a ‘kinetic coefficient’ \( \lambda \) (\([\text{cm s}^{-1}]\)) dependent on the physical properties of the protein. As a consequence, using mass balance (see, e.g., Rosenberger, 1986), and assuming a linear dependence of the growth rate by the interface supersaturation (see, e.g., Lin et al., 1995), in terms of the aforementioned kinetic coefficient, the surface incorporation conditions read:

\[
\left( \frac{D}{\rho_p - \rho_c C_i / \rho_S} \right) \frac{\partial C}{\partial n} = \lambda(\hat{n}) \left( \frac{C_i - S}{S} - \delta_o \right)
\]

where \( C_i \) is the concentration of the protein at the crystal surface, \( D \) is the related diffusion coefficient, \( S \) is the solubility (its value is a function of the local concentration of the precipitant agent and/or of temperature), \( \rho_p \) and \( \rho_c \) are the protein mass density and the total mass density in the crystal, \( \rho_S \) is the total density of the solution, \( \delta_o \) is the width of the supersaturation zone in which no growth occurs. The parameter \( \delta_o \) takes into account the so-called ‘dead zone’, that according to many investigators, in the case of organic substances, is due to the absorption of impurities that lead to strong retardation of the growth kinetics. In the case of protein substances, such ‘impurities’ are not necessarily extrinsic contaminants, being often the result of protein ‘microheterogeneity’ (Monaco and Rosenberger, 1993).

It is worthwhile to stress how in eq.(1), the parameter \( \lambda \) behaves as a function of \( \hat{n} \) (the unit vector perpendicular to the crystal surface pointing into the liquid in a reference frame fixed to the crystal).

The rate of growth, in fact, often is determined by the nature of the growing crystal surface. Addition of molecules to a rough surface requires less energy than addition to a smooth surface, where surface nucleation is required for addition. According to the Periodic Bond Chain Theory, (Boistelle and
Astier, 1988), different types of growth surfaces exist: flat faces and stepped faces. Flat faces require two dimensional nucleation (the formation of growing sheets of molecules) in order to induce growth, and thus grow the slowest. Stepped faces grow as columns of molecules, which requires only one dimensional nucleation, and thus have intermediate growth rates. Stepped faces typically occur as a result of a crystallographic screw axis causing spiral growth patterns to occur at the surface of the crystal. Growth is also possible owing to kinked sites which do not require local nucleation to promote further growth, and therefore grow faster than the other two surface types. Thus the type of the growth site strongly influences the rate at which crystal growth occurs.

From mass balance the solution for the dimensional velocity \( q_n \) of the advancing crystal interface is:

\[
\overline{q}_n = \left( \frac{\rho_c}{\rho_s} \right) \overline{\vartheta}
\]  

(2)

where the dimensional growth rate (see, e.g., Pusey et al., 1986) reads:

\[
\overline{\vartheta} = \lambda \left( \frac{\beta}{\nu} - 1 - \delta_{\alpha} \right)
\]

(3)

with \( \vartheta \) satisfying eq.(1).

The parameter \( \overline{\vartheta} \) is a measure of the rate of absorption of the protein at the crystal surface (it is proportional to \( \partial C / \partial n \)) and can be seen also as a measure of the velocity of the advancing solid/liquid interface. It is called "growth rate" by organic crystal growers and usually is expressed in dimensional form as \([\text{Å s}^{-1}]\). According to the present model it can be computed on the basis of eq. (3) after the computation of the protein concentration at the crystal surface according to eq. (1).

The purpose of any crystallization technique is to favor crystal growth by driving the feeding solution from undersaturation to supersaturation (this is the actual driving force). The classical procedure is to "gradually" increase the level of saturation of a precipitant agent (e.g., a salt). By these techniques a ‘suitable’ limited degree of supersaturation can be achieved. In very concentrated solutions, in fact, the macromolecules may aggregate as an amorphous precipitate. This result is to be avoided if possible and is indicative that supersaturation has proceeded too extensively or too swiftly.
Once crystals grow, the concentration of soluble macromolecules and the supersaturation decrease. After some time, an equilibrium is reached, growth ceases and the concentration of soluble macromolecules becomes equal to the solubility:

\[ C_i = S \]  \hspace{1cm} (4)

As long as \( C_i < S \), more solid material dissolves if any. If, on the other hand, \( C_i > S \), material condenses on any material *already existing* and augment its size. The ‘growth regime’ may be very complex and non-linear (see, e.g., Vekilov, Alexander and Rosenberger, 1996). Its features depend on several parameters and in particular it is a function of the ratio between surface-attachment kinetics (modeled by eq.(1)) and mass transport in liquid phase (diffusive or convective), see Lappa (2003b).

The nucleation of new material is something else and the features of this phenomenon are out the scope of the present work (for theoretical analysis of this phenomenon see the excellent paper of McPherson, 1990; for numerical models dealing with the phenomena of protein nucleation see, e.g., Lappa, Castagnolo and Carotenuto, 2002 and Lappa and Castagnolo, 2003); the term ‘precipitation’ refers, in fact, to the composite phenomenon of nucleation and subsequent growth. Growth can take place at concentrations lower that those needed for nucleation, as long as \( C_i > S \). The solution is said to be *supersaturated* when the solute content is greater than \( S \), and the degree of supersaturation \( \sigma \) is defined by \( \sigma = C/S \).

3. The OCGLSET - Organic Crystal Growth Level Set Method

3.1 General properties

This method, first introduced by Osher and Sethian (1988), is conceptually similar to a phase-field model in that the solid–liquid interface \( \Gamma \) is represented as the zero contour of a level set function, \( \varphi(r,t) \), which has its own equation of motion. The movement of the interface is taken care of implicitly through an advection equation for \( \varphi(r,t) \). Unlike the phase-field model, however there is no arbitrary interface width introduced in the level set method; the sharp-interface equations can be solved directly
and, as a result, no interface reconstruction techniques (e.g., PLIC) are required (Kim, Goldenfeld, Dantzig, 2000).

The goal is to compute and analyze the subsequent motion of $\Gamma$ under a velocity field $q$ (Osher, Fedkiw, 2002). This velocity can depend on position of the interface of the organic crystal ($\Omega$) and the external physics (for the case under investigation it depends on the surface incorporation kinetics i.e. on the mechanisms of attachment of solute molecules to the growing solid surface i.e. eq. (1)). The interface is captured for later time as the zero level set of the function $\varphi(r,t)$, i.e., $\Gamma(t) = \{ r : \varphi(r,t) = 0 \}$.

In practice the level set function is defined as the signed normal distance from the solid–liquid interface such that $\varphi$ is positive in the liquid phase, negative in the crystal, and zero at the solid/feeding-solution interface:

$$
\begin{align*}
\varphi(r,t) > 0 & \text{ for } r \not\in \Omega \\
\varphi(r,t) = 0 & \text{ for } r \in \partial \Omega = \Gamma(t) \\
\varphi(r,t) < 0 & \text{ for } r \in \Omega 
\end{align*}
$$

Thus, the interface is to be captured for all later time, by merely locating the set $\Gamma(t)$ for which $\varphi$ vanishes. Its motion is analyzed by convecting the $\varphi$ values (levels) with the velocity field $q$:

$$
\frac{\partial \varphi}{\partial t} + q \cdot \nabla \varphi = 0
$$

Here $q$ is the desired velocity on the interface, and is arbitrary elsewhere.

Actually, only the normal component of $q$ is needed: $q_n = q \cdot \frac{\nabla \varphi}{|\nabla \varphi|}$ so eq. (6) becomes

$$
\frac{\partial \varphi}{\partial t} + q_n \frac{\nabla \varphi}{|\nabla \varphi|} = 0
$$

Integrating Eq. (7) for one time step results in moving the contours of $\varphi$ along the directions normal to the interface according to the velocity field $F$, which varies in space. $F$ is constructed to be an extension of the interface velocity, $q_n$, such that $F=q_n$ for points on the interface and the lines of
constant $F$ are normal to the interface (see eq. (9)). Thus, advecting $\varphi$ according to Eq. (7) moves the front with the correct velocity.

After solving Eq. (7) for one time step, the level set function will no longer be equal to the distance away from the interface. It is necessary to reinitialize $\varphi$ to be a signed distance function. This step is accomplished by solving

$$\frac{\partial \varphi}{\partial \tau} + sqn(\varphi) \left[ (\nabla \varphi) \cdot \nabla \right] F_{sqn} = 0$$  \hspace{1cm} (8)

$$\varphi(r,0) = \varphi(r,t)$$

Following Osher and Fedkiw (2002), in order to define the distance $\varphi$ in a band of width $\varepsilon$ around $\Gamma(t)$, eq. (8) is solved only for $\tau = O(\varepsilon)$.

The basic level set method concerns a function $\varphi(r,t)$ which is defined throughout space. Clearly this is useless if one only cares about information near the zero level set. The local level set method defines $\varphi$ only near the zero level set. In practice, eq. (7) is solved in a neighborhood of $\Gamma(t)$ of width $m\Delta r$, where $m$ is typically 5 or 6. Points outside of this neighborhood need not be updated by this motion.

The function $\varphi$ is reinitialized to be signed distance to $\Gamma(t)$, only near the boundary, smoothly extending the velocity field $q_n$ off of the front $\Gamma(t)$ (see eq. (9)) and solving equation (7) only locally near the interface $\Gamma(t)$, thus lowering the complexity of this calculation by an order of magnitude (Osher and Fedkiw, 2002). This makes the cost of level set methods competitive with other techniques.

With regard to the smooth extension of the quantity $q_n$ on $\Gamma(t)$ to a neighborhood of $\Gamma(t)$, this step is accomplished by solving:

$$\frac{\partial F}{\partial t} + sqn(\varphi) \left[ \frac{\nabla \varphi}{|\nabla \varphi|} \cdot \nabla F \right] = 0$$  \hspace{1cm} (9)

$$F(r,0) = q_n(r,t)$$

Again, this equation is solved only for $\tau = O(\varepsilon)$ in order to extend $q_n$ to be constant in the direction normal to the interface in a region of width $\varepsilon$. 
For the case under investigation \( q_n \) is given by eqs (2) and (3) with \( C \) at the crystal surface satisfying eq. (1). It is worthwhile to stress how the function \( q_n \) is not given "a priori" but has to be computed at any instant as part of the problem (it dynamically changes during the growth process according to the residual protein available in liquid phase, and according to the steepness of the concentration gradient at the solid/liquid interface that in turn is changed by the motion of the fluid surrounding the macromolecular seed). For further details on the computation of \( q_n \) see the next section.

### 3.2 Governing field equations:

In presence of convection, the flow is governed by the continuity, Navier-Stokes and species equations, that in non-dimensional conservative form read:

\[
\nabla \cdot V = 0 \tag{10}
\]

\[
\frac{\partial V}{\partial t} = -\nabla p - \nabla \cdot [V V] + Sc \nabla^2 V + Sc Ra \left( \frac{C}{C_{(o)}} - 1 \right) i_g + \frac{D_{salt}}{D} Sc Ra_{salt} \left( \frac{C_{salt}}{C_{salt(o)}} - 1 \right) i_g \tag{11}
\]

where \( v \) is the kinematic viscosity, \( Sc = \frac{v}{D} \), \( Ra = \frac{g \beta_{prot} C_{(o)} L^3}{vD} \) and \( Ra_{salt} = \frac{g \beta_{salt} C_{salt(o)} L^3}{vD_{salt}} \) (the Boussinesque approximation is used to model the buoyancy forces, \( \beta_{prot} \) and \( \beta_{salt} \) are the solutal expansion coefficients related to organic substance and salt respectively);

\[
\frac{\partial C}{\partial t} = \left[ -\nabla \cdot (V C) + \nabla^2 C \right] \tag{12}
\]

\[
\frac{\partial C_{salt}}{\partial t} = \left[ -\nabla \cdot (V C_{salt}) + \frac{D_{salt}}{D} \nabla^2 C_{salt} \right] \tag{13}
\]

where \( V \) and \( p \) are the non-dimensional velocity and pressure. The non-dimensional form of the equations results from scaling the lengths by a reference distance (L), the time by \( L^2/D \); the initial value of protein and precipitant agent (salt) are \( C_{(o)} \) and \( C_{salt(o)} \) respectively. Note that concentrations are not posed in non-dimensional form ([g cm\(^{-3}\)]). The reference velocity and pressure are \( D/L \) and
\( \rho_s D^2/L^2 \) respectively. If solubility modulation is induced by temperature control, eq. (13) must be replaced by the energy equation.

Equations (10)-(13) are not solved for the domain occupied by solid phase since there convective velocities are zero and since macromolecular solute and salt cannot diffuse through the lattice; nutrient is "incorporated" (attached) at the crystal surface \( \Gamma(t) \) according to eq. (1) i.e. the crystal surface behaves as a sink for the protein available in liquid phase. The rate of absorption for the above-mentioned phenomenon is usually measured by the so-called "growth rate" \( \theta \) (that is proportional to the velocity of the moving solid/liquid interface).

On the surface of the crystal \( (\varphi=0) \), protein concentration must satisfy the kinetic condition that in non-dimensional form reads:

\[
\left( \frac{1}{\rho_p - \rho_c C_s / \rho_s} \right) \frac{\partial C}{\partial n} = \tilde{\lambda} (\sigma, -1 - \delta_o)
\]

(14)

where \( \tilde{\lambda} = \lambda L / D \), \( \sigma \) is a function of the local precipitant concentration;

since \( \frac{\partial C}{\partial n} = \nabla C \cdot \tilde{n} = \alpha \frac{\partial C}{\partial x} + \beta \frac{\partial C}{\partial y} \), (hereafter the subscript ‘i’ is omitted) where

\[
\tilde{n} = \frac{\nabla \varphi}{|\nabla \varphi|} = (\alpha, \beta)
\]

(15)

\[
\alpha = \frac{\partial \varphi}{\partial x} / \sqrt{\left( \frac{\partial \varphi}{\partial x} \right)^2 + \left( \frac{\partial \varphi}{\partial y} \right)^2}, \quad \beta = \frac{\partial \varphi}{\partial y} / \sqrt{\left( \frac{\partial \varphi}{\partial x} \right)^2 + \left( \frac{\partial \varphi}{\partial y} \right)^2}
\]

(16)

equation (14) can be written as

\[
\alpha \frac{\partial C}{\partial x} + \beta \frac{\partial C}{\partial y} = \tilde{\lambda}(\alpha, \beta)(\rho_p - \rho_c C_s / \rho_s)(C / S - 1 - \delta_o)
\]

(17)

where \( \tilde{\lambda}(\rho_p - \rho_c C_s / \rho_s)(C / S - 1 - \delta_o) \) represents the mass exchange flux between solid and liquid phase (i.e. crystal and protein solution).
The non-dimensional growth rate (see, e.g., Pusey et al., 1986) is computed as:

$$\theta = \left( \frac{1}{\rho_p - \rho_C C / \rho_s} \right) \frac{\partial C}{\partial n} = \tilde{\lambda} \left( \sigma - 1 - \delta_o \right)$$  \hspace{1cm} (18)

with C satisfying eq.(14).

Finally \( q_n = (\rho_c / \rho_s) \theta \).

3.3 Solution procedure

The solution procedure is summarized in the flow-scheme below; it proceeds in 5 major stages:

[i] advancing the interface (eq. (7)), [ii] reinitializing the level set function to be a signed distance function (eq.(8)), [iii] solving for the new concentration and velocity fields in the liquid (eq. 10-13),

[iv] adjourning of the local values of C at the crystal/feeding-solution interface (eq. (17), this stage accounts for solute depletion), [v] computing the surface growth rate distribution (the growth velocity is not directly imposed but it results from internal conditions related to solute transport).

The Navier Stokes equations can be solved using the SMAC method (it is not described here since it is well-known, for further details see Lappa, 2003b). The domain can be discretized with a uniform mesh with the flow field variables defined over a staggered grid.

3.4 Discussion and comparison with the OCGVOF method

It is worthwhile to compare the present OCGLSET with the corresponding "phase field" method introduced by Lappa (2003b) in order to show analogies and differences, advantages and limitations.

The OCGVOF method is a "volume of fraction" method. It accounts for the solid mass stored in the generic computational cell by assigning an appropriate value of a phase field variable (\( \phi \)) to each mesh point (\( \phi=1 \) crystal, \( \phi=0 \) feeding solution and \( 0<\phi<1 \) for an interfacial cell). The key element for the OCGVOF method is its technique for adjourning \( \phi \). Upon changing phase, the \( \phi \)-value of the cell is adjusted to account for mass release or absorption, this adjustment being reflected in the protein concentration distribution as either a source or sink. The modeling of these phenomena leads to the
introduction of two differential equations, strictly related, from a mathematical point of view, to the ‘kinetic conditions’ used to model mass transfer at the crystal surface. The first equation is eq. (17), the second one is a phase field equation where the mass exchange flux
\[ \lambda (\rho_p - \rho_c C / \rho_s ) (C / S - 1 - \delta_o) \]
between crystal and protein solution is used to update the value of \( \phi \) in the computational cell located "astide" the crystal surface (see Lappa, 2003b).

According to these equations, if the protein concentration is locally depleted, correspondingly, the solid mass stored in the computational cell grows and the phase variable is increased; on the other hand if mass stored in the cell begins to re-dissolve, protein is released in solute phase and the local value of protein concentration is increased. These phenomena are driven by the attachment kinetic condition, i.e. the existing deposit grows if local protein concentration is larger than \( S \) and the mass exchange is proportional to the local value of the orientation-dependent kinetic coefficient; in the opposite situation, i.e. in case protein concentration becomes smaller than \( S \), deposit begins to re-dissolve.

The present technique (OCGLSET) leaves aside mass exchange phenomena i.e. it does not take into account the solid mass stored in each computational cell and the evolution equations for this quantity. Rather the surface attachment kinetics are directly used to compute the normal velocity at the crystal/solution interface (i.e. the "growth rate"). Then this velocity is used to advect \( \phi \) and calculations of this function are performed only in a narrow region around the interface.

If the protein concentration is locally depleted, correspondingly, the concentration gradient in eq. (14) and the associated growth rate in Eq. (18) are positive and the interface \( \Gamma \) is transported outward; on the other hand if mass stored in the cell begins to re-dissolve, protein is released in solute phase and the concentration gradient in eq. (14) and the associated growth rate in Eq. (18) are negative (the interface \( \Gamma \) is transported inward). These phenomena are driven by eq. (7) and (17).

Although the volume of fraction model has led to very interesting results for the case of protein crystallization (see Lappa, 2003b, 2003c), there are still some limitations in this approach. The proper use of these models requires, in fact, that an asymptotic analysis be performed in order to obtain a
mapping between the parameters of the phase-field equations and the sharp-interface equations i.e. a very accurate "interface reconstruction technique is required" (for further details on this aspect see the excellent paper of Kim, Goldenfeld, Dantzig, 2002 and Lappa, 2003b). Moreover computationally, the grid spacing must be small enough to resolve the interfacial region. The multiphase region (region where phase change occurs i.e. region where the "growth units" are added to the pre-existent crystalline structure) is, in fact, defined by the condition 0<\phi<1 and is therefore associated to a somehow arbitrary thickness (the "width" depends on the resolution of the computational mesh).

A reconstruction is a geometrical approximation of the true solid/liquid interface (to be applied in the computational cells where 0<\phi<1), and various techniques are available in literature for this purpose. For the OCGVOF method described in Lappa (2003b), the interface was approximated by a straight line of appropriate inclination in each cell (PLIC piecewise linear interface approximation): the slope of the line is given by the interface normal (gradient of the volume fraction \phi), and the intercept follows from invoking volume conservation (see, e.g., Lappa, 2003b). The reconstructed interface then was used to compute the fluxes (i.e. for the case of growth from supersaturated solutions the mass flux of "growth units" that are incorporated by the crystal) necessary to integrate the volume evolution equation.

These techniques are very laborious and not easy to implement (for a technical description of the PLIC approach see the excellent analysis of Gueyffier et al., 1999). On the contrary, the level set computational approach has the capability to track the motion of the interface without resorting to mathematical manipulations and complex reconstruction techniques. Moreover there is not any arbitrary thickness associated with the region where liquid turns to solid since the interface is captured as the zero level set of the function (it is sharp). Shape changes, corner and cusp development, and accurate determination of geometric properties are naturally obtained in this setting. Moreover topological merging and breaking are well defined and easily performed.

The results provided by the present level set method have been compared with simulations of crystal growth performed using the corresponding VOF model (that in turn was validated through comparison
with the results of Lin et al., 1995). As an example figs.1 show level-set-based simulations for the same conditions dealing with the growth of a lysozyme seed that was simulated by Lappa (2003b). The results exhibit a satisfactory agreement.

4. Conclusions
In conclusion the level set method should be considered as an interesting alternative to the use of volume of fraction models for the simulation of problems dealing with the crystallization of organic crystals and the related slow attachment kinetics. The level set method offers significant advantages in terms of versatility, robustness and simplicity and exhibits wide capabilities to provide all the parameters of interest for organic crystal growers.

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6. References


Fig. 1: Isothermal protein crystal growth system under normal gravity conditions, rectangular crystal of initial dimensions $400 \, \mu m \times 600 \, \mu m$ positioned at the bottom of a 1 [mm] high and 6 [mm] wide growth cell, protein is lysozyme, $C_{\text{lyso}}=5.0 \times 10^{-2} \, [g \, cm^{-3}]$: a) velocity field at $t = 4 \, [h]$ to be compared with the corresponding figure in Lappa (2003b), b) Growth habit simulation and snapshots of the crystal shape versus time ($\Delta t = 6.6 \times 10^2 \, [s]$).