Powder Technology 190 (2009) 112–117

Contents lists available at ScienceDirect

Powder Technology

journal homepage: www.elsevier.com/locate/powtec

Mechanism of formation of lysozyme crystals in concentrated ammonium sulfate solution from concentration profiles and equilibria: Influence of the 2nd osmotic virial coefficient

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ARTICLE INFO

Available online 3 May 2008

Keywords:
Moment of population
Protein
Crystallization
Second virial coefficient

ABSTRACT

We have modelled the nucleation of the orthorhombic crystal form of lysozyme and subsequent crystal growth from concentration profiles established during the measurement of equilibrium in concentrated solutions of ammonium sulphate. A BCF mechanism for the crystal growth has been assumed. The second osmotic virial coefficient is used to calculate the activity coefficient of the protein in solution. A steep decrease of B_{22} is predicted for salt molalities higher than 6.8 m.

1. Introduction

During the determination of the solid–liquid phase diagram for lysozyme/ammonium sulfate solutions, the concentration profiles of lysozyme after the mixing of protein and ammonium sulfate solutions have been measured for salt molalities ranging between 3 and 8.5 m at pH 8 and 25 °C [1,2]. The concentration decreases very quickly initially to attain a first plateau (in a few seconds). At this point two phases are in equilibrium: a liquid containing ammonium sulfate, water and protein, and a solid (dense) phase containing amorphous protein, water and ammonium sulfate. After some hours, the concentration begins to decrease toward a second plateau. During the second decrease in concentration, a crystal phase detected by X-ray analysis is formed. At the end of the experiment, the supernatant concentration is assumed to be equal to the equilibrium solubility of the crystal phase (orthorhombic structure). Three phases are present in the vessel: crystal phase, dense phase and liquid phase. Two types of experiments have been performed. At low molality (I<5.5 m), only a crystal phase is formed. At high molality (I>5.5 m) an amorphous phase appears immediately, and after a few hours the crystal phase is formed.

The aim of this study is to try to understand the mechanism of formation of the crystal phase and model it. The classical theories of nucleation and growth have been used. The knowledge of the kinetics of lysozyme crystallization provides information that can aid in understanding the interactions involved during crystallization and permits identification of favourable crystallization conditions for the design of large scale crystallization processes.

2. Model

2.1. Nucleation and growth rates

Solution of the population and mass balances permits calculation of the concentration profile as a function of time. The population balance has been written in terms of the moments of distribution; equations in terms of the normalized moments are given in the Appendix. Solution of these equations requires knowledge of the supersaturation ratio S and the nucleation and growth rates of the crystal phase (J and G).

In the following calculations, the crystals are considered to be spherical in shape.

The nucleation rate J has been calculated using [3]:

$$J = zf^*C_0\exp\left(-\frac{\Delta G^*}{k_BT}\right)$$

with ΔG^* the critical free enthalpy, $\Delta G^* = \frac{16\pi v_0^2\sigma^3}{3(k_BT)^2\ln^2 S}$, z the Zeldovich factor, $z = \left(\frac{W^*}{3\pi k_BT(n^*)^2}\right)^{1/2}$. f^* is the monomer attachment frequency, which can be described, for attachment controlled by volume diffusion, by $f_{\text{diffusion}}^* = (48\pi^2 v_0)^{1/3}DC'_{\text{eq}}Sn^{*1/3}$, and, for attachment controlled by interfacial transfer, by $f_{\text{interface}}^* = (6\pi^2 v_0)^{1/3}DC'_{\text{eq}}Sn^{*2/3}$ with C_0 the concentration of nucleation sites, C'_{eq} the equilibrium molar concentration of protein, D the diffusion coefficient of the protein in the medium, T the temperature, n^* the number of molecules in the critical cluster, v_0 the molecular volume of the protein in the solid phase, and σ the crystal/solution surface energy.

A Burton–Cabreara–Frank (BCF) mechanism has been chosen to describe the crystal growth. The growth rate G is expressed by $G = \frac{K_{sc1}}{K_{sc2}}(S-1)^2\tanh\left(\frac{K_{sc2}}{(S-1)}\right)$ where K_{sc2} is a function of surface energy, temperature, diameter of the growth units, number of cooperating spirals

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doi:10.1016/j.powtec.2008.04.085

Table 1
B22 values, growth rate parameters from $\sigma = -K\left(\frac{k_B T}{(d_0/2)^2}\right)\ln\left(\frac{\phi_s}{m}\right)$ for the surface energy, and mean volume diameters

Parameters	Frequency of monomer attachment: Control by diffusion		Frequency of monomer attachment: Control by interfacial transfer	
	With amorphous phase	Without amorphous phase	With amorphous phase	Without amorphous phase
K_{SC1} (m/h)	4.469 10^{-8}	1.240 10^{-7}	3.209 10^{-8}	7.340 10^{-8}
K_{SC2}	0.385	0.3490	0.3420	0.3457
K	0.0492	0.0492	0.050	0.050
		$d(4,3)$ (μm)		
I=5.8 m		6.2		3.8

and K_{sc1} of temperature, retardation factor during adsorption of the growth unit into a kink site in the step, shape of the spiral, number of growth units per unit volume of solution and activation energy of dehydratation.

The supersaturation ratio has been calculated by the classical expression, $S = \frac{\gamma^L C'}{\gamma^L_{eq} C_{eq}}$.

The activity coefficient of the protein has been expressed in terms of the osmotic virial coefficients [4]

$$\ln\gamma^L = 2B_{22}C' + \frac{3}{2}B_{222}C'^2 + 0\left(B_{2222}C'^3\right) \approx 2B_{22}C'$$

where C' is the protein molar concentration and the standard state for the protein is taken such that $\gamma^L \to 1$ as $C' \to 0$, B_{22} and B_{222} are the second and third osmotic virial coefficients respectively. In a dilute solution, binary interactions are much more probable than ternary interactions so it usually suffices to examine only interaction involving pairs of particles. When B_{22} is negative, the net interaction between protein molecules is attractive and when B_{22} is positive, the net interaction is repulsive.

Combining the equations for activity coefficients and supersaturation ratio gives an estimate of the thermodynamic driving force for crystallization:

$$S = \exp\left[\ln\left(\frac{C}{C_{eq}}\right) + 2B_{22}M_{protein}\left(C - C_{eq}\right)W_{water}\rho_{solution}\right]$$

with C the concentration of protein expressed in molality (kg protein/kg water), $M_{protein}$ the molar weight of protein, w_{water} the weight fraction of water in the solution and $\rho_{solution}$ the density of the solution.

2.2. Model assumptions

In the model, we have assumed that:

- One parameter permits description of the surface energy for experiments in the presence or absence of the amorphous phase. The surface free energy can be expressed in terms of the interactions between protein molecules [5]. In the case of low solubility, the authors proposed

$$\sigma = -K\left(\frac{k_B T}{(d_0/2)^2}\right)\ln\left(\frac{\phi_s}{m}\right)$$

where K is a constant function of the stacking of molecules in the crystal lattice z (for instance, K=0.042 for z=6), ϕ_s is the volume

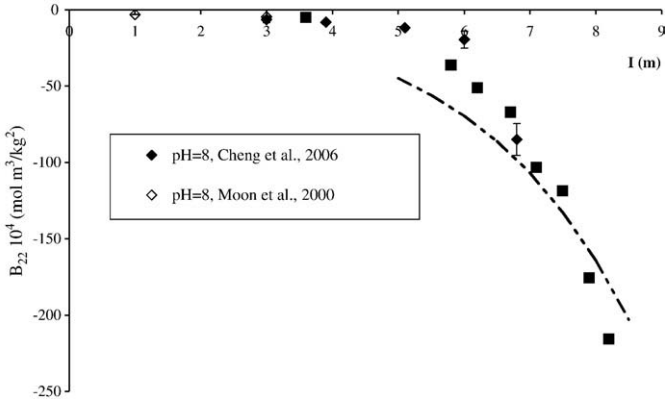

Fig. 1. Evolution of the measured second osmotic virial coefficients (◆ and ◇) as a function of the molality (parameter values in Table 1), the calculated second osmotic virial coefficients from crystallization model (■), and the calculated second osmotic virial coefficients from the correlation of Haas et al. [6] with $A = 1.47 \cdot 10^{-5}$ and z=2 (- - - -).

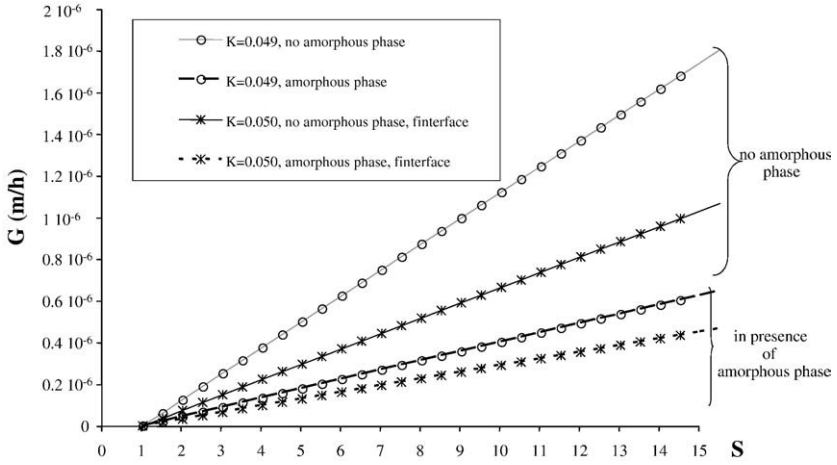

Fig. 2. Growth rate with and without amorphous phase as function of supersaturation ratio.