CIBTech Journal of Biotechnology ISSN: 2319–3859 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/cjb.htm
2017 Vol. 6 (2) April-June, pp.23-27/Nikbakht and Manteghian

Research Article

DETERMINATION OF NUCLEATION MECHANISM AND INDUCTION TIME OF CRYSTALLIZATION OF CASEIN NANOPARTICLES AND THE EFFECT OF SURFACE ACTIVE AGENTS

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ABSTRACT

Induced crystallization is one of the methods applied to separate the particles from solution phase. In this method a secondary solvent (anti-solvent) in which the solubility of the solute is negligible, is added to the solution and by reducing the solubility of solute, it is removed from the solution. This method has the advantage of being energy-saving. Determining the solubility and kinetic of nucleation is the first step in designing any type of crystal manufacturing device. Induction time is used to determine the nucleation mechanism and parameters affecting the nucleation. In this research, the induction time of casein nanoparticle formation in induced crystallization process is measured. The ability to control the amount of anti-solvent, allows a more accurate induction time determination.

Keywords: *Induced Crystallization, Kinetic of Nucleation, Induction Time, Casein Nanoparticles, Anti-Solvent*

INTRODUCTION

In Crystallization system, the time from super saturation to the first changes in the physical properties of the system due to the emergence of new particles is defined as induction time (Sohnel and Garside, 1992). Induction time is the total of the time needed for getting to the metastable state in order to distribute clusters and the time needed for forming a stable core and the time needed for nuclear growth becoming partly determinable.

Induction time contains the information about nucleation kinetics of the new phase and has an inverse relation with nucleation rate.

Super-saturation and temperature are the two most important factors in determining the induction time so that when temperature or Super-saturation increases, induction time decreases. It is also influenced by external factors such as impurities and seeds (Mullin, 2001; Pokrovsky, 1998; Verdoes *et al.*, 1992; Tai and Chien, 2003; Abdolkarimi and Manteghian, 2008).

In this paper casein nanoparticles have been by produced induced crystallization method and in the presence of stabilizers as to measure the induction time for various number of super saturation. The size of these is controlled by nanoparticles by controlled crystallization kinetics. In this regard, induction time can be used to as a tool to detect particles nucleation (Baldaga and Jasinska, 2009; Creighton *et al.*, 1989, Cohen *et al.*, 2001).

Moreover, the registration of nucleation event and induction time is a useful method for determining surface tension (Colton and Suh, 1987; Clifford and Wen-Chen, 2002; El-Sayed, 2001). Because of the agreement between the *classical* nucleation theory and experimental data of induction time, the surface tension of casein nanoparticle is calculated using the classical nucleation relationship (Frawely *et al.*, 2002; Gutsch *et al.*, 2012).

MATERIALS AND METHODS

Theory of the Research

Classical homogeneous nucleation theory assumes that clusters are formed in a solution by incremental mechanism to reach the critical size. The degree of nucleus formation through this mechanism can be expressed as follows:

$$B=B^0\exp(-\frac{\Delta G}{KT}) \quad (1)$$

According to the maximum value of ΔG (Gibbs free energy) at the critical nucleuses based on the minimum size of sustainable nucleuses and clusters which are based on Gibbs-Thomson equation, Nucleation rate in equation (1) is as follows:

$$B=B^0\exp(-\frac{16\pi\gamma^3V_m^2}{3(KT)^3(\ln S)^2}) \quad (2)$$

$s=\frac{C}{C^*}$ is the super saturation, V_m is the Molecular Size, K is the Boltzmann constant, and T is the temperature. As mentioned in the introduction, induction time can be inversely proportional to the rate of nucleation, $\frac{1}{B}\propto t_{ind}$:

$$t_{ind}=K\exp(\frac{16\pi\gamma^3V_m^2}{3(KT)^3(\ln S)^2}) \quad (3)$$

Taking the natural logarithm of both sides of the equation 3:

With $\ln t_{ind}=\ln K+(\frac{16\pi\gamma^3V_m^2}{3(KT)^3(\ln S)^2})$ (4)

So, for a given temperature $\ln t_{ind}$ graph versus $\frac{1}{T^3(\ln s)^2}$ is a straight line with a slope A_1:

$$A_1=\frac{16\pi\gamma^3V_m^2}{3K^3} \quad (5)$$

If the data of induction time properly express homogeneous, we can also calculate surface tension.

RESULTS AND DISCUSSION

First, we separately solved an amount of casein, according to the table (1), in 20 ml of normal sodium hydroxide 0.6 (Na OH). Then, we solved 0.08 grams of surfactant sodium dodecyl sulfate (SDS) in 100 ml of distilled water and added one milliliter of it to casein sodium and hydroxide solution. Solution was stirred by magnetic stirrer with constant stirring speed of 200 rpm. Although, increasing the temperature helps to dissolve the casein into sodium hydroxide solution, in all experimentations temperature was kept constant at 25 ° C. Then, we poured an amount of hydrochloric acid drop by drop into the burette and added it to the solution and simultaneously we measured time with a stopwatch, so that, after pouring every drop of anti-solvent we waited about 5 minutes until the solution becomes opaque. Then, the exact time of solution becoming opaque, which is the induction time, was written. Opaque solution was placed into a sampling container and we waited about 12 hours to completely separate two soluble phases. Then, 10 ml of a transparent solution was taken and put in the oven, then, we weighed net grams of casein and showed it with C *, which is the same as Saturation concentration of solution. Using this amount along with Solubility we can attain super saturation. Furthermore, in order to ensure the formation of casein particles caused by adding surfactant SDS, we took photos of the sample by using scanning electron microscopy (SEM) and in Figure 1 it can be seen.

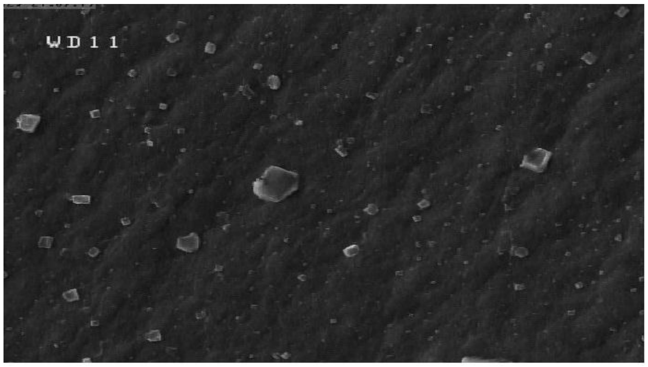

Figure 1: SEM Image of Nano-Particles of Casein in the Presence of Sodium Dodecyl Sulphate

Table 1: The Induction Time of Super Saturation and Volume Consumption of Hydrochloric Acid in the Presence of Sodium Dodecyl Sulphate (2mM)

The Number of Experimentation	The Initial Concentration ($\frac{g}{cc}$)	The Equilibrium Concentration ($\frac{g}{cc}$)	The Volume of Anti-Solvent (cc)	Super Saturation $S=\frac{c}{c^*}$	Induction Time (S)	$\ln t_{ind}$	LnS	$\frac{1}{(\ln S)^2}$
1	0.00055	0.000369	14.3	1.4904	14.1	2.646	0.3991	6.2782
2	0.00091	0.000519	13.61	1.7524	10.60	2.361	0.561	3.1774
3	0.00127	0.000691	13.11	1.8378	10.17	2.320	0.6086	2.6996
4	0.00152	0.000812	12.76	1.8764	9.984	2.301	0.6294	2.524
5	0.00177	0.000878	11.91	2.0157	9.115	2.210	0.701	2.0349

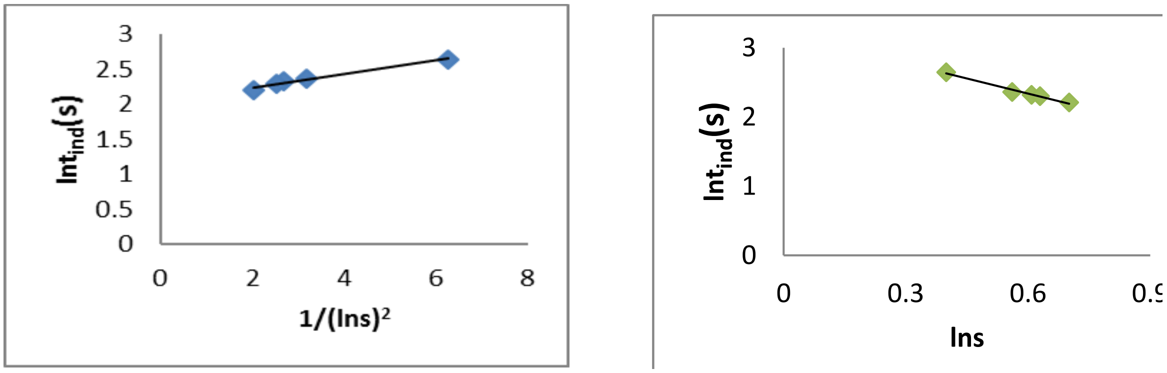

Figure 2: Drawing Induction Time against the Super Saturation for Experimentations 1 to 5

Then the results of experimentations were compared with the classical theory of nucleation. The Classical nucleation theory predicts a linear relationship between $\ln t_{ind}$ and $\frac{1}{(\ln s)^2}$. Therefore, these two quantities were drawn in figure (2) a linear relationship was observed. As shown in figure (2) the initial correlation coefficient of nucleation is more than the secondary one, so initial nucleation takes precedence over secondary nucleation. Nucleation power can also be acquired by induction time data as well. If we consider that the nucleation rate can be presented as

$$B=K_bS^n \qquad (6)$$

Then according to $t_{ind} \propto \frac{1}{B}$ we will have:

$$\ln t_{ind} = \ln k_1 - n \ln S \qquad (7)$$

And thus the slope of the straight line that is drawn between $\ln t_{ind}$ and lnS determines the nucleation power which equals 1.4506.

Calculation of the Surface Tension

By measuring the induction time, the surface tension of particles can be calculated. (2) The Surface tension at 25 °C, from the slope of the line (A1) in Figure 3, is calculated according to the following equation:

$$\gamma = K\left(\frac{3A}{16\pi V_m^2}\right)^{0.33} \qquad (8)$$

In equation (8) Boltzmann constant $k = 1.3805 \times 10^{-23}$ j k^{-1} and the volume of Casein atom is vm = 4.2636 $\times\ ^{28}$ m^3 which is measured by the $v_m=\frac{M}{\rho.N_A}$) equation. $N_A= 6.023 \times 10^{23}$ is Avogadro's number and $\rho = 1250 kgm^3$ is Casein density and $M=0.231\frac{Kg}{mol}$ is its Molecular mass.

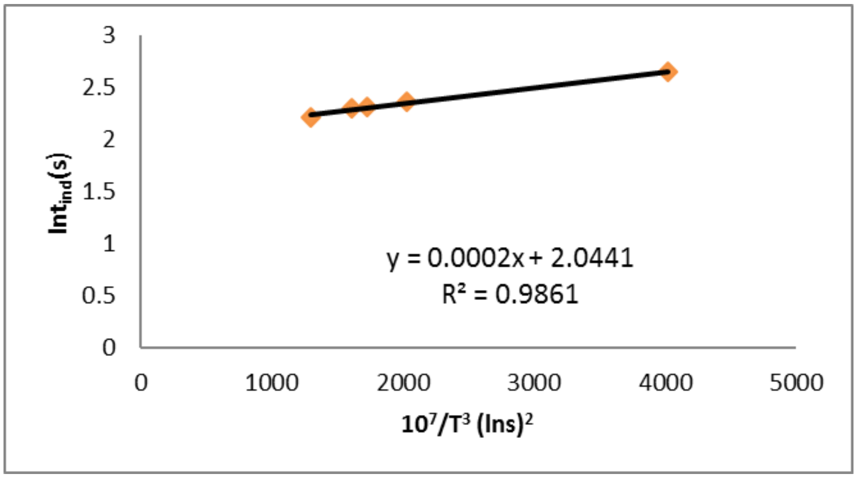

Figure 3: Drawing $\ln t_{ind}$ against $\frac{10^7}{T^3 \ln S^2}$ in Order to Calculate the Surface Tension of Particles

So, according to the equation (8), the surface tension is equal to $\gamma = 3/380113 \frac{mJ}{m^2}$

Conclusion

The Induction time of the casein nanoparticles produced by induced crystallization method was measured for various number of super saturation at a constant temperature of 25°C. As understood by the experimental data, when temperature or Super-saturation increases, induction time decreases. The outcomes of measuring the induction time, as predicted by the classical theory of nucleation, were measured.

ACKNOWLEDGEMENT

We sincerely thank the laboratory staff of Dashtestan city health network for their cooperation.

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