Organic Process Research & Development **2006**, *10,* 1212–1218

Technical Notes

Crystallization of Amoxicillin Trihydrate in the Presence of Degradation Products

Shaohua Feng,* Ning Shan, and Keith J. Carpenter

Institute of Chemical and Engineering Sciences, 1 Pesek Road, Jurong Island, Singapore 627833

Abstract:

Amoxicillin is an important semi-synthetic antibiotic produced in large quantities around the world, and developing a highly cost-effective crystallization process for amoxicillin is of significant importance. In this study an in situ turbidity meter is used to measure the induction period for the onset of nucleation in the pH-controlled crystallization of amoxicillin from aqueous solutions. Amoxicillin trihydrate is crystallized at pH 4.7 at 4 °C. It is demonstrated that turbidimetry is an effective technique for detecting nucleation in industrial crystallization. The induction period decreases with an increase in supersaturation, and the degradation products inhibit the nucleation process. The powder X-ray diffraction patterns show that the resulting products purified by crystallization are confirmed as being identical to amoxicillin trihydrate standard material, and high-pressure liquid chromatography analysis demonstrates that the purity of amoxicillin obtained in the crystallization is at least as high as that of the standard material.

1. Introduction

Crystallization is widely used for manufacturing specific, active ingredients during final and intermediate stages of purification and separation, and this process determines chemical purity and physical properties of active ingredients, such as crystal morphology, size distribution, and crystal structure.[1] These properties of crystalline solids can have important effects on their flowability, filterability, tableting behavior, bioavailability, and stability.[1] Crystallization processes are regulated by both thermodynamic properties and crystallization kinetics, and understanding the crystallization processes is fundamental in better controlling and optimizing existing processes and designing new processes.[2]

Generally, a crystalline solid is formed as a consequence of nucleation and crystal growth. In the stage of nucleation, molecules in solution aggregate and form crystal embryos (nuclei); subsequently in the stage of crystal growth, nuclei grow into macroscopic crystals and achieve a certain size within a given time. The kinetics and mechanism of crystallization are governed by solubility, supersaturation, diffusivity, temperature, and the presence of impurities.[2] In the absence of seeding, nucleation is often the determining stage in crystallization processes.[3] The induction period (t_{ind}), defined as the time elapsed between the onset of supersaturation and the first change in the physical properties of crystallization system due to the formation of a crystalline phase, is frequently used as a measure of nucleation kinetics and can be considered to be inversely proportional to nucleation rates.[2] Although the induction period (which depends on the technique applied to detect the formation of crystalline solids) is not a fundamental property of crystallization systems, experimental observation of the induction period can provide important information about the kinetics and mechanism of the formation of the crystalline phase and the growth process, from critical nuclei to detectable crystals. Whenever crystals are formed and grown in crystallization media that have optical properties different from that of the surrounding liquid medium, light is scattered by these suspended crystals, and consequently, the turbidity of the crystallization medium will change. In the past decade, turbidimetry, based on measurement of light transmission through a suspension, has become a convenient and inexpensive technique for determining the induction period of nucleation processes.[4,5]

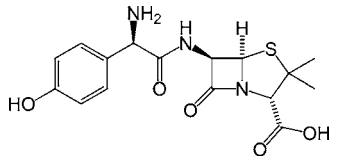


Figure 1. **Molecular structure of amoxicillin.**

Amoxicillin (shown in Figure 1), a semi-synthetic antibiotic produced in large quantities around the world, was selected as the model compound. Amoxicillin is crystallized as the trihydrate form from aqueous solutions by the pH-controlled crystallization method, and a recent patent claims that the bulk density, particle size distribution, and dissolution rate of crystalline amoxicillin trihydrate powder can be

* Corresponding author. Telephone: (65) 6796-3850. Fax: (65) 6316-6183. E-mail: shaohua@ices.a-star.edu.sg.

(1) Myerson, A. S. *Handbook of Industrial Crystallization*, 2nd ed.; Butterworth-Heinemann: Oxford, 2002.
(2) Mullin, J. W. *Crystallization*, 4th ed.; Butterworth-Heinemann: Oxford, 2001.
(3) Rodriguez-Hornedo, N.; Murphy, D. *J. Pharm. Sci.* **1999**, *88*, 651.
(4) Prisciandaro, M.; Lancia, A.; Musmarra, D. *AIChE J.* **2001**, *47*, 929.
(5) Lancia, A.; Musmarra, D.; Prisciandaro, M. *AIChE J.* **1999**, *45*, 390.

Published on Web 09/13/2006

10.1021/op060078l CCC: $33.50

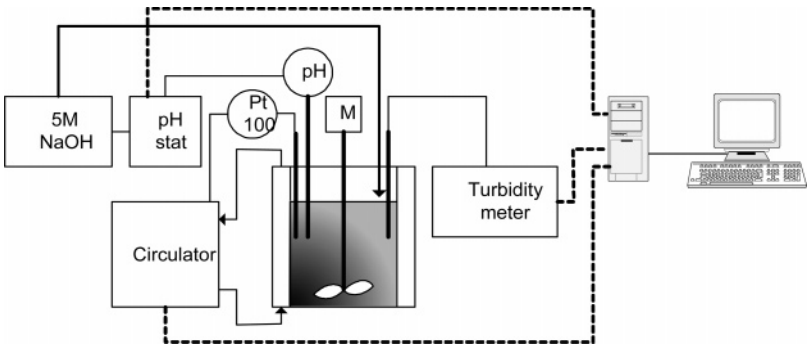


Figure 2. **Experimental apparatus for amoxicillin crystallization.**

improved by optimizing the crystallization conditions.[6] It is easy to hydrolyze and degrade amoxicillin in acid and alkali solutions, and many degradation products are formed, including penicilloic acids and the dimer and trimer of amoxicillin.[7,8] The existence of these degradation products not only reduces the yield of the synthesis but also affects downstream purification and separation processes. It was reported that impurities have a clearly negative influence on the nucleation and growth-rate kinetics of the crystallization of ampicillin,[9] which has a structure similar to that of amoxicillin. In addition, the concentrations of impurities influence the nucleation rate of vanillin significantly.[10] In view of the massive production of amoxicillin around the world and the marked influence of impurities on crystallization, understanding the influence of degradation products on the nucleation of amoxicillin and developing cost-effective crystallization techniques to separate amoxicillin from degradation products are potentially of significant importance.

In this paper, the crystallization process with an in situ turbidity meter was developed, and the induction periods of the crystallization of amoxicillin from aqueous solutions at different supersaturations and with different degrees of degradation were monitored. The crystallization products were characterized by powder X-ray diffraction (PXRD), high-pressure liquid chromatography (HPLC), and scanning electron microscopy (SEM) analyses.

2. Materials and Experimental Methods

2.1. Materials. Amoxicillin trihydrate raw material (CP95) was obtained from North China Pharmaceutical Inc., Hebei, China. Amoxicillin trihydrate standard material (purity ≥ 97%), methanol, sodium hydroxide, and hydrochloric acid were purchased from Sigma-Aldrich, Singapore. Deionized water was used in the preparation of all solutions.

2.2. Solubility Measurements. The solubility of amoxicillin was determined in various pH buffers. Glass flasks with screw caps were immersed in a Thermo Haake C23P refrigerated circulator, whose temperature was controlled at 4 °C. An excess amount of amoxicillin trihydrate raw material was added to the flask to ensure saturation, and then a fixed volume of pH buffer was added. The suspensions in glass flasks were stirred for at least 6 h by magnetic stirring bars. After equilibration, the suspensions were allowed to settle for about 30 min. Then samples of saturated amoxicillin solutions were withdrawn by syringes, separated by 0.2 μm nylon membrane filters, and diluted 10 or 20 times with the buffer. The concentrations of amoxicillin in the samples were analyzed by HPLC, and the pH values of saturated solutions were measured by a pH meter.

2.3. HPLC Analysis. The concentration of amoxicillin was analyzed by an Agilent 1100 HPLC system consisting of a variable wavelength detector, an automatic injector, and a quaternary pump. The reverse-phase column was a Zorbax SB-C18 (4.6 mm × 75 mm with a pore size of 3.5 μm). The mobile phase consisted of 10% v/v methanol and 90% v/v 70 mM Na_2HPO_4, which was brought to a pH of 3 with H_3PO_4. The flow rate of the mobile phase was 1 mL/min. The absorbance was measured at 230 nm.

2.4. Crystallization Experiments. Figure 2 shows the crystallization equipment used in this study. Amoxicillin trihydrate raw material, 1.80 to 5.00 g, was first dissolved in 300 mL of 1 M HCl. Two dissolution conditions were applied to prepare amoxicillin solutions with different degrees of degradation. For the experiments of a low degree of degradation, amoxicillin trihydrate raw material was dissolved in 1 M HCl at 4 °C for 1 h, while for those of a high degree of degradation, amoxicillin trihydrate raw material was dissolved in 1 M HCl at 25 °C for 30 min.

Amoxicillin solutions were then filtered to remove undissolved particles by the 0.2 μm Whatman Nylon membrane. (In the preliminary studies, it was observed that the filtration step before the nucleation experiments efficiently removed tiny crystal seeds in the solution, and therefore this step is crucial for the reliability of nucleation results, as evidenced by repeating the experiments performed.) The supernatant was charged into the 0.5-L jacketed reactor, and 5 M NaOH was dosed by a pH stat (718 STAT Titrino, Metrohm AG, Switzerland) until the pH value of the solution reached 4.7 (the isoelectric point of amoxicillin). The volume of 5 M NaOH used was approximately 56 mL. Subsequently, the sample in the reactor was withdrawn using a syringe, and the concentration of amoxicillin was measured by HPLC. In the entire crystallization process, the temperature of the reactor was controlled at 4.0 °C by a Thermo Haake C23P

(6) Groenendaal, J. W.; Leenderts, E. J. A. M.; Van der Does, T. PCT Int. Appl. WO2004/082661A1, 2004.
(7) Tsuji, A.; Nakashima, E.; Hamano, S.; Yamana, T. *J. Pharm. Sci.* **1978**, *67*, 1059.
(8) Bundgaard, H. *Acta Pharm. Suec.* **1977**, *14*, 47.
(9) Ottens, M.; Lebreton, B.; Zomerdijk, M.; Rijkers, M. P. W. M.; Bruinsma, O. S. L.; van der Wielen, L. A. M. *Ind. Eng. Chem. Res.* **2004**, *43*, 7932.
(10) Pino-Garcia, O.; Rasmuson, A. C. *Cryst. Growth Des.* **2004**, *4*, 1025.

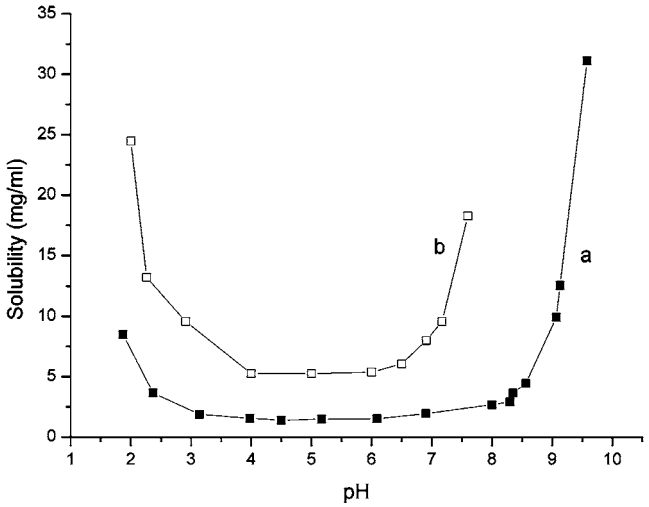


Figure 3. **Solubility of amoxicillin at different pH values: (a) Experimental results at 4 °C and (b) literature results at 37 °C.[7]**

refrigerated circulator, and the stirring speed of the impeller was set at 250 rpm. The turbidity of the solution was monitored in situ by a Brinkmann PC-720 turbidity meter coupled with a fiber-optic probe. The data of the temperature, pH, and turbidity were recorded and stored in a personal computer.

2.5. SEM Analysis. The crystallization products were collected by filtration and then dried at ambient temperature. The morphologies of the crystals were examined by SEM analysis. SEM images were acquired by a JSM-6700 scanning electron microscope (JEOL, Japan). Samples were coated with gold for 1 min using an ion sputter coater (Cressington 208HR, Ted Pella Inc., U.S.A.).

2.6. PXRD Analysis. The crystal structure of the crystallization products was identified by D8 Discover powder X-ray diffractometer (Bruker AXS GmbH, Germany). The voltage and current applied were 40 kV and 40 mA, respectively. The 2θ ranged from 5° and 40°. The PXRD patterns of crystallization products were compared with that of the standard material to confirm the crystal structure of the crystallization products.

3. Results and Discussion

3.1. Solubility. The solubilities of amoxicillin at 4 °C in different pH values are shown in Figure 3. The pH-solubility curve is U-shaped, with the minimum solubility at the pH value near to the isoelectric point (pH 4.7) of amoxicillin. As in this work the solubility of amoxicillin is measured at 4 °C, the solubility values at the entire pH range are much lower than those of literature results at 37 °C;[7] however, the trend of solubility value varying with pH value is consistent with that of literature results. It is noted that the solubility of amoxicillin decreases significantly, the supersaturation is generated, and subsequently the crystallization occurs by changing the pH value of the aqueous solution from acid solution to the isoelectric point of amoxicillin. On the basis of the solubility data, the nucleation study is conducted at pH 4.7 in the following study.

3.2. Determination of Induction Periods. The induction period is determined by monitoring the change of the

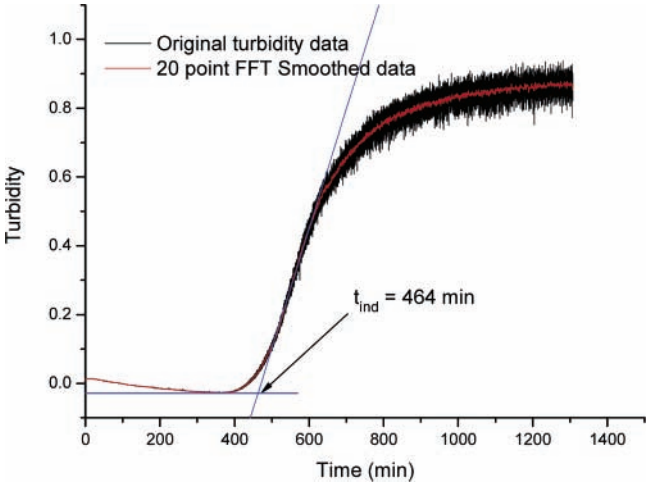


Figure 4. **Graphic method of determining the induction period of amoxicillin nucleation via turbidity data.**

turbidity of the solution; the initial time is defined as the moment when the pH value in the reactor reaches 4.7.

Figure 4 refers to the result of the turbidity as a function of time in a typical experiment. It is observed that, in the first stage (from 0 min to approximate 400 min), turbidity decreases slowly with the increase of time. From the blank experiment of water, it is confirmed that such a minor decrease of turbidity is caused by the signal drift of the turbidity meter. In the second stage (from approximate 400 min to 1000 min), crystals nucleate and grow, and as a result turbidity relevant to the optical properties in the suspension increases. In the third stage (from approximate 1000 min to the end of the experiment), the suspension becomes very dense, and turbidity reaches a plateau due to the onset of multiple scattering. Graphical and numerical methods are adopted to determine the induction period.[5] In the graphical approach, the original data are first smoothed by means of 20-point fast Fourier transformation (Origin 7, OriginLab, U.S.A.). The turbidity curve is first sketched by a horizontal line, which represents the unchanged optical properties of the solution in the absence of solid phase, and by a second line which refers to the period of linear increase with time, as caused by the crystallization phenomenon. The intersection of these two lines gives the induction period (t_{ind}); for the data reported in Figure 4, it is 464 min. In the numerical approach, the time at which the turbidity value is 0.01 units higher than that of its initial value is defined as the induction period. In Figure 4, the turbidity value of the clear solution before nucleation is −0.027, and the induction period determined by the numerical method is 411 min when the turbidity value reaches −0.017. The error of the induction time determined by the two methods (graphical and numerical) in this experiment is ±11%, and the error of t_{ind} for all of the experiments in the paper is ±9%. As the induction time determined by the two methods is quite similar, the average value is used for further discussion.

3.3. Impact of Supersaturation and Degradation on Crystallization. The influence of supersaturation and the degree of degradation on induction periods was investigated.

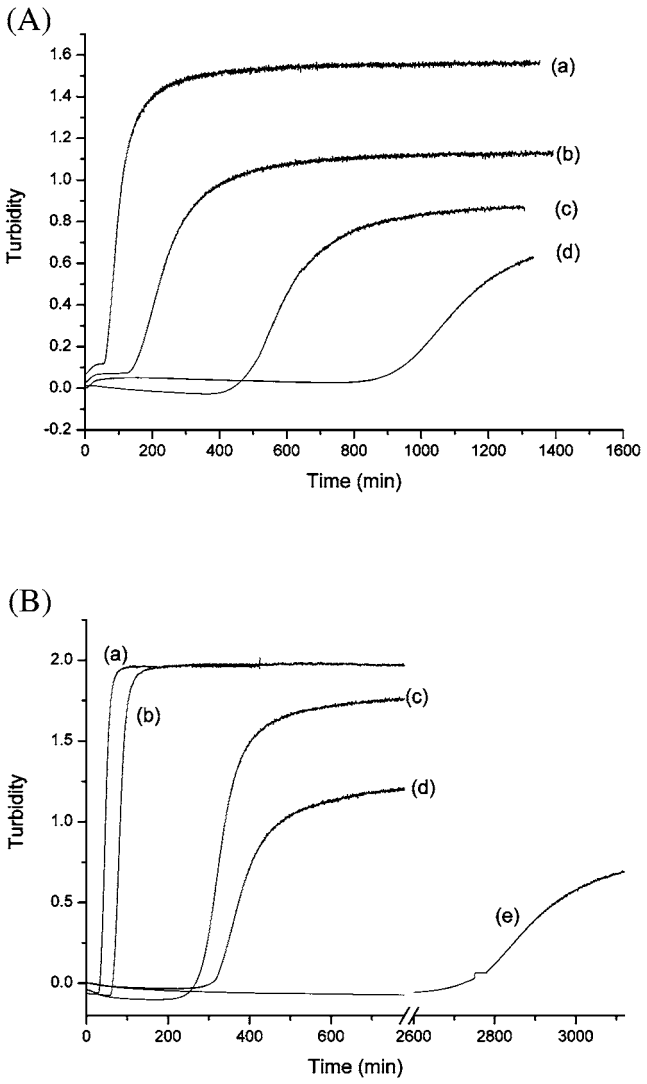


Figure 5. **Turbidity curve vs time in the crystallization of amoxicillin. (A) Low degrees of degradation: (a) $s = 3.239$, (b) $s = 2.807$, (c) $s = 2.590$, and (d) $s = 2.430$. (B) High degrees of degradation: (a) $s = 4.278$, (b) $s = 3.971$, (c) $s = 3.113$, (d) $s = 3.090$, and (e) $s = 2.493$.**

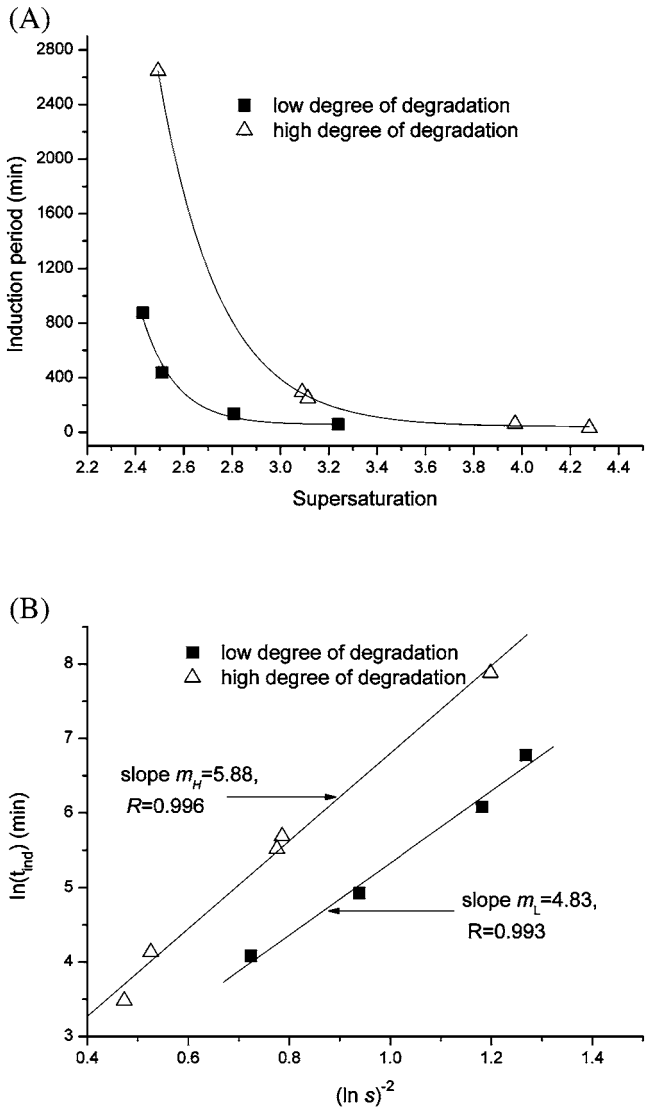


Figure 6. **(A) The induction period as a function of supersaturation in low and high degrees of degradation. (B) $\ln(t_{ind})$ vs $(\ln s)^{-2}$ in low and high degrees of degradation.**

Supersaturation, the driving force of crystallization, is defined as

$$s = \frac{c_0}{c_s} \tag{1}$$

where c_0 is the concentration of amoxicillin before the onset of nucleation, and c_s the solubility of amoxicillin at pH 4.7. c_0 and c_s were measured by HPLC analysis.

Most of impurities presented in amoxicillin solutions come from the degradation process of amoxicillin. However, the degradation process of amoxicillin in the aqueous solution is very complex, and diverse degradation products, including penicilloic acids and the dimer and trimer of amoxicillin,[7,8] are formed. According to the previous report,[11] the half-lives of amoxicillin at 37 °C in aqueous solutions of pHs of 1, 4, and 5 are 5.2, 176.9, and 176.9 h respectively. It can be inferred that the degradation rate of amoxicillin at pH 4.7 is quite slow compared to that at the acidic condition of the dissolution process; thus, the degradation products generated in the crystallization process (pH 4.7 and 4 °C) can be ignored when compared to those formed in the dissolution process. In order to simplify the research, the total effect of all degradation products on crystallization is considered. The percentage of degradation products (P_{dp}), indicating the degree of degradation, is defined as

$$P_{dp} = \left(1 - \frac{m_0}{m_i}\right) \times 100\% = \left(1 - \frac{c_0 v_0}{m_i}\right) \times 100\% \tag{2}$$

where m_i is the amount of amoxicillin dissolved, m_0 the amount of amoxicillin before the onset of nucleation, and v_0 the volume of amoxicillin before the onset of nucleation.

Two levels of P_{dp} were investigated in this study. The average value of the P_{dp} in the experiments of a low degree of degradation is 14.5%, whereas that in the experiments of a high degree of degradation is 36.3%. In Fig-

(11) Erah, P. O.; Goddard, A. F.; Barrett, D. A.; Shaw, P. N.; Spiller, R. C. *J. Antimicrob. Chemother.* **1997**, *39*, 5.

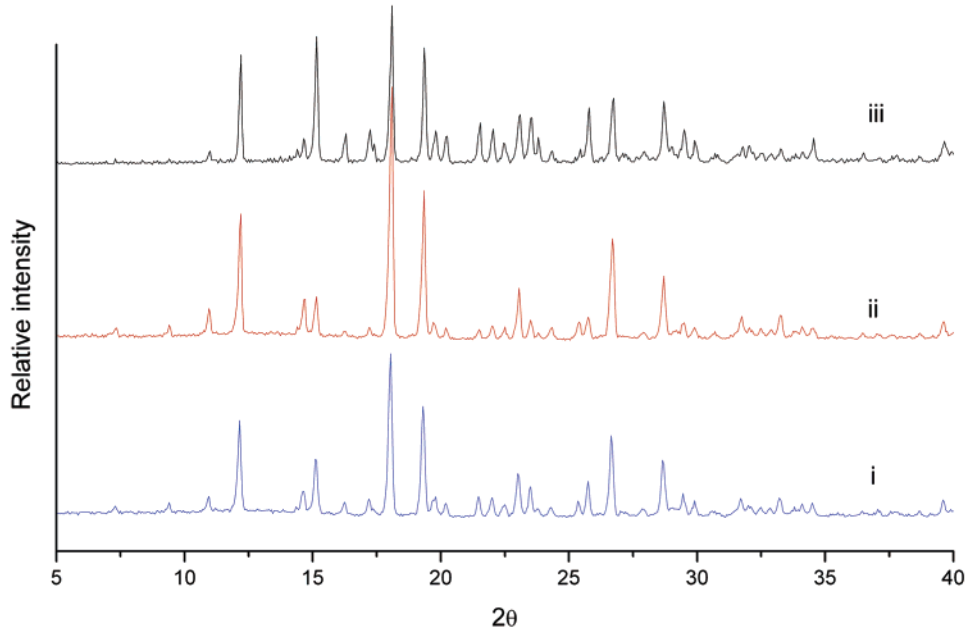


Figure 7. **PXRD pattern of amoxicillin trihydrate: (i) the standard material, (ii) crystallization product obtained in A(a) of Figure 5, and (iii) crystallization product obtained in B(a) of Figure 5.**

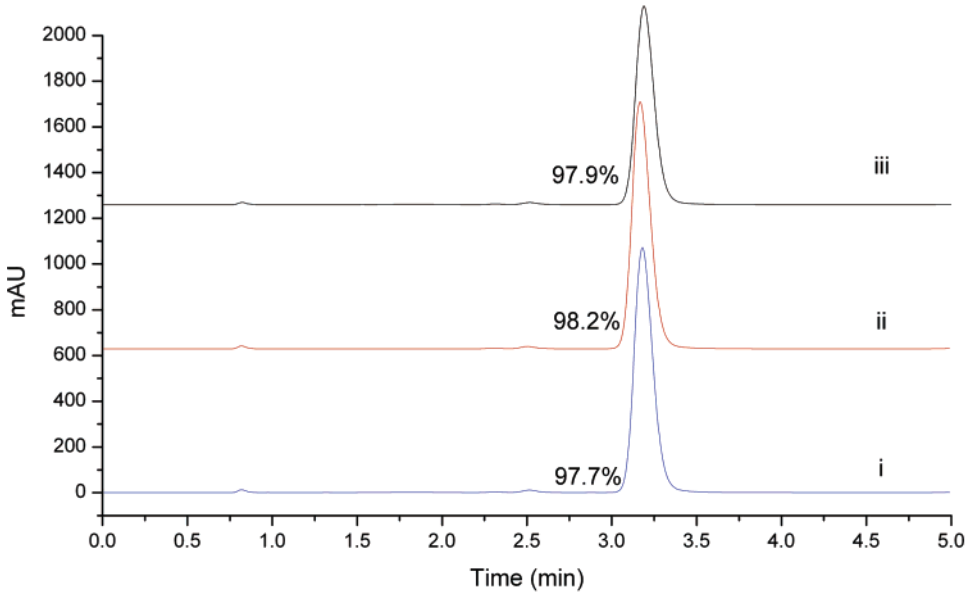


Figure 8. **Chromatograms of amoxicillin trihydrate: (i) the standard material, (ii) crystallization product obtained in A(a) of Figure 5, and (iii) crystallization product obtained in B(a) of Figure 5.**

ure 5A and B, the turbidity is reported as a function of time for four different supersaturations at a low degree of degradation, and for five different supersaturations at a high degree of degradation. The figures clearly show that the turbidities of the solutions increase considerably with the nucleation and growth of amoxicillin; hence, turbidimetry is an efficient technique to detect the nucleation of amoxicillin.

Figure 6A illustrates the dependence of the induction period on the supersaturation at low and high degrees of degradation. It shows that the induction period increases with decreasing supersaturation and such an increase is particularly strong in low supersaturation for high and low degrees of degradation. In the low degree of degradation, the induction period varies from 59.1 to 875.2 min as the supersaturation varies from 3.239 to 2.430, whereas in the high degree of degradation, the induction period varies from 32.6 to 2646.8 min as the supersaturation varies from 4.278 to 2.493. It can be seen that the induction periods of amoxicillin are quite long, and the turbidity meter is a very convenient and effective tool to detect such slow nucleation processes. It is also observed that the induction periods at high degrees of degradation are higher than those at low degrees of degradation, assuming the supersaturation is same. Since the induction period of unseeding crystallization of amoxicillin could take 2646.8 min (1.8 days), developing an appropriate seeding technique to shorten the induction period will be of significant importance.

According to the classical nucleation theory,[2] the rate of primary nucleation can be expressed as

$$J = K \exp\left(\frac{-f\beta\gamma^3\Omega^2}{(kT)^3(\ln s)^2}\right) \quad (3)$$

where K is the kinetic factor, k the Boltzmann constant, β the shape factor, γ the surface free energy, Ω the molecular volume, s the supersaturation, and f the correction factor for heterogeneous nucleation.

For a given volume of the solution, the rate of nucleation (J) is inversely proportional to the induction period (t_{ind}), so the relationship between t_{ind} and s can be derived as eq 4.

$$\ln t_{ind} = A + \frac{f\beta\gamma^3\Omega^2}{k^3T^3} \times \frac{1}{(\ln s)^2} \quad (4)$$

where A is a constant.

The linear dependence of $\ln t_{ind}$ on $(\ln s)^{-2}$ gives the slope

$$m = \frac{f\beta\gamma^3\Omega^2}{k^3T^3} \quad (5)$$

from which, γ, surface free energy, can be estimated.

Figure 6B shows the dependence of $\ln t_{ind}$ on $(\ln s)^{-2}$ obtained from the experiments for high and low degrees of degradation. It is found that the experimental results fit very well with a linear relationship. The slope of the straight line for high degree of degradation (m_H) is 5.88, while that for a low degree of degradation (m_L) is 4.83.

According to eq 5 and assuming that all parameters except γ are kept constant,

$$\frac{\gamma_H}{\gamma_L} = \sqrt[3]{\frac{m_H}{m_L}} = \sqrt[3]{\frac{5.88}{4.83}} = 1.068$$

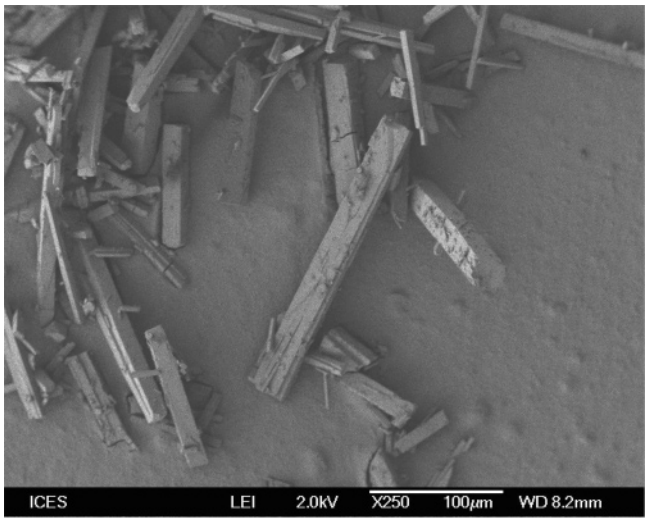
where γ_H is the free surface energy for the high degree of degradation, and γ_L is the free surface energy for the low degree of degradation.

The above calculation results show that the free surface energy increases when the total concentration of degradation products increases. Thus, nucleation at a high concentration of degradation products will be delayed much more than that at a low concentration. Similar influences of impurities or degradation products have been observed in the crystallization of ampicillin.[9,12]

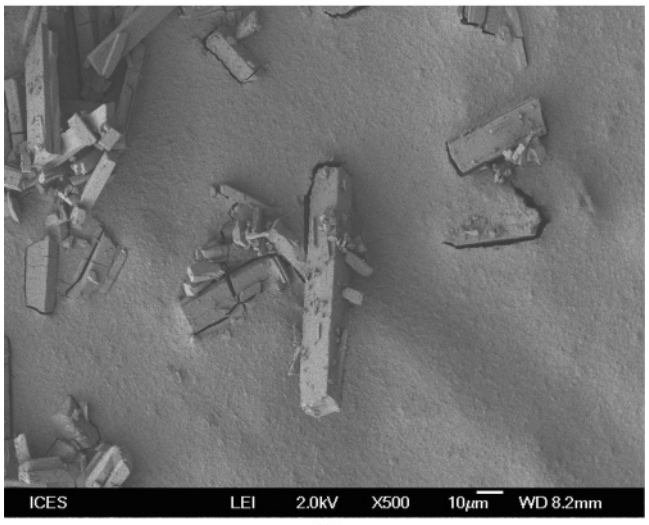
3.4. Characterization of Crystallization Products. Figure 7 shows that the PXRD patterns of the crystallization products are consistent with that of amoxicillin trihydrate standard material. Thus, it can be confirmed that the crystals obtained in this paper are crystalline amoxicillin trihydrate solids.

Figure 8 shows that the area percentages of amoxicillin in the HPLC analysis are respectively 97.7%, 98.2%, and 97.9% for amoxicillin standard material and those obtained in the crystallization, and it means that the purity of amoxicillin obtained in the crystallization is not lower than that of amoxicillin trihydrate standard material (the purity of amoxicillin standard material claimed by Sigma-Aldrich is higher than 97.0% dried material). Although a high concentration of degradation products exists in the crystallization media, the high purity of amoxicillin trihydrate products shows that the crystallization is an efficient process for purifying amoxicillin from a mixture with diverse degradation products and other impurities.

Typical morphologies of amoxicillin trihydrate crystals obtained in both high and low degrees of degradation are shown in Figure 9A and B. The amoxicillin crystals are prismlike with an aspect ratio ranging from 4.0 to 8.0. The morphology difference between crystals obtained from a high degree of degradation and those from a low degree of degradation cannot be detected. Such images shows that the degradation products have no significant influence on the morphology of amoxicillin trihydrate.



(i)



(ii)

Figure 9. **SEM image of amoxicillin trihydrate: (i) crystallization product obtained in A(d) of Figure 5 and (ii) crystallization product obtained in B(d) of Figure 5.**

(12) Ottens, M.; Lebreton, B.; Zomerdijk, M.; Rijkers, M.; Bruinsma, O. S. L.; van der Wielen, L. A. M. *Ind. Eng. Chem. Res.* **2001**, *40*, 4821.

Concluding Remarks

The in situ turbidity meter is applied to the study of the nucleation of amoxicillin, and the experimental results demonstrate that turbidimetry is an effective technique to monitor the nucleation of amoxicillin. The induction period, which is obtained by measurement with the turbidity meter, decreases with an increase in supersaturation and increases with an increase in the concentration of degradation products, which inhibits the nucleation of amoxicillin. As evidenced by HPLC and PXRD analyses, amoxicillin trihydrate crystals obtained from the experiments have a purity grade similar to that of the standard material. Future work will be focused on the study of the recovery yield of amoxicillin trihydrate products due to its importance in the crystallization process of amoxicillin.

Acknowledgment

We thank Dr. Chia-Ni Lim for useful discussions.

Received for review April 10, 2006.

OP060078L