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Solvent effects on the crystallization of avermectin B1a

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Abstract

Effects of different solvents including methanol, ethanol, and *n*-butylalcohol on the crystallization of avermectin B1a were studied in this paper. In pure crystallization system, solvent effects on nucleation rate and crystal morphology were studied by varying relative supersaturation ratio, which was 1.3–2.8 for methanol, 1.8–2.8 for ethanol, and 2.8–4.5 for *n*-butylalcohol respectively. Results showed that the nucleation rate of avermectin B1a decreased in the order of crystallization solvents methanol, ethanol, and *n*-butylalcohol, and the crystal morphology transited from needle like to distorted cube shape. Purification effect of the crystallization process was studied through the segregation coefficient of B2a. The study was carried out by varying the supersaturation ratio as well as the amount of impurity (B2a, 8.7–26.1%, w/w). The segregation coefficient study indicated that the purification effect of the crystallization decreased with increasing supersaturation and purity of the crystallization system. In addition, crystallization with methanol and *n*-butylalcohol could remove impurities better than with ethanol.

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1. Introduction

Avermectins, macrolytic lactones produced by the fungus *Streptomyces avermitilis*, have found wide application as pesticides and antiparasitic drugs for humans and animals. Natural avermectins have eight components: A1a, A2a, B1a, B2a, A1b, A2b, B1b and B2b. The most extensively used compounds of this class are avermectin B1a and its synthetic derivative, ivermectin [1]. The natural avermectins need to be purified to enhance the content of B1a. The purification process used to isolate avermectin B1a includes extraction, concentration, crude crystallization and recrystallization, among which crystallization is the main step. Two or three recrystallizations are needed to get the final product, leading to the high cost for the purification process in industry. In our previous work [2], impurity effects on the crystallization of avermectin B1a were investigated and the mechanism through which the impurities could be incorporated into product crystals was proposed. We will continue discussing factors that affect the crystallization of avermectin B1a in this paper.

It has long been known that solvents have coequal important effects on the crystallization process. Plenty of research has been conducted on solvent effects including influencing the solubility and the crystallization kinetics [3–5], modifying the crystal habit [5–9] etc. within various crystallization system. However, the role played by solvent-surface interactions is still not completely resolved. Despite the difficulties in considering these solvent effects theoretically, their potential importance should always been borne in mind. In pharmaceutical purification industry, solvent is an especially important factor used to modify the physicochemical characteristics of product crystals, such as crystal size, crystal shape, melting point, density, flow properties, dissolution characteristics, and so on [10–12].

To our knowledge, there is still no report of studies on solvent effects on the crystallization of avermectin B1a,

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though it is especially important for industrial crystallization. Therefore, this paper aimed to investigate into different solvent effects on nucleation, crystal morphology, and the segregation coefficient of impurity. Solvents investigated included methanol, ethanol, and *n*-butylalcohol. Solvent effects on nucleation rate and crystal morphology were studied with pure crystallization system. Crystallizations were also carried out with levels of impurity (B2a) present in the crystallization system in order to study the solvent effects on the segregation coefficient of the impurity, which will be especially helpful for industrial crystallization control in return.

2. Experiment and method

2.1. Crystallization experiments

The experimental crystallization apparatus was the same as described in the former study [2]. An appropriate amount of avermectin crystal (B1a: 98.17%, B2a: 0.86%, A1a: 0.47%, A2a: 0.19%, other compounds: <0.31%) was added to the crystallizer which contained 10 ml ethanol at 20 °C, the mixture was stirred and heated to 55 °C to make a saturated or undersaturated solution. After equilibration, the solution was cooled to 20 °C in one minute to give a relative supersaturation S ($S = C_0/C_e$), where C_e was the equilibrium solubility at 20 °C, and C_0 was the concentration of original solution at 55 °C.

The process of nucleation was detected by vision. The time lapse between the solution reaching the cooling temperature (20 °C) and the onset of opacity was taken as the induction time, and was measured by stopwatch. The crystallization system was kept at 20 °C and a stirring rate of 300 rpm for 1 h. The suspension was filtered, and determination of purity of the crystals and concentration of the mother liquor was carried out using high-performance liquid chromatography (HPLC, HP1100 Agilent Technologies, USA).

Experiments were carried out over a supersaturation ranging from 1.8 to 2.8. The crystallization processes were repeated using methanol ($1.3 < S < 2.8$) and *n*-butylalcohol ($2.8 < S < 4.5$) as crystallization solvents.

The crystallization processes were also repeated with levels of B2a, which was isolated from avermectin crude powder using silica gel adsorption chromatography, present in the crystallization system. The experiments were carried out with varying initial relative supersaturation ratio ($1.8 < S < 2.8$ for ethanol, $1.3 < S < 2.8$ for methanol, and $2.8 < S < 4.5$ for *n*-butylalcohol) at fixed impurity concentration (B2a, 10%, w/w), as well as with varying initial content of B2a (8.7–26.1%, w/w) at fixed supersaturation ($S = 2.3$ for ethanol, $S = 1.5$ for methanol, and $S = 4.0$ for *n*-butylalcohol).

2.2. Characterization method

The method for characterizing the morphology of product crystals and determining the purity of the crystals and concentration of mother liquor was the same as described in the previous paper [2].

3. Results and discussion

3.1. Nucleation rate

Nucleation rate was studied through the induction time (τ), which is defined as a period of time lapse between the achieving of supersaturation and the appearance of crystals. Based on the classical crystallization theory, the rate of homogenous nucleation can be expressed as

$$J = K \exp\left[-\frac{16\pi\gamma^3 \upsilon^2}{3k^3T^3(\ln S)^2}\right], \quad (1)$$

where K is frequency constant (which is known as the pre-exponential factor and has a theoretical value of 1030 nuclei/m^3 s), k is the Boltzmann constant (1.38×10^{-23} J/K), γ is the surface free energy or interfacial tension characterizing the energy needed for the creation of a new crystal surface from the solution (J/m^2), υ is the molecular volume (1.315×10^{-27} m^3 for avermectin B1a, which was calculated from the crystallographic data as determined by Springer [13]), T is the absolute temperature (K), and $S = C/C_e$ (C and C_e are the concentrations of the supersaturated and saturated solutions at given temperature, T), is the relative supersaturation ratio.

For a given volume of the solution, the rate of nucleation, J, is inversely proportional to the induction time, τ (min). The Eq. (1) may be written as

$$\ln \tau = A + \left[\frac{16\pi\gamma^3 \upsilon^2}{3k^2T^3(\ln S)^2}\right], \quad (2)$$

where A is a constant (dimensionless). There is a linear dependence of $\ln \tau$ versus $(\ln S)^{-2}$ when the temperature is fixed. Surface free energy, γ, can be estimated from the slope of the linear relationship. This can only be justified, however, if the data relate to true homogeneous nucleation [14].

Nucleation periods of crystallization with different solvents (methanol, ethanol, and *n*-butylalcohol) were depicted in Fig. 1. For the three alcohols investigated, the induction time all decreased with the increase of supersaturation level. In addition, when compared at the same relative supersaturation ratio, the induction time increased in the order of the crystallization solvents methanol, ethanol, and *n*-butylalcohol. On the basis of the theoretical considerations above (Eq. (1)), nucleation rate should increase with the increase of supersaturation and solid–liquid surface free energy.

The relationship between the induction time and the relative supersaturation was re-plotted as shown in Fig. 2. The experimental variation of the crystallization in methanol can be divided into two linear dependences of high gradient (0.356) in high supersaturation range ($2.0 < S < 2.8$) and low gradient (0.0878) in low supersaturation range ($1.3 < S < 1.8$). These regions have been