PEPTIDES CRYSTAL APPLIED IN THE FOOD AND PHARMACEUTICAL INDUSTRIES: RESEARCH AND DEVELOPMENT OF COUETTE-TAYLOR CRYSTALLIZER SYSTEM

Đến toà soạn 02-07-2024

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TÓM TẮT

TINH THỂ PEPTIDES ỨNG DỤNG TRONG CÁC NGÀNH THỰC PHẨM VÀ DƯỢC PHẨM: NGHIÊN CỨU PHÁT TRIỂN HỆ KẾT TINH COUETTE-TAYLOR

Hệ kết tinh Couette-Taylor được nghiên cứu phát triển nhằm thu hồi các sản phẩm tinh thể Peptide chất lượng cao đạt chuẩn dược điển của Mỹ (FDA) và Châu Âu (EMA). Kết quả nghiên cứu cho thấy hình thái học và kích thước của sản phẩm tinh thể được kiểm soát tốt bởi hệ Couette-Taylor. Chúng tôi nhận thấy hình thái học lăng kính của sản phẩm tinh thể không thay đổi tại các điều kiện hoạt động của hệ Couette-Taylor. Trong khi đó, kích thước trung bình của sản phẩm tinh thể phụ thuộc vào chế độ thủy động lực học của hệ Couette-Taylor. Tinh thể có kích thước lớn hơn sẽ được kết tinh ở cường độ thủy động lực học cao hơn. Đối với cấu trúc tinh thể, chúng tôi nhận thấy cấu trúc tinh thể ổn định không thay đổi ở cường độ thủy động lực học từ 360 vòng/phút đến 500 vòng/phút. Tuy nhiên, cấu trúc tinh thể sẽ bị thay đổi và chuyển sang dạng cấu trúc khác khi cường độ thủy động lực học tăng hơn 700 vòng/phút.

Từ khoá*: mầm tinh thể, phát triển tinh thể, đa cấu trúc tinh thể, hình thái học, kích thước, hệ kết tinh Couette-Taylor.*

1. INTRODUCTION

Cooling crystallization is often employed to control the properties of crystalline products, including purity, structure, shape, size, and size distribution. This is an essential issue in the food and pharmaceutical industries because these properties will directly affect human health [1-6].

A reality in production is that more than 50% of solid pharmaceuticals have polymorphism (crystal structure) phenomena [7-10]. Polymorphism is the phenomenon of the same initial solute, but during the crystallization process, it can produce many different crystal structures depending on the operating conditions. The polymorphism phenomenon is very complex and challenging to control during crystallization. Differences in crystal structure will lead to changes in the chemical-physical-biophysical properties of crystal products; here, they will differ in solubility, directly affecting the biological activity and therapeutic value of solid pharmaceuticals. Controlling the polymorphism phenomenon during crystallization has become a complex and

significant problem for the pharmaceutical industry over the past 25 years. For example, Ritonavir, an antiretroviral drug against HIV, came to market in 1998. However, the drug showed no therapeutic effect because the stable α-form was crystallized instead of needing to crystallize the less stable β-form as desired [10]. Not only is the polymorphism phenomenon of crystal products necessary, but other crystal properties such as morphology, size, and size distribution are also important because they also directly affect the dissolution rate of the drug as well as subsequent processing stages such as filtering, drying, mixing to create drug formulas, pressing tablets, packaging [11-12].

Crystals used in pharmaceuticals must meet strict quality requirements and fully meet pharmacopeia standards in terms of purity, polymorphism (crystal structure), shape, size, and size distribution. The properties of crystalline products will be evaluated, licensed, and monitored by reputable pharmaceutical and food industry agencies in the US (FDA) and Europe (EMA) before being commercialized on the world market. Therefore, research and development of crystallization technology processes to create high-quality crystal products to meet these high standards is a critical issue in the pharmaceutical industry [1-12].

Peptide crystal products are essential in the food and pharmaceutical industries [13-18]. Their global revenue exceeded 50 billion USD in 2019, with an average growth rate of 7.7%. For example, diglycine peptide crystals have many different applications and are widely used in cosmetics, food additives, and biochemical research. The global market for this crystal product is estimated to reach 5 billion USD annually.

The Couette-Taylor crystallization system has been previously studied and shown to have many outstanding advantages regarding mass transfer, shear rate, and energy [19]. Therefore, the Couette-Taylor crystallization system has been applied to many different crystallization fields [19-24], especially in the food and pharmaceutical industries. Unlike previous studies, we are developing a new Couette-Taylor crystallization system to improve the efficiency of the production process of peptide crystal products. Here, we will investigate the effect of crystallization on the morphology, size, and crystal structure of peptides.

2. EXPERIMENT

High purity (≥98%) homopeptide diglycine crystalline material was purchased from Sigma-Aldrich Company without further purification. The feed solution was prepared by dissolving the crystalline material in distilled water at a concentration of ~330 mg/L and ~60 °C. The batch Couette-Taylor crystallization system is illustrated in Figure 1. The crystallization system is comprises two coaxial cylinders made of stainless steel and glass. The outer cylinder is made of glass and remains stationary, while the inner cylinder is made of stainless steel and is rotated by a motor. Here, the crystallizer's length is L=44 mm, while the inner and outer cylinders' diameters are D1=20 mm and D2=24 mm, respectively. The solution was fed to the gap between the two cylinders. The sensor and a laser thermometer directly measured the solution temperature in the gap cylinders. The rotation speed of the inner cylinder can be varied from 360 rpm to 700 rpm. The volume of the crystallization system is 6.0 mL with a cooling rate of ~4.0 ^{o}C/min until the solution temperature drops to 30 ^{o}C and

the liquid-solid equilibrium is maintained at this temperature.

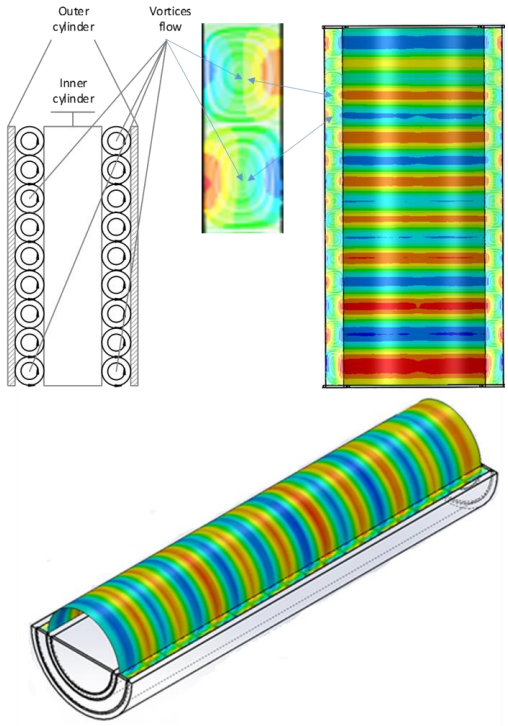


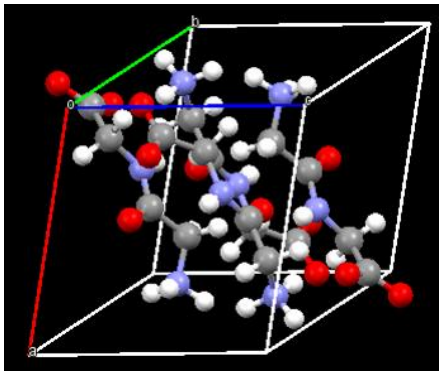
Figure 1. Fluid hydrodynamics in the batch Couette-Taylor crystallization system

Solute concentration was determined by UV-Vis spectroscopy (UV-Vis 1800 Shimadzu, Japan) using an absorption band at 210 nm [25]. Solubility is determined by dissolving the product solid in water at different temperatures until a saturated solution is achieved, then filtering the solution and determining the solute concentration. The crystalline products were separated by vacuum filtration, washed with water, and then dried in a desiccator over silica gel. The crystals were observed and analyzed using an optical microscope (AmScope 40X-2500X) and Raman spectroscopy (HR320 LabSpec6). In addition, the structure of the crystals was determined by X-ray diffraction (MAC Science, M18XHF-SRA). The molecules in the unit cell and mophology of crystal were simulated by the software programs Mercury and Material Studio [19]. Here, we used the Morphology module (BFDH, quality medium) in the Material Studio software. The fluid hydrodynamics of the batch Couette-Taylor crystallization system was simulated by computational fluid dynamics using flow simulation software SolidWorks [19].

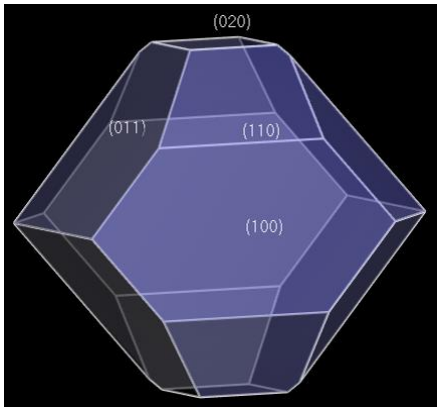
3. RESULTS AND DISCUSSION

The peptide crystal has a prismatic morphology, as shown in Figure 2(a). The unique morphology of this crystal system is based on its distinct structure; here, the structure of this crystal belongs to the monoclinic system with space group P 21/c. The number of molecules in a unit cell is Z = 4, and the parameters of a unit cell are a = 7.97980 Å, b = 9.52010 Å, and c = 7.76430 Å with angles $\alpha = 900$, $\beta = 106.150$, and $\gamma = 900$, as shown in Figure 2(b). We also simulated the morphology of this crystal and observed surfaces such as (110), (100), (011), and (010) through the BFDH module in Material Studio software, as shown in Figure 2(c). Furthermore, the characteristic of the crystal structure expressed through the distinct arrangement of molecules in a unit cell was also identified by X-ray diffraction, as shown in Figure 3.

(a)



(b)



(c)

Figure 2. (a) Experimental peptide crystal, (b) a unit cell of the peptide crystal, and (c) simulated morphology of the peptide crystal

The phase diagram of the crystal versus temperature was determined to estimate the solid-liquid phase separation to recover the solid product, as shown in Figure 4. We found that the crystal's solubility increases as the solution temperature increases (see Figure 4). Therefore, crystallization by cooling can be applied to conduct solid-liquid phase separation to recover peptide crystal products.

The fluid hydrodynamics in the batch Couette-Taylor crystalline system have been computationally simulated. Figure 1 shows the crystalline system's cross-section and longitudinal section. The ratio between the fluid's centrifugal force and viscous force is expressed through the dimensionless Taylor number (Ta), as in equation (1).

$$Ta = \frac{R_I \omega d}{\upsilon}\left(\frac{d}{R_I}\right)^{0.5} \qquad (1)$$

Where ω (rad/s) and υ (m2/s) are the angular velocity of the inner cylinder and the kinematic viscosity of the fluid, respectively. R_I (m) and d (m) are the radius of the inner cylinder and the gap between the inner and outer cylinders, respectively.

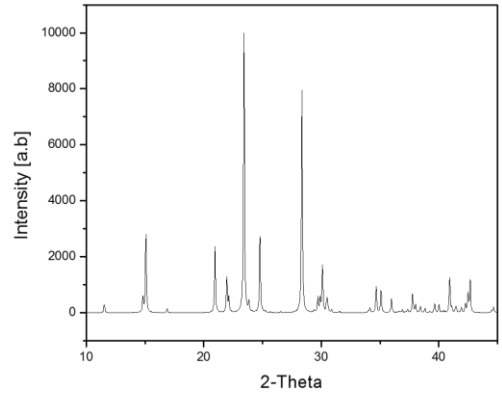


Figure 3. X-ray diffraction of the peptide crystal product

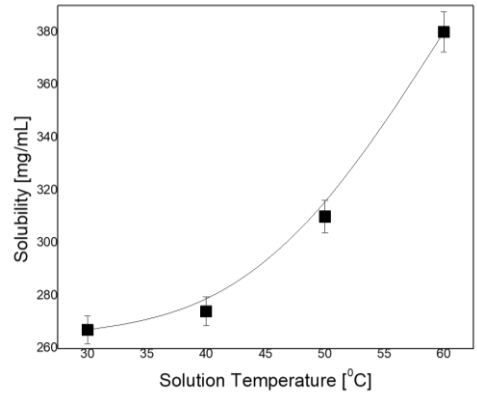
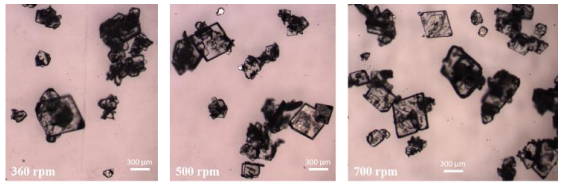


Figure 4. Solubility of peptide crystal versus temperature

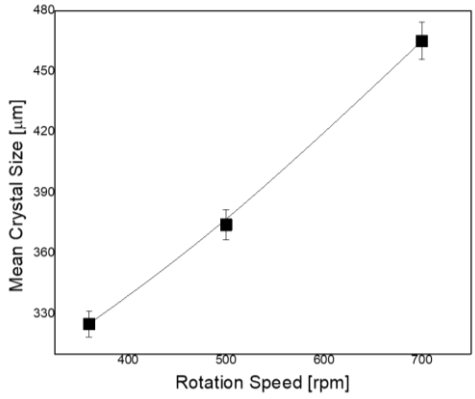
In general, when the Taylor number is low (Ta<48), the fluid flow is laminar. When the Taylor number reaches the critical value Ta_c=48.6, the characteristic Taylor vortex will appear in the

crystalline system (see Figure 1). During the crystallization process, when the hydrodynamic regime changes from 360 rpm to 700 rpm, the calculated Taylor number has a value much larger than the critical value Ta_c=48.6. Therefore, peptide crystallization is always carried out in a Taylor vortex. However, it should be noted that the crystallization process will be susceptible to changes in the Taylor number (Ta) because a change in this number will lead to a change in the fluid flow velocity as well as the number of Taylor vortexes in the crystallizer system.

When the crystallization process was performed at different hydrodynamic regimes from 360 rpm to 700 rpm, the crystalline products were recovered and analyzed through optical microscopy, as shown in Figure 5(a). We found that all crystal products under these operating conditions were similar in prismatic shape (see Figure 5(a)). However, the average size of the crystalline product varies according to the hydrodynamic regime; the average size is increased with increasing the intensity of fluid flow, as shown in Figure 5(b). The average size will increase from 328 µm to 425 µm when increasing the intensity of fluid motion from 360 rpm to 700 rpm. Increasing the intensity of the fluid hydrodynamics enhanced crystal size, which could be explained by the growth process determined by the solution's mass transfer [4]. Here, the growth process of crystals occurs via the convection, diffusion, and integration of the growth unit, such as molecules, nanocrystals, or nanodroplets, to the interface of crystals [4]. The increasing intensity of fluid motion promotes the mass transfer of the solution that accelerates the growth process of crystals, resulting in the enhancement of crystal size. Thus, the batch Couette-Taylor crystallization system can control the crystal product's morphology and average size.



(a)



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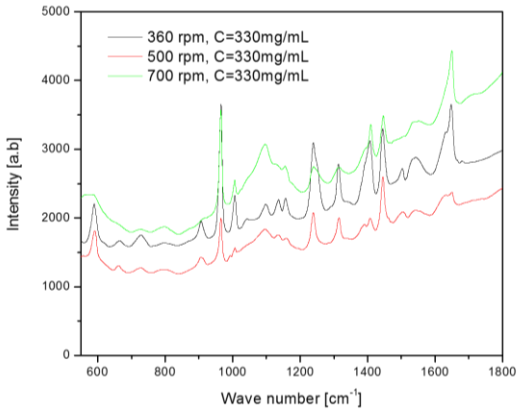

(c)

Figure 5. Effect of Taylor vortex on crystalline products: (a) crystal morphology, (b) average crystal size, and (c) peptide crystal structure

The structures of the peptide crystal products at different operating conditions were also confirmed through Raman analysis, as shown in Figure 5(c). The study showed that the crystal products' structures were similar when the hydrodynamic regime changed from 360

rpm to 500 rpm. However, when the Taylor vortex intensity increased above 700 rpm, the structure of the crystalline product was altered (see green line in Figure 5(c)). Thus, a new structure of crystalline products was created by increasing the intensity of fluid flow in the crystallization system. This result is explained through the random arrangement of solute molecules in the pre-nucleation stage; here, the pre-nucleation stage is determined by the movement of the fluid flow, causing the solute molecules to collide, attach, and interact with each other in various ways to create different crystal structures [19].

4. CONCLUSION

Initial research has demonstrated the success of the newly developed Couette-Taylor crystallization system through the cooling crystallization method. Experimental results show that the properties of the peptide crystal products are well controlled. Here, the prism-like morphology of the crystal product is always stable. Meanwhile, the crystalline product's average size increases as the fluid flow intensity increases. For the crystal structure, we found that the crystal structure will not change when the Taylor vortex intensity is low, from 360 rpm to 500 rpm. However, the structure of the crystalline product will change when the Taylor vortex intensity increases above 700 rpm.

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