CHEMICAL ENGINEERING TRANSACTIONS
VOL. 47, 2016
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.aidic.it/cet
Guest Editors: Angelo Chianese, Luca Di Palma, Elisabetta Petrucci, Marco Stoller

ISBN 978-88-95608-38-9; **ISSN** 2283-9216
DOI: 10.3303/CET1647072

Preparation and Characterization of Nanoparticles of Amorphous Cefmenoxime Hydrochloride

Jinxiu Wang[a], Chuang Xie[ab], Qiuxiang Yin[ab], Linggang Tao[c], Jun Lv[c], Yongli Wang[ab], Hongxun Hao[*ab]

[a]School of Chemical Engineering and Technology, Tianjin University Tianjin 300072, People's Republic of China
[b]Collaborative Innovation Center of Chemical Science and Chemical Engineering of Tianjin, Tianjin University
[c]Lionco Pharmaceutical Group, Haikou, Hainan 570100, China
hongxunhao@tju.edu.cn

Poor water solubility of cefmenoxime hydrochloride greatly limits its bioavailability and clinical application. The decreasing of the particle size could significantly enhance the solubility of the solute. In this work, nanoparticles of amorphous cefmenoxime hydrochloride with nano-size were successfully prepared by the methods of cooling crystallization, anti-solvent crystallization and emulsion crystallization. Different stabilizers, such as Tween 80 and Polyvinylpyrrolidone, were tried to prevent particles from agglomeration and growth. The final products were characterized by scanning electron microscopy, transmission electron microscopy and powder X-ray diffraction. Results show that different preparation methods have significant impacts on morphology of particles. Products prepared by cooling crystallization are smooth spherical with a central hole while products prepared by anti-solvent crystallization are agglomerated seriously and products prepared by emulsion crystallization have not specific shapes. Although the addition of stabilizers don't show an effective effect on preventing particle growth or enhancing particles stability, they have effect on the morphology of the particles, especially for Tween 80.

1. Introduction

Cefmenoxime hydrochloride ($C_{32}H_{35}ClN_{18}O_{10}S_6$, CAS registry No: 75738-58-8), one of third-generation cephalosporins, is widely used for treatment of gonorrhea and inflammation caused by postoperative, respiratory, urinary infection (Matsumoto et al., 1983; Tsuchiya et al., 1981). The third-generation cephalosporins are considered to be one of the most significant therapeutic entities (Jones, 1994). It was reported that cefmenoxime hydrochloride has broader antibacterial spectrum (Miyagawa et al., 1984) and shows the highest antimicrobial activity against both gram-negative and gram-positive bacteria in comparison with other cephalosporins (Kurashige et al., 1982).The chemical structure of cefmenoxime hydrochloride is shown in Figure 1.

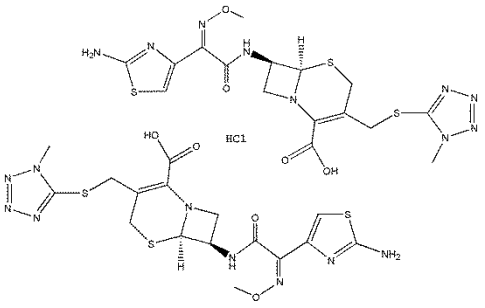

Figure 1： Chemical structure of cefmenoxime hydrochloride

Please cite this article as: Wang J., Xie C., Yin Q., Tao L., Lv J., Wang Y., Hao H., 2016, Preparation and characterization of nanoparticles of amorphous cefmenoxime hydrochloride, Chemical Engineering Transactions, 47, 427-432 DOI: 10.3303/CET1647072

Although it has been put into market since 1983, the problem of poor solubility of cefmenoxime hydrochloride in water hasn't been solved. Actually, it is estimated that approximately more than 40% of drugs or drug candidates are poorly soluble in water, which greatly limits their bioavailability and absorption (Chen et al., 2011). Synthesis of prodrugs, formation of salts or co-crystals, polymorphs or pseudopolymorphs and complex formation with cyclodextrins are traditional approaches to overcome the challenges posed by the poor solubility in water (Chen et al., 2011; Patravale and Kulkarni, 2004). Recently, nanoparticles technology have emerged and attracted much attention in the chemical field (Schiavi et al., 2015). And based on extensive literature screening, few publications about preparation of cefmenoxime hydrochloride in nano-size can be found. Reduction in particle size results in an increase in high effective surface area which, in turn, increases the solubility and dissolution rate of drug (Chan and Kwok, 2011). Generally, the methods of nanoparticles preparation can be divided into two categories: top-down and bottom-up (Chan and Kwok, 2011). In top-down methods, large particles are broken down to particles in micro or nanoscale by high energy or pressure. In contrary to top-down methods, micro or nanoparticles can be obtained from growth of drug molecular in solution. The methods of cooling crystallization, anti-solvent crystallization and emulsion crystallization used in this work are classified as bottom-up approaches. At the same time, it has been acknowledged that the solubility of amorphous phases of drug is higher than that of crystalline phase. Therefore, in this work, nanoparticles of amorphous cefmenoxime hydrochloride were prepared. In addition, the effects of several factors including the preparation methods, the cooling rate, and the surfactants on the morphology and size of particles, are also investigated.

2. Experimental section

2.1 Material

Cefmenoxime hydrochloride was supplied by China Union Chempharma (Suzhou) Co. Ltd. and the distilled–deionized water was supplied by Nankai University, China. The organic solvents (isopropanol, methanamide and acetone) used in the experiments were purchased from Tianjin Jiangtian Chemical Co., Ltd. (Tianjin, China), which are analytical grade reagents with purity higher than 99.5% in mass fraction without any further purification. Tween 80 (≥99.5% in mass fraction) was purchased from Tianjin Guangfu Fine Chemical Research Institute. Polyvinylpyrrolidone (≥99.5% in mass fraction) was purchased from Tianjin Damao and Docusate sodium (≥96% in mass fraction) was purchased from Shanghai Macklin Biochemical Co. Ltd. All Chemicals used in this work were determined by gas chromatography without further purification.

2.2 Preparation of nanoparticles

1. Cooling crystallization

In this work, cooling crystallization was chosen as one of the methods of preparing nanoparticles. At first, a certain amount of cefmenoxime hydrochloride weighed by electronic analytical balance (type AB204, Mettler-Toledo, Switzerland with accuracy of ± 0.0001 g) was added into binary solvent mixtures of isopropanol + water where the mole fraction of isopropanol is 0.2. Then suspension was heated to 313.15 K in a crystallizer which is a double-jacketed vessel and was kept for several hours until the solid remaining in the solution was completely dissolved. The laser monitoring system composed of a photoelectric transformer, a laser generator, and a light intensity display was used to judge the endpoint of dissolution of the particles. The temperature of crystallizer was controlled by a thermostat (SW23, Julabo, Germany) with temperature uncertainty of ±0.01 K. It is noted the amount of the drug added into the solvent is based on the solubility data from the previous experiments. At last, the clear solution was cooled down at different rate. Centrifugal separation was used to separate the solids from the suspension. And the solid was kept in the vacuum drying chamber to remove residual solvents. In addition, different surfactants were added into the solution before it was cooled down to investigate their effect on the morphology and size of final products.

2. Anti-solvent crystallization

Different from cooling crystallization, another solvent needs to be added into the solution containing the cefmenoxime hydrochloride in anti-solvent crystallization. Cefmenoxime hydrochloride should be slightly soluble or insoluble in the second solvent. In this work, cefmenoxime hydrochloride was firstly dissolved in methanamide at room temperature and then isopropanol which is chosen as the second solvent was added into the solution by peristaltic pump to get the nanoparticles. The drying process of the products obtained by anti-solvent crystallization was the same with cooling crystallization.

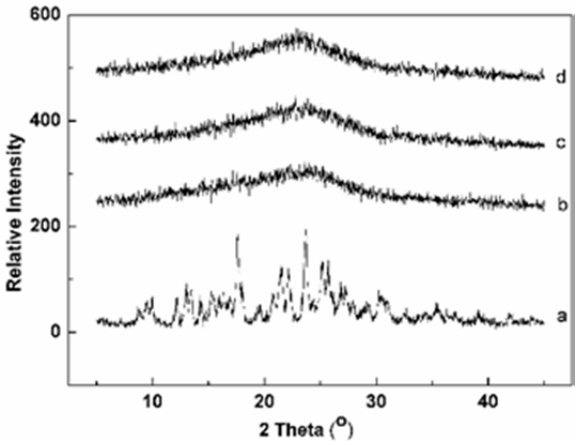

Figure 2: Powder X-ray diffraction patterns of raw material and the final products prepared by different methods: (a) raw material, (b) products from cooling crystallization, (c) products from anti-solvent crystallization, (d) products from emulsion.

3. Emulsion crystallization

Different from anti-solvent crystallization, emulsion is a heterogeneous system consisting of two immiscible liquids in which one liquid is dispersed as droplets in another liquid. In this work, the solution of the binary water + acetone solvent mixtures containing the raw material was seen as the aqueous phase. Isooctane containing docusate sodium was seen as the oil phase. The aqueous phase was added into the oil phase at low temperature under vigorous stirring. And the mole fraction of binary water + acetone solvent mixtures was 0.2.

2.3 Characterization methods

1. Powder X-ray diffraction

Powder X-ray diffraction data of the original material and the products between 5 to 50°was collected on a powder X-ray diffractometer (D/MAX 2500, Rigaku, Tokyo, Japan) using Cu Kα radiation, with a step size of 0.02° and operated at a voltage of 40 kV.

2. Morphology investigation

The morphology of the original material and products was characterized by scanning electron microscope (Hitachi X650) and transmission electron microscope (JEOL JEM2100F) under an accelerating voltage of 200 kV.

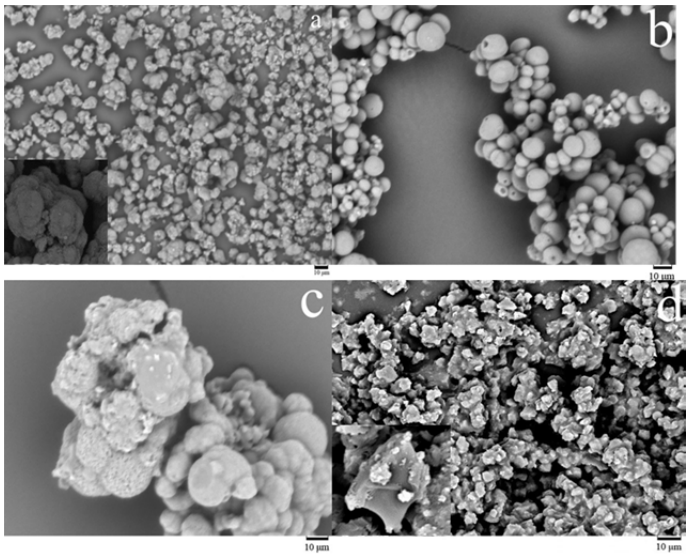

Figure 3: Scanning electron microscope microphotographs of raw material and the final products prepared by different methods: (a) raw material, (b) products from cooling crystallization, (c) products from anti-solvent crystallization, (d) products from emulsion.

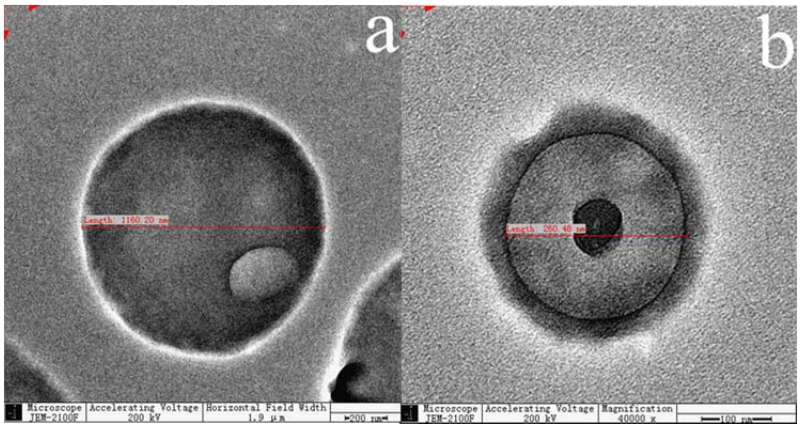

Figure 4: Transmission electron microscope microphotographs of nanoparticles from cooling crystallization: (a) lateral view of particle from cooling crystallization at -0.4 K/min of cooling rate, (b) front view of particle from cooling crystallization at -3 K/min of cooling rate.

3. Results and discussion

3.1 Influence of different preparation methods on the morphology

The final products prepared by different methods including cooling crystallization, anti-solvent crystallization and emulsion are characterized by powder X-ray diffraction. The results are shown in Figure 2. As shown in Figure 2, the final products don't have characteristic peak while the raw material of cefmenoxime hydrochloride has several characteristic peaks. It proves that the final products are amorphous. In addition, scanning electron microscope and transmission electron microscope are used to further characterize the morphology. The results of scanning electron microscope are shown in Figure 3. Transmission electron microscope was used to characterize the morphology of particles from cooling crystallization and the result was shown in Figure 4. It can be seen that compared with the raw material of cefmenoxime hydrochloride, morphology of the products prepared by different methods changed and different preparation methods have different effects on the morphology of the products. The particles obtained from cooling crystallization are smooth sphere with a central hole while particles with rough surface prepared by anti-solvent crystallization are agglomerated seriously and particles prepared by emulsion are not sphere but specific shapes. Nanoparticles of cefmenoxime hydrochloride with size of 260 nm were successfully prepared by cooling crystallization method. It can be found from literature the morphology of many drugs in nanoscale is spherical. But the morphology of cefmenoxime hydrochloride in this work is unique as the particles are hollow after cooling crystallization. The formation mechanism of this special shape need to be further investigated.

3.2 The influence of cooling rate on the size of particles in cooling crystallization

In cooling crystallization, operating factors play an important role to get products with high quality including size distribution, morphology and polymorph. In this paper, the effect of cooling rate on the particle size was investigated. Transmission electron micrographs of the products obtained from different cooling rate were shown in Figure 4. It can be seen from Figure 4, the particle size is 1160 nm at -0.4 K/min of cooling rate and the particle size is about 260 nm at -3 K/min of cooling rate. The particle size decreases as the cooling rate increases. This phenomenon can be explained by basic principles of solvent precipitation. The process of the precipitation can be divided into three steps including nucleation, solute diffusion and particle growth (Chan and Kwok, 2011). Nucleation rate can be expressed by the following equation:

$$\frac{dN}{dt} = K_n(C_i - C^*)^a \quad (1)$$

where K_n is the solute nucleation constant, C_i and C^* refer to the solute concentration and saturation concentration, respectively. Here, ΔC (=C_i -C^*) can be defines as supersaturation. a is the parameter of this equation. The diffusion rate of solute to the particle surface can be expressed by

$$\frac{dm}{dt} = K_d(C - C_i) \quad (2)$$

where K_d is the solute diffusion rate constant and C refers to the bulk solution concentration. The growth rate of particle is

$$\frac{dl}{dt} = K_g (C_i - C^*)^b \quad (3)$$

where K_g is the particle growth rate constant. b refers to the parameter of this equation which increases with the temperature. From the equations above, it can be found that both the nucleation and particle growth depend on the level of supersaturation. Generally, C_i can be considered as C when the micro-mixing is sufficient. Particles in nano-size range can be obtained by enhancing nucleation while suppressing particle growth. Therefore, with the increasing of cooling rate, the instant supersaturation rapidly rise which promotes the nucleation of particles and suppresses particle growth.

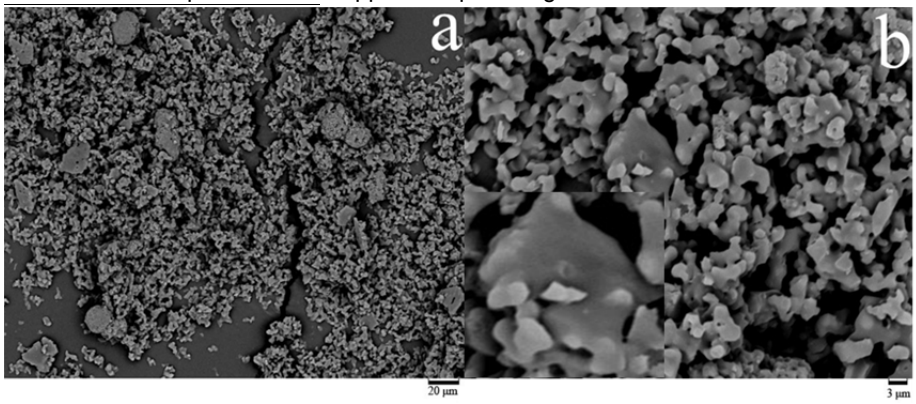

Figure 5: Scanning electron microscope microphotographs of products in cooling crystallization after adding Tween 80.

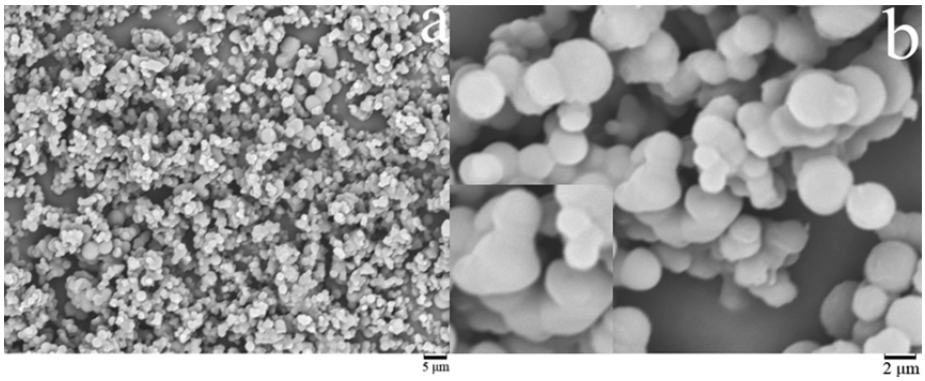

Figure 6: Scanning electron microscope microphotographs of products in cooling crystallization after adding Polyvinylpyrrolidone

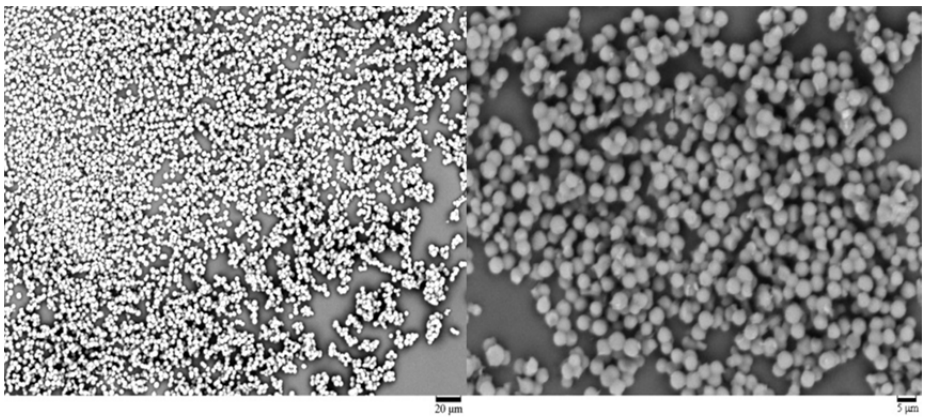

Figure 7: Scanning electron microscope microphotographs of products in cooling crystallization in absence of surfactants

3.3 Influence of different stabilizer on the morphology of particles

It has been acknowledged that surfactants can be employed to stabilize the nanoparticulate suspension in many researches (Oppenheim et al., 1978). In this work, the surfactants of Tween 80 and Polyvinylpyrrolidone were chosen as the stabilizer. The results are shown in Figures 5 and 6, and the particles prepared by cooling crystallization are also shown in Figure 7 to compare the effect of surfactants on the morphology of the particles. It can be seen from Figures 5,6 and 7 that the surfactants used in this work have not apparent effect on preventing particle growth or enhancing particles stability since the sizes of final products' particles are similar. But from Figures 5 and 7, it can be seen the morphology of the particles drastically change when compared with the products from cooling crystallization in absence of surfactants. After adding Tween 80 in the process of cooling crystallization, the morphology of the particles changes from sphere to irregular plate. This indicates the surfactants can be employed not only to prevent particle growth or enhance particles stability but also to change the morphology of the particle.

4. Conclusions

In this work, nanoparticles of amorphous cefmenoxime hydrochloride with size of 260 nm were successfully prepared by cooling crystallization. It shows that different preparation methods have significant impacts on the morphology of particles. In addition, the impacts of cooling rate and surfactants on morphology of particles were also studied. The results show that high cooling rate tends to obtain smaller particles. Although the chosen surfactants didn't have obviously effect on preventing particle growth or enhancing particles stability, Tween 80 show clear effect on the morphology of cefmenoxime hydrochloride particles.

Acknowledgements

This research is financially supported by National Natural Science Foundation of China (No. 21376165) and Key Project of Tianjin Science and Technology Supporting Program (No. 13ZCZDNC08900).

References

Chan, H.-K., Kwok, P.C.L., 2011. Production methods for nanodrug particles using the bottom-up approach. Adv. Drug Delivery Rev. 63, 406-416.

Chen, H., Khemtong, C., Yang, X., Chang, X., Gao, J., 2011. Nanonization strategies for poorly water-soluble drugs. Drug Discov. Today 16, 354-360.

Jones, R., 1994. The antimicrobial activity of cefotaxime: comparative multinational hospital isolate surveys covering 15 years. Infection 22, S152-S160.

Kurashige, T., Wakiguchi, H., Kitamura, I., Ozaki, H., Nishibayashi, Y., Hamawaki, M., 1982. Therapeutic effects of cefmenoxime in the treatment of various infections on infants and children. Jpn. J. Antibiot. 35, 2579-2588.

Matsumoto, K., Nagatake, T., Uzuka, Y., Harada, T., Rikitomi, N., 1983. Laboratory evaluation of cefmenoxime: a new cephalosporin. Chemotherapy 29, 80-88.

Miyagawa, I., Goto, H., Nakashita, E., Fukuda, K., Hamamoto, R., Inoue, A., Miyake, S., Nishida, H., Umano, I., Nakamura, I., 1984. Clinical studies on cefmenoxine concentration in prostatic tissue and bladder wall. Jpn. J. Antibiot. 37, 787-790.

Oppenheim, R.C., Marty, J.J., Speiser, P., 1978. Injectable compositions, nanoparticles useful therein, and process of manufacturing same. Google Patents.

Patravale, V., Kulkarni, R., 2004. Nanosuspensions: a promising drug delivery strategy. J. Pharm. Pharmacol. 56, 827-840.

Schiavi P.G., Altimari P., Pagnanelli F., Moscardini E., Toro L., 2015, Synthesis of cobalt nanoparticles by electrodeposition onto aluminium foils, Chemical Engineering Transactions, 43, 673-678.

Tsuchiya, K., Kondo, M., Kida, M., Nakao, M., Iwahi, T., Nishi, T., Noji, Y., Takeuchi, M., Nozaki, Y., 1981. Cefmenoxime (SCE-1365), a novel broad-spectrum cephalosporin: in vitro and in vivo antibacterial activities. Antimicrob. Agents Chemother. 19, 56-65.