Preparation method of penicillin V potassium β-cyclodextrin inclusion

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Abstract: In order to conceal the unpleasant smell of pharmaceutical raw materials of Penicillin V potassium, we use β-cyclodextrin (β-CD) as a drug carrier. The Penicillin V potassium β-CD inclusion complex was prepared by saturated aqueous solution method; the characterization of penicillin V potassium β-CD inclusion complex is determined by scanning electron microscopy, Differential Scanning Calorimeter (DSC) and X-ray Diffraction (XRD), the formation constants of the complexes were determined by UV spectrophotometry; based on the results obtained from the orthogonal experimental design, the optimum preparation process are summarized as follow: β-CD: Penicillin V potassium = 4: 1 (molar ratio), stirring temperature 50 centigrade, stirring time 12h, the encapsulation efficiency is 29.40%.

Keywords: Penicillin V potassium, β-cyclodextrin, clathrate, orthogonal design.

INTRODUCTION

Cyclodextrin is a cyclic glucose, which is composed of six to twelve glucose molecules connected together, β-cyclodextrin (β-CD) is a cyclic oligosaccharide compound formed by 1, 4 glycosidic bond. β-cyclodextrin has optimum hole size, the lowest cost, so it is the most commonly material inclusion used in the inclusion process (Wakao 2002, Hedges 1998). The characteristics of β-CD is internal hydrophobic and external hydrophilic, it has a three-dimensional hollow structure, the different drugs can be inclusion selective spontaneously as guest molecule, which can increase drug stability, solubility, covering bad smell. Some cyclodextrins also have slow release, emulsification, anti-oxidation, anti-decomposition, thermal insulation, moisture and other functions (Swaldo 1989, Ágnes 2000).

The formation process of β cyclodextrin clathrate is a physical process, the inclusion complex molecule is formed stability by van der Waals forces between the subject and object. At present, preparation of cyclodextrin inclusion compound are: saturated solution method (co-precipitation method); ultrasonic method; grinding; freeze-drying method; spray drying method and so on (Moyano *et al*., 1998, Shaikh *et al*., 2017).

Penicillin was first discovered by Flemming in the early twentieth century, Penicillin is the first antibiotics apply in clinic, and it is still the drug of choice as one of the clinical resistance to infection. For nearly a century of clinical applications, penicillin as a representative drug of antibiotics, has strong antibacterial activity, lower toxicity and side effects (Moller 1992; Aldaihani and Alenezi 2017). Penicillin V potassium was discovered by Behrens in 1947. Penicillin V potassium is penicillin antibiotics, antibacterial spectrum are the same as penicillin which have antibacterial activity to most Gram-positive bacteria, Gram-negative bacteria, Gram-negative bacilli individual (e.g., Haemophilus), spirochetes and actinomycetes. Compared to penicillin G, penicillin V has an oxygen atom added to a side chain, although only a very small structure changes, but some of its nature change tremendously. In acidic aqueous solution, penicillin V is degraded slowly, after three hours, about eighty percent of the active ingredient remains, the acid does not easily damage their structures, so Penicillin V potassium can be used as an oral preparation (Milton 1993, Cho 2017). WHO believes that the resistance of children is weak, impact on children caused by some diseases is more serious. Despite the great demand for children's drugs, but pharmaceutical formulations suitable for children are limited. Antibiotics are used 180,000 tons per year (including medical and agricultural) in China. Daily outpatient visits are nearly 1 million people in Beijing Children's Hospital, one third of the children need the infusion which is mainly antibiotics (Akay *et al*., 2017, Nawaz *et al*., 2017). Penicillin V potassium tablet is a national essential drug, its effectiveness and safety has been verified. Since the drug has a special taste, children have poor medication compliance, so the purpose of this study is to conceal the unpleasant smell of Penicillin V potassium using β cyclodextrin as a drug carrier, preparation of Penicillin V potassium β-cyclodextrin inclusion complex (Hamad 2018; Xie 2018; Ishaq and Jafri 2017).

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MATERIALS AND METHODS

Penicillin V potassium (North China Pharmaceutical Co., Ltd. Lot number: B067114, Content 89.6%); Standard Penicillin V potassium (National Institute for Food and Drug Control, Beijing, China); β-cyclodextrin (Sinopharm Reagent Co., Ltd.); Anhydrous ethanol (AR, Beijing Chemical Reagent Company) and other reagents were of analytical grade.

UVmini-1240 spectrophotometer (Puxi Radio Factory);T-214 electronic balance (Beijing sartorius Instrument System Co. Ltd); 85-1 thermostat magnetic stirrer (Jiangsu jintan Instrument Co., Ltd.); HW-10 infrared oven (Beijing Xing contention equipment factory); SHB-III circulating water pumps (Zhengzhou Great Wall Industry and Trade Co., Ltd.); BX1-general microscope (Japan Olympus Company); HH-6-type constant temperature water bath (Guohua Electric Appliance Co., Ltd.); Differential scanning calorimeter: DSC 200 F3(Beijing Physical and Chemical Analysis and Testing Center); S4800 Field-Emission Scanning Electron Microscope, D8 advance X-ray diffractometer (Beijing Physical and Chemical Analysis and Testing Center).

Preparation method

B-CD completely dissolved in distilled water to form a saturated solution, in a water bath at 40-50 centigrade, Penicillin V potassium accurately weighed, placed in 10ml of distilled water, then injected slowly into the stirring solution of β-CD saturated solution, continue stirring for 3-12hours, cooled to room temperature, and then add 50ml ethanol, placed at 4°C overnight, the inclusion complex so formed is filtered, washed with ethanol to remove the untrapped feed and was dried in an oven at infrared oven 2-4hours, finally obtained white crystals.

$$\text{Encapsulation rate} = \frac{\text{1–the quality of free Penicillin V potassium}}{\text{Penicillin V potassium total inputs}} \times 100\% \quad (1)$$

STATISTICAL ANALYSIS

UV analysis was applied to determine the content of Penicillin V potassium. Dissolved 2.0mg Penicillin V potassium in 20ml water, then separately weigh 1.0, 2.0, 3.0, 4.0, 5.0ml solution and diluted with water to 10ml, Penicillin V potassium concentration was calculated using the standard sample as the calibration standard. A good linear relationship was obtained over the range of 10 μg/ml < C50 μg/ml, and the regression is y = 0.0074x + 0.0285 (R2=0.9996), where y is the concentration of Penicillin V potassium (μg·ml-1), x is the absorbance at 267nm and R is the regression coefficient.

RESULTS

The experiment of β-cyclodextrin inclusion complex of Penicillin V potassium

Penicillin V potassium β-CD inclusion complex is prepared by saturated aqueous solution method. There are three factors which were the molar ratio of β-CD and Penicillin V potassium, the temperature of the inclusion and inclusion time influence the effect of inclusion and encapsulation rate in Eq. (1). L_9 (3^3) orthogonal experiment was used to determine the optimal preparation, experimental design is shown in table 1, the experimental results are shown in table 2.

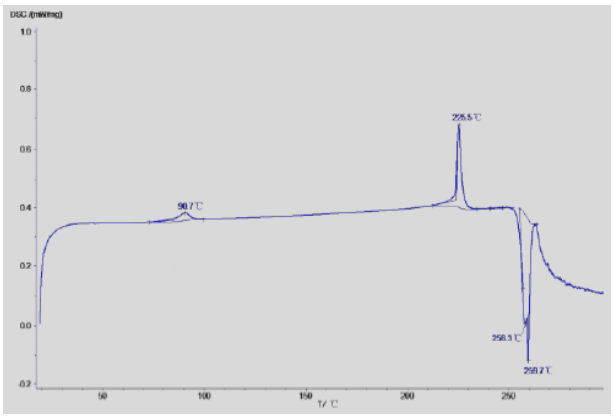

Fig. 1: DSC of Penicillin V potassium (heating rate is 10°C/min)

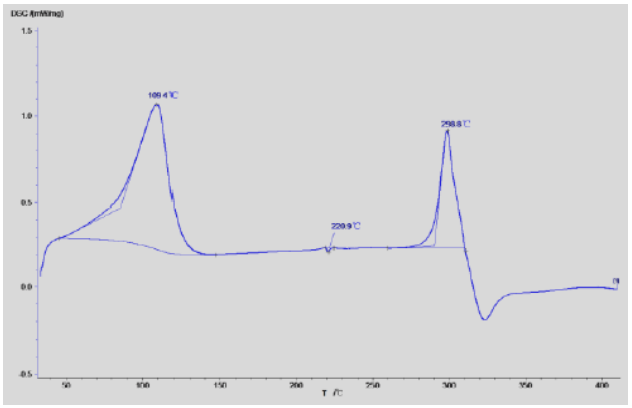

Fig. 2: DSC of β-CD (heating rate is 5°C/min)

From table 2 we can see that the factors influencing the encapsulation rate are listed in decreasing order as follows A > C > B according to the R value, the molar ratio of β-CD and Penicillin V potassium is the key parameter of the inclusion process. The optimum inclusion process parameters should be A3B3C3 which are the molar ratio of β-CD and Penicillin V potassium=4: 1, inclusion temperature 50°C, inclusion time stirring 12hours, the maximum encapsulation rate can be achieved 29.40%.

Table 1: 3 levels and 3 factors graph

Level	The molar ratio of β-CD and Penicillin V potassium A	Temperature (°C) B	The inclusion time (h) C
1	1: 1	40	3
2	2: 1	45	6
3	4: 1	50	12

Table 2: Results and calculations of $L9(3)^3$

Test	A	B	C	Encapsulation rate (%)
1	1: 1	40	3	5.24
2	1: 1	45	6	6.27
3	1: 1	50	12	7.24
4	2: 1	40	6	10.35
5	2: 1	45	12	12.20
6	2: 1	50	3	11.15
7	4: 1	40	12	22.77
8	4: 1	45	3	19.57
9	4: 1	50	6	21.40
K1	18.75	38.36	35.96	
K2	33.70	38.04	38.02	
K3	63.74	39.79	42.21	
R	44.99	1.75	6.25	

Identification of inclusion complexes

Identification inclusion compound by differential scanning calorimeter

Small amount of Penicillin V potassium, β-CD, a mixture of β-CD and Penicillin V potassium, Penicillin V potassium inclusion complexes was taken in aluminum crucibles respectively, cover with tie holes, N2 60mL/min, heating rate was 5-10°C/min. The pictures of four substances DSC are shown in fig, 1-4. We can see Penicillin V potassium has a melting peak at about 225.5°C in fig. 1, β-CD have two peak at 109.4°C and 298.8°C which is dehydration endothermic peak and melting peak respectively in fig. 2, the mixture of β-CD and Penicillin V potassium maintained the endothermic peak which is the superposition of single compound spectrum substantially in fig. 3, but in fig. 4 the position and shape of the peaks have changed in the inclusion complex spectrum, suggesting that the inclusion compounds has formed.

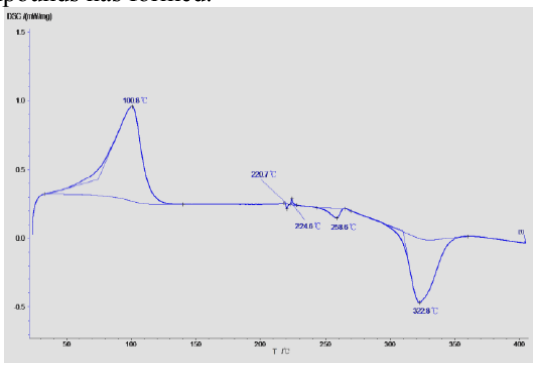

Fig. 3: DSC of mixture of β-CD and Penicillin V potassium (heating rate is 5oC/min)

Identification inclusion compound by SEM

S4800 Field-Emission Scanning Electron Microscope: 5.0KV9.7mm×50.0 SE (M), 15KV15.1mm×20.0k SE (M). In fig. 5 we can see Penicillin V potassium is a rectangular pillar-shaped crystal, the polymer block of β-CD is irregular, loose in fig. 6. There is no crystalline Penicillin V potassium, form tight polymer in fig. 7 that shows the scanning electron microscope of Penicillin V potassium inclusion complexes, speculate that Penicillin V potassium dispersed in the β-cyclodextrin, formation of irregular aggregates, the specific surface area increases.

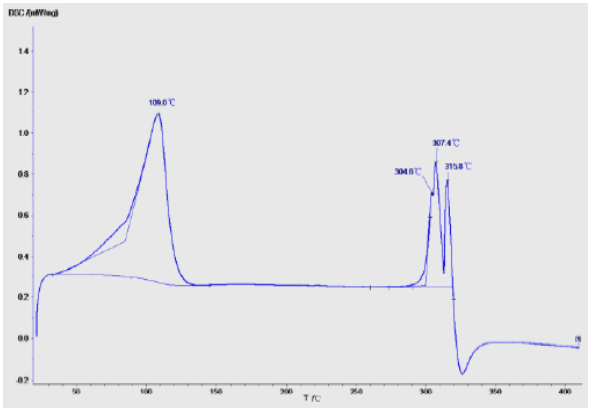

Fig. 4: DSC of Penicillin V potassium inclusion complexes (heating rate is 5°C/min)

Identification inclusion compound by XRD

We identify the inclusion compound by X-ray diffraction (XRD) with D8 advance X-ray diffractometer (German Bruker axs company), 40kv, scan speed 0.1sec/step, increment 0.02.

The results showed in fig. 8-9 that the penicillin is rendered crystalline state, but in the inclusion complex diffraction pattern, the diffraction peaks of Penicillin V potassium disappeared, that prove the Penicillin V potassium inclusion complexes formed.

Fig. 5: Scanning Electron Microscope of Penicillin V potassium.

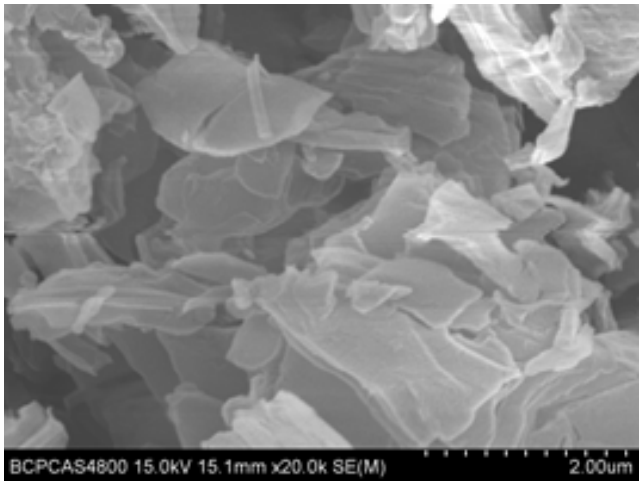

Fig. 6: Scanning Electron Microscope of β-CD.

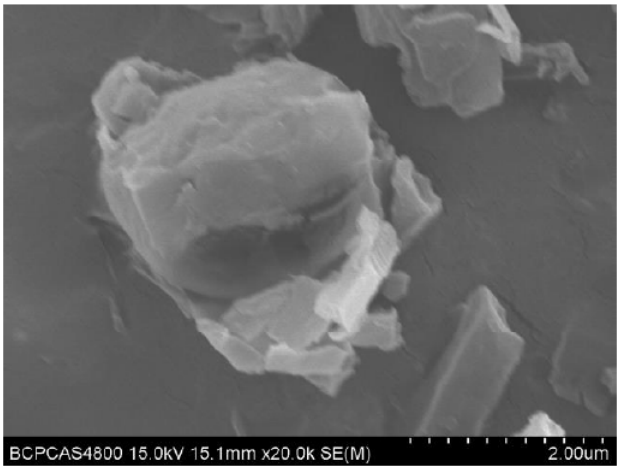

Fig. 7: Scanning Electron Microscope of Penicillin V potassium inclusion complexes.

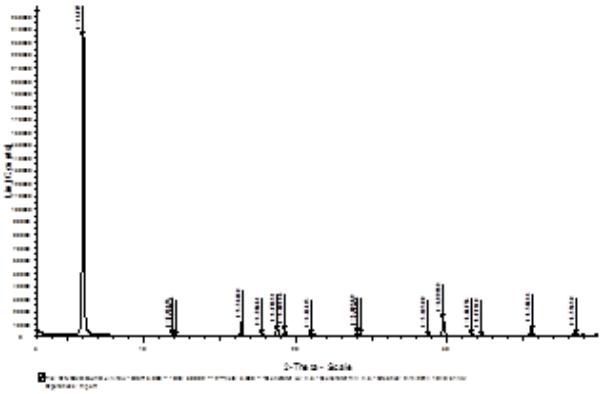

Fig. 8: XRD of Penicillin V potassium.

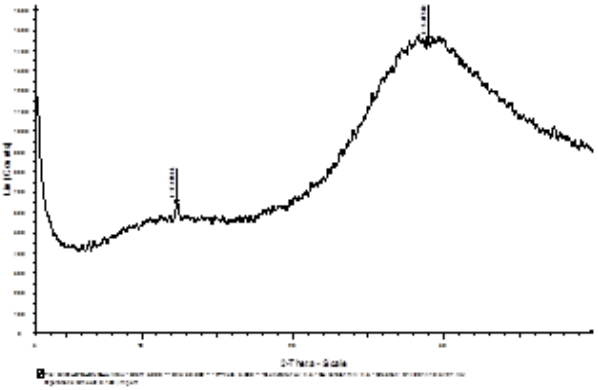

Fig. 9: XRD of Penicillin V potassium inclusion complexes.

DISCUSSION

The hydrolysis rate of Penicillin V potassium is slowest compared to other penicillins, so oral solid preparations can be made. However, the drug has a special taste, the patient's medication compliance is poor especially for children. In this study, we used β-cyclodextrin as a drug carrier. The preparation of Penicillin V potassium β-CD inclusion complex was prepared by saturated aqueous solution method, conceal the unpleasant smell of pharmaceutical raw materials of Penicillin V potassium (Shareef and Akhtar 2018). Based on the results obtained from the orthogonal experimental design, the encapsulation efficiency is 29.40%, the encapsulation rate was not high, this may be due to Penicillin V potassium is water soluble drugs, the characteristics of β-CD is internal hydrophobic and external hydrophilic, so it is difficult for Penicillin V potassium to enter the interior of β-CD (Khan *et al*., 2017). Penicillin V potassium inclusion complex's the position and shape of the peaks have changed in the inclusion complex spectrum, this may be due to the fact that the inclusion associations of cyclodextrin changes the crystal structure and molecular environment, which generate the formation of polymer and increase the specific area around penicillin V potassium, so its fluorescence quantum and intensity get change and it

generates the position and shape of Penicillin V potassium inclusion complexes. The series of changes bring the inclusion compounds into being

CONCLUSION

In this paper, the Penicillin V potassium inclusion complex with β-cyclodestrin (β-CD) is prepared by saturated aqueous solution method, The results of encapsulation rate has conducted to determine preparation conditions: aqueous as solvent, β-CD as carrier, the inclusion molar ratio of drug-carrier was 4: 1, The preparation temperature was 50°C, stirring time was 12h, add ethanol, placed at 4 centigrade overnight, filter and the samples were washed 3 times with water, encapsulation rate is 29.40% which is not high as we expected, but in this process, the unpleasant smell of pharmaceutical raw materials of Penicillin V potassium can be concealed with security β-CD and the process is simple. This research laid the foundation for future in-depth study of Penicillin V potassium inclusion complex.

ACKNOWLEDGEMENTS

This work was financially supported by National Natural Science Foundation of China (Grant No. 11475020 and 8151114029), Chaoyang District collaborative innovation project (Grant No. 2016-8), and Graduate program of Beijing Union University (Grant No.2018).

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