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Polymorphism of flucloxacillin sodium

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The polymorphism of flucloxacillin sodium has not been discussed sufficiently so far. Flucloxacillin sodium which was crystallized with different solvents, was found to exist in amorphism and three crystal forms (I, II, III). This results were confirmed by infra-red (IR) spectra, thermogravimetry (TG), X-ray diffraction analysis (XRD) and equilibrium solubility. It is noticed that form III has very good solubility in phosphate buffer solution, with an average solubility of 0.86 g (20–40 °C). However, more efforts are needed to carry out and decide whether this form can be used for industrial production.

1. Introduction

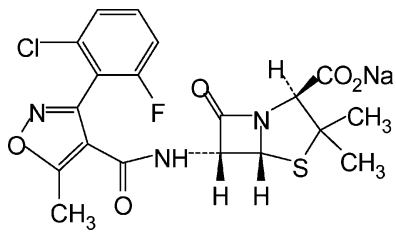
Flucloxacillin sodium is a β-lactam antibiotic, belonging to the isoxazolyl family of penicillins. It shows a broad spectrum of activity and is relatively resistant to most of the bacterial β-lactamases (Refat and El-Didamony 2006).

Some organic compounds, for example, pindolol (Nunes et al. 2004) and mebendazole (de Villiers et al. 2005), can present different spacestructures in the solid state. It is also well known that different spacestructures of one drug can give rise to different chemical and physical properties. Solubility and bioavailability are also influenced (Vega et al. 2007; Raw et al. 2004; Llinas and Goodman. 2008), so it is vital to select the right polymorph in industrial production. Modern technical analysis (Kauffman et al. 2008; Vickery et al. 2002; Blanco et al. 2006) have been widely used in distinguishing different crystal forms of drugs. In this article, different organic solvents were used to crystallize flucloxacillin sodium and the isolated crystal forms were characterized by melting point method, IR spectra, thermal analysis, X-ray powder diffraction patterns and equilibrium solubility.

2. Investigations, results and discussion

2.1. Melting point test

The melting point of amorphous substance was 160–180 °C. It was difficult to get accurate melting points of the three crystal samples, because all of them broke down at a temperature above 175 °C and the decomposing rate increased. At the same time, their colour changed which illustrated decomposition. The test results for the three crystal forms were: for thermochromic temperature, form I is 175 °C, form II is 175 °C, it is very interesting that form III is 165 °C; form I starts melting at 206 °C, form II and form III at 191 °C already; melting was finished for form I at 208 °C, for form II and form III at 193 °C and 192.5 °C, respectively.



Scheme 1: Flucloxacillin sodium

2.2. Thermal analysis

Figure 1 shows the thermal analysis results for the four polymorphic forms of flucloxacillin sodium. The thermal decomposition of form I was a two-stage process. The first mass-loss stage appeared at 34–175 °C which is attributed by the loss of adsorbed and bound water. The sample of form I lost adsorbed water at 34–85 °C and bound water at 85–175 °C, respectively. The rate of lost bound water weight was 3.87% (in theory 3.64%), indicating the loss of a molecule of bound water. Furthermore, the DTG-curve showed a small weight loss peak at about 150 °C. We could observe the second mass-loss stage at temperatures up to 175 °C, because the sample of form I broke down at this temperature. There was a super peak of weight losing between 175 and 360 °C on the DTG-curve. We deemed that the main temperature interval of decomposition is from 175 to 360 °C. The residue is approximate 40.2% of the total weight, and the theoretical value is 21.46% (The residue was Na_2CO_3, if it decomposed completely), so this implies that form I did not break down completely at the experimental temperature, what could also be confirmed from the TG-curve, which did not descend to a horizontal line.

The thermal decomposition of form II and form III followed also a two-stage process, and were very similar to each other, but amorphism had only one-stage process of thermal decomposition. The thermal decomposition data are shown in Table 1.

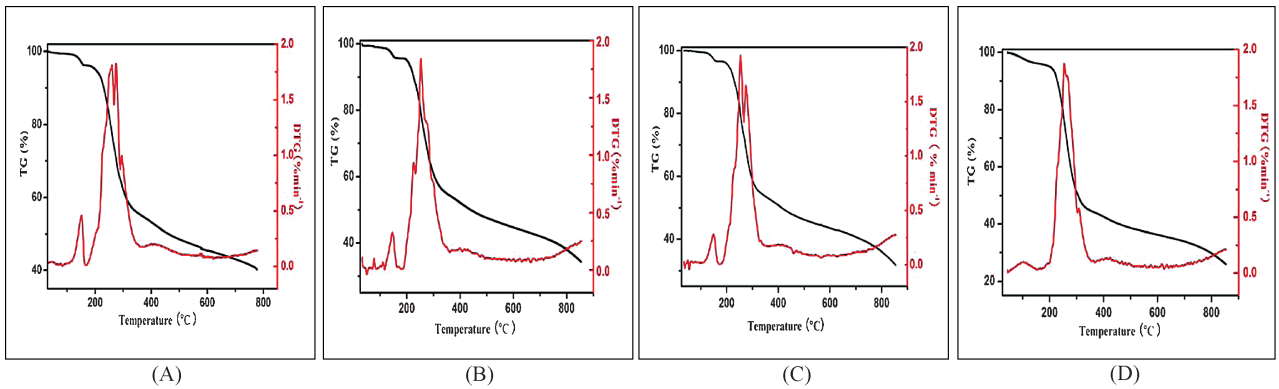


Fig. 1: The chart of TG-DTG of four polymorphs of flucloxacillin sodium. A. form I; B. form II; C. form III; D. amorphous

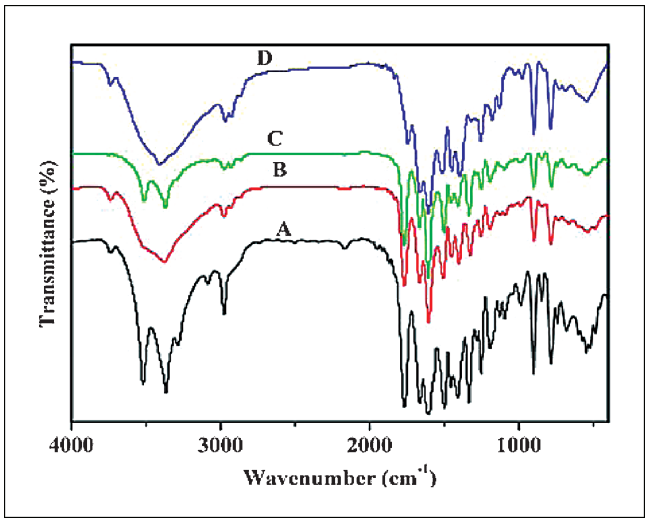


Fig. 2: IR spectra of the four polymorphs of flucloxacillin sodium. A. form I; B. form II; C. form III; D. amorphous

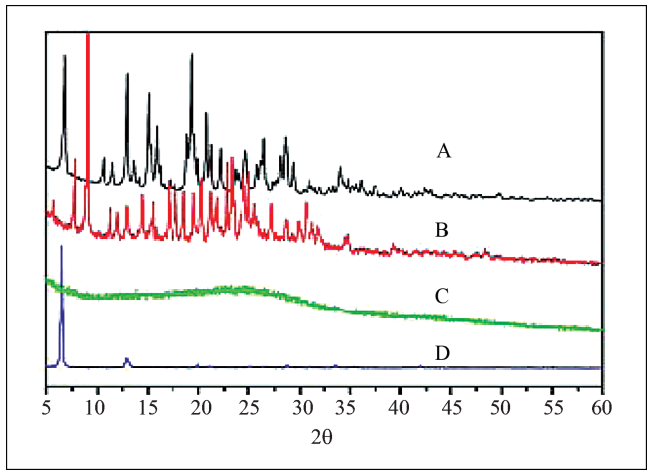


Fig. 3: XRD diagram of four polymorphs of flucloxacillin sodium. A. form I; B. form II; C. form III; D. amorphism

2.3. *IR spectra*

The IR spectra for four polymorphs are compared and shown in Fig. 2. Significant differences exist in the IR spectra of the four polymorphs. An additional peak at 1335 cm^{-1} was observed from forms I-III which is missing in the amorphous state. Another peak at 1765 cm^{-1}, the vibration intensity of the form I-III was larger than that in amorphism. The two peaks can be observed through standard IR spectra (British pharmacopoeia 2009). There are two small peaks at 2160 and 3089 cm^{-1} in form I only. We can get more information above 2750 cm^{-1} to differentiate crystal forms of flucloxacillin sodium. Between 3238 and 3575 cm^{-1}, there are three peaks show in form I, and two peaks show in form III, only one peak can be found in form II and amorphism.

2.4. *X-ray diffraction*

The XRD diagrams of crystal forms I-III and amorphous form are shown in Fig. 3. It is very easy to see the differences among the four forms in XRD diagrams. Amorphism shows an especial peak at approx. $2\theta = 10–30°$. It is the essential feature for amorphism to be distinguished from forms I-III. The characteristic peak ($I/I_0 = 100$) position of form III at $2\theta = 6.4861°$ is different from that of form I and form II, at approx. $2\theta = 20.3427°$ and 8.9733°, respectively. Besides, form I shows a peak at approx. $2\theta = 14.3606°$, which is absent in form III and form II. Furthermore, there are still some differences at other positions with form I-III, such as at $2\theta = 11.2168°$, 21.2438°, 24.9677°, 27.2465°, 30.8213°.

2.5. *Equilibrium solubility*

Interestingly, the crystal forms I-III and amorphous form of flucloxacillin sodium exhibit different solubility. The solubility and dissolution velocity of amorphous flucloxacillin sodium are both better those of the crystal forms. In particular, amorphous drug has the best solubility and dissolution velocity at 35 °C. This may be related to crystal lattice, because amorphous drug does not have crystal lattice and has a higher free energy. From Table 1, we can see the sequence of solubility form III > form I > from II. The average solubility of each polymorph of flucloxacillin sodium is: amorphous 0.836 g; form I 0.446 g; form II 0.33 g; form III 0.86 g. So solubility is a very useful tool to distinguish the polymorphs of flucloxacillin sodium.

2.6. *Conclusions*

We obtained different crystal forms of flucloxacillin sodium by recrystallizing from different organic solvents. The conspicuous characteristics of these crystal forms were detected by modern analysis technology, such as XRD, IR spectra etc. The results of melting point tests showed that above a temperature of 175 °C, all three crystal forms decompose. Different crystal forms of flucloxacillin sodium expose different melting points. All polymorphs of flucloxacillin sodium contain a molecule of bound water except for amorphism. Finally, amorphous drug and form III own a better solubility at normal temperature, and it can be speculated that they are more feasible for industrial production processes than the other two crystal forms.

Table 1: Thermal decomposition data of four polymorphs of flucloxacillin sodium

Polymorph	Form I		Form II		Form III		amorphous	
	Temperature (°C)	Weight loss rate (%)	Temperature (°C)	Weight loss rate (%)	Temperature (°C)	Weight loss rate (%)	Temperature (°C)	Weight loss rate/%
Adsorbed water	34–85	0.53	34–105	1.21	34–100	0.51	34–175	3.89
Bound water	80–175	3.34	105–170	3.31	100–172	3.14		
Decomposition	175–850	55.94	170–850	61.21	172–850	64.54	175–850	70.26

3. Experimental

3.1. Materials

Flucloxacillin sodium, pharmaceutical grade, was purchased from Guilin Pharmaceutial Corp, China. Isopropanol (The First Reagent Factory, Shanghai, China), acetone, tetrahydrofuran, methanol, ethanol, ethyl acetate, n-butanol (Xilong Chemicals, Shantou, China) were used as received. All water used in this experiment was double-distilled.

3.2. Methods

3.2.1. Preparation of flucloxacillin sodium polymorphic forms

Form I: To 1.0 g flucloxacillin sodium, 17 mL of isopropanol (or 10 mL of ethylacetate) was added with stirring. The liquid was slowly heated to 50 °C, then filtered while hot, and the filtrate was allowed to cool to room temperature. The crystals were collected, dried under vaccum and kept in a desiccator over silica gel until used.
Form II: Flucloxacillin sodium (2.0 g) was dissolved in 20 mL of boiling ethanol, then filtered while hot, and the filtrate was allowed to cool slowly. The crystals were collected and treated as above.
Form III: To 1.0 g of flucloxacillin sodium, 13 mL of acetone was added with stirring, and heated to boiling, filtered while hot, and the filtrate was allowed to cool to room temperature. The crystals was collected and treated as similar as form I.
Amorphous: Flucloxacillin sodium (1.0 g) was dissolved in 17 mL of boiling tetrahydrofuran (or 6 mL of methanol), filtered while hot, and the filtrate was allowed to cool to room temperature. The collected product was treated similar to form I.

3.2.2. Micro melting point apparatus

Micro melting point apparatus (XT4A, Keyi, China) contains hot-stage, microscope and digital display controlling instrument which was used for determining the melting points of the compounds.

3.2.3. Thermal analysis

Thermogravimetric studies were performed with a STA-499 thermogravimetric apparatus (Netzsch, Germany) between 30 °C to 850 °C at a heating-rate of 10 °C/min using nitrogen as purge gas.

3.2.4. IR spectra

Infra-red (IR) spectra were obtained on a Shimadzu-8400 system (Shimadzu, Japan). Samples were dispersed in KBr powder and pellet was made for measurement. No polymorphic changes were observed to be induced by grinding or compressing flucloxacillin sodium raw material for sample preparation.

3.2.5. X-ray powder diffraction analysis

X-ray powder diffraction (XRPD) data was obtained on a X'Pert PRO system (PANalytical, Holland) with Cu, $K\alpha_{1,2}$ radiation (wavelength = 1.54060 Å, 1.54443 Å), tube powered at 40 kV and 40 mA. The powder was ground, spread onto a zero background quartz plate and scanned at a rate of 5 °/min. The diffraction patterns were recorded in the range of 2θ angles from 5 to 60 °.

3.2.6. Solubility measurement

Comparison products were dried to constant weight at 105 °C. 100 mg comparison products (weighing accurately) and 50 mL phosphate buffer solution were added into a 100 mL beaker, dissolved, and then dropped the solution into a 100 mL volumetric flask, after that, made up to the mark with phosphate buffer solution (PBS, pH = 6.8). The scan range of ultraviolet-spectrophotometer (UV-2450, Shimadzu, Japan) was 200–800 nm with PBS (pH = 6.8) as reagent blank. Test results showed that the maximum absorption wavelength is 286 nm.

Table 2: Solubility of flucloxacillin sodium at different temperatures (phosphate buffer solution, pH 6.8)

T/K		293	298	303	308	313
Solubility (g/mL)	Form I	0.28	0.37	0.46	0.54	0.58
	Form II	0.08	0.22	0.33	0.46	0.56
	Form III	0.72	0.79	0.86	0.93	1
	Amorphism	0.58	0.71	0.84	0.96	1.09

A standard solution of 0.1 g/L∼0.5 g/L was prepared, whose absorbance was determined by UV spectrophotometry at 286 nm. The calibration curves were constructed and the equations of the calibration curves were y = 0.61301x-0.08546, r = 0.9989, where y is solubility of flucloxacillin sodium and x is absorbance.
The saturated solutions of each polymorphs of flucloxacillin sodium in phosphate buffer solution (pH = 6.8) were prepared at 20 °C, 25 °C, 30 °C, 35 °C and 40 °C, respectively. After filter of these saturated solution, absorbency of filtrate was measured and recorded at 286 nm by UV, the gained equilibrium solubility were calculated according to the standard curve which were shown in Table 2.

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