ELSEVIER

International Journal of Pharmaceutics 220 (2001) 33–41

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

# Microwave-enhanced dehydration and solvent washing purification of penicillin G sulfoxide

Xuejun Pan, Huizhou Liu *, Zhentao An, Jing Wang, Guoguang Niu

*Young Scientist Laboratory of Separation Science and Engineering, State Key Laboratory of Biochemical Engineering, Institute of Chemical Metallurgy, Chinese Academy of Sciences, Beijing 100080, People's Republic of China*

Received 8 September 2000; received in revised form 9 January 2001; accepted 28 February 2001

**Abstract**

In the present study, a new microwave-enhanced dehydration and solvent washing purification of penicillin G sulfoxide technique has been developed. The results show that microwave irradiation can dehydrate penicillin G sulfoxide from a water content of 14–26 to below 0.5% in 40–60 min in $N_2$ or air. After washing with ethyl acetate to remove impurities and residual water, the penicillin G sulfoxide can be used to synthesize cephalosporanic acid. The recovery of cephalosporanic acid was equal to and the purity of cephalosporanic acid was higher by 2% than that of the current dehydration technique. FTIR spectroscopy was used to study the process of microwave-enhanced dehydration and solvent washing purification of penicillin G sulfoxide. 

*Keywords:* Penicillin G sulfoxide; Dehydration; Microwave-enhanced; Washing; FTIR spectroscopy

## 1. Introduction

7-aminodesacetoxycephalosporanic acid (7-ADCA) is an important intermediate in the synthesis of cephalosporins. It is prepared by several step reactions including oxidation and ring-expansion from penicillin G (Chauvette et al., 1971; Chow et al., 1962; Harrison and Hodge, 1976; Koning et al., 1975; Morin et al., 1969). Penicillin G sulfoxide (Fig. 1) is the intermediate oxidized form from penicillin G (Koning et al., 1975).

The sulfoxide was centrifuged from water solution and the content of water was about 14–26%, in which about 9% were the crystalline-bound water, and the other 5–17% was free water. The ring-expansion reaction from penicillin G sulfoxide to cephalosporanic acid must be performed with anhydrous materials and solvents as the recovery and the purity of cephalosporanic acid are greatly influenced by water content and the purity of penicillin G sulfoxide. The water content must be lower than 0.2% and the purity of penicillin G sulfoxide be about 99% in the ring-expansion synthetic technique.

Penicillin G sulfoxide is easy to decompose in high temperature ($>60°C$) because it has an un-

* Corresponding author. Tel.: +86-10-62554264; fax: +86-10-62561822.

*E-mail address:* hzliu@home.icm.ac.cn (H. Liu).

0378-5173/01/$ - see front matter 
PII: S0378-5173(01)00647-0

saturated S→O bond (Fig. 1). It is very difficult to dehydrate crystalline-bound water from penicillin G sulfoxide at low temperature ( < 60°C). According to TGA analysis of sulfoxide (Fig. 2), the average dehydration temperature of free water was about 55.6°C, and the average dehydration temperature of crystalline-bound water (about 9.34%) was about 124.0°C. If the traditional heat conduction, heat convection and heat radiation were used for dehydration, penicillin G sulfoxide will decompose before dehydration.

In the current dehydration technique, the sulfoxide and solvent were mixed in high liquid/solid ratios (about 7:1–10:1) and were treated at vacuum for 15–20 h. Crystalline penicillin G sulfoxide can be obtained after the mixture was centrifuged. The water content of penicillin G sulfoxide is typically lower than 0.3% and the purity of penicillin G sulfoxide is about 99%. But the current dehydration technique needs a long time, has high solvent consumption, high cost of solvent recovery and low recovery of penicillin G sulfoxide. The yield of 7-ADCA was limited.

The heating pattern of sample with microwave energy will depend, in part, upon the dissipation factor of the sample (tan $\delta$). The dissipation factor is a ratio of the sample's dielectric loss or 'loss' factor ($\varepsilon''$) to its dielectric constant ($\varepsilon'$), $\tan\delta = \varepsilon''/\varepsilon'$ (Kingston and Jassie, 1988).

The rate at which the temperature will rise due to electromagnetic irradiation is determined by the following equation (Meek, 1987):

$$\frac{\delta T}{\delta t} = \frac{K\varepsilon'' f E_{\mathrm{rms}}^2}{\rho C_{\mathrm{p}}} \quad (1)$$

Where $E$ is the electric field intensity, $\rho$ is the density of the material, $C_{\mathrm{p}}$ is the specific heating capacity, and $f$ is the frequency of electromagnetic field in hertz.

This in situ mode of energy conversion depends only on the dielectric properties of the material. It allows the selection of target-specific molecules and deposition of the energy in the whole volume of the sample without the usual limitations of heat conduction and convection.

The dielectric constant ($\varepsilon'$) of water is 80 and dielectric loss or 'loss' factor ($\varepsilon''$) is about 0.2 (2450 MHz), therefore, microwave energy is readily absorbed. The dielectric constant ($\varepsilon'$) and dielectric loss or 'loss' factor ($\varepsilon''$) of penicillin G sulfoxide are very low, it absorbs little microwave energy compared with water. Water greatly absorbs microwave energy with resulting local superheating, promoting loss of crystalline-bound water. At the same time, short heating time is needed, the heat transfer is lessened from water vapor to penicillin G sulfoxide by conduction. If the water vapor and the residual heat can be removed rapidly, the temperature of penicillin G sulfoxide will not exceed 60°C. Decomposition of penicillin G sulfoxide is reduced.

## 2. Materials and methods

### 2.1. Materials

Sulfoxide:water content K.F. = 14–26%, about 1–3% of penicillin sulfones, penicillin and other impurities, provided by North China Pharmaceutical Corporation (NCPC); Anhydrous penicillin G sulfoxide:the content of penicillin G sulfoxide is about 99%, water content K.F. < 0.2%, provided by North China Pharmaceutical Corporation (NCPC); Ethyl acetate:analytical reagent.

### 2.2. Analysis methods

Water content of penicillin G sulfoxide and ethyl acetate were analyzed by Karl–Fischer titration method (Pande, 1974).

The purity of penicillin G sulfoxide and cephalosporanic acid were analyzed by high performance liquid chromatography (HPLC equipment, Waters). In the present work, HPLC analy-

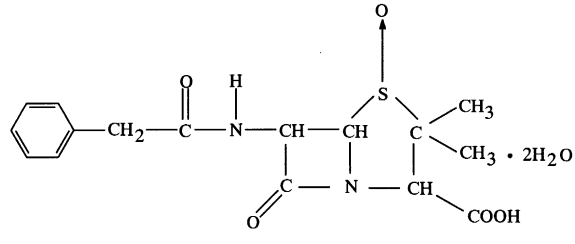


Fig. 1. Molecular structure of penicillin G sulfoxide including two molecular crystalline bound water.

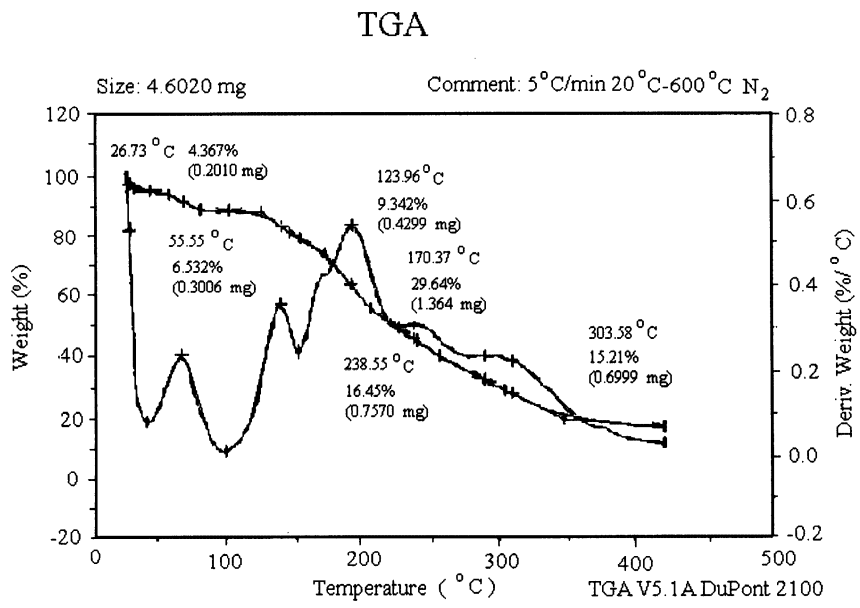


Fig. 2. TGA of sulfoxide.

sis was achieved on ODS-2S (5 μm, 4.6 × 200 mm, Du Pont) column and was eluted with $CH_3CN$-buffer solution (pH 6.0) (18:82, v/v) at UV 230 nm at a flow rate of 1.4 ml $min^{-1}$. Retention time was about 5 min.

In the present work, the purity of penicillin G sulfoxide or cephalosporanic acid was defined as follows:

$$\text{The purity of penicillin G sulfoxide or cephalosporanic (\%)} = \frac{\text{The content of penicillin G sulfoxide or cephalosporanic aicd analysis by HPLC}}{\text{1-water content of penicillin G sulfoxide or cephalosporanic ( K.F.\%)}}$$

### 2.3. Microwave-enhanced dehydration

A household microwave oven (full power 500 W) was modified in our laboratory with the addition of time controlling (Fig. 3). About 10 g of sulfoxide was scattered evenly on the bottom of glass bottle. The gas flux was about 0.3–0.4 $m^3$ $h^{-1}$ measured by flowmeter. The sulfoxide was irradiated under microwaves for the preset procedure (5 min power on for heating, 1 min power off for cooling), till the presetting microwave irradiation time.

### 2.4. Washing purification with ethyl acetate

About 20 g of penicillin G sulfoxide, after microwave-enhanced dehydration, and ethyl acetate were mixed and agitated for 30 min according

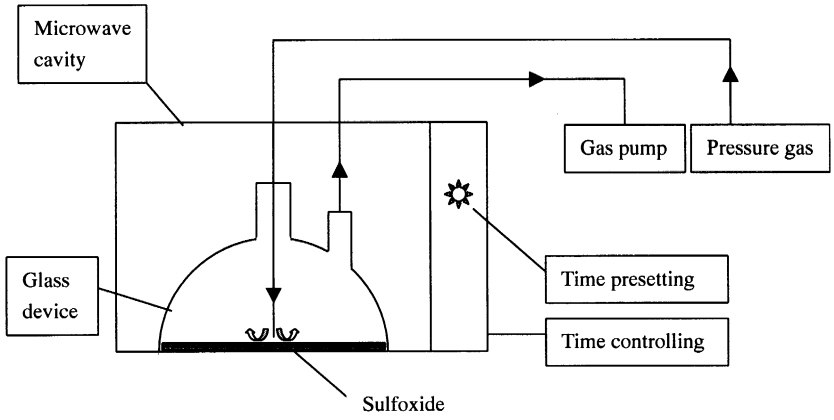


Fig. 3. Schematic diagram of the reactor for microwave-enhanced dehydration.

to different liquid/solid ratios, and then cooling for 15 min below 0°C. The penicillin G sulfoxide was separated from solvent.

### 2.5. FTIR measurements

FTIR spectra (KBr) with a resolution of 4 $cm^{-1}$ were recorded on a Bruker Vector 22 FTIR spectrometer.

## 3. Results and discussions

### 3.1. The results of microwave-enhanced dehydration from oxide

Figs. 4 and 5 show that water content of the sulfoxide was decreased and the percentage deweight of sulfoxide was increased with the increase of microwave irradiation (MR) time. Water content of the sulfoxide can reach about 0.5–0.2% in $N_2$ when microwave irradiation time was 50–60 min (Fig. 4). Water content of the sulfoxide can reach about 0.5% in air when microwave irradiation time was 40 min (Fig. 5).

The results of Table 1 show that water content of penicillin G sulfoxide can be decreased from 14–26 to below 0.4% after microwave irradiation for 60 min. The purity of penicillin G sulfoxide remains about 98%.

The penicillin G sulfoxide after microwave irradiation for 60 min (K.F. = 0.33%, the purity of penicillin G sulfoxide: 97.95%) was used to synthesize cephalosporanic acid. The recovery and purity of cephalosporanic acid were 83.15% (the current dehydration methodology: > 84%) and 93.86% (the current dehydration methodology: > 94%), respectively. They were little lower than that of the current dehydration technique.

### 3.2. The results of washing purification with ethyl acetate

There are penicillin sulfones, penicillin and other impurities in the sulfoxide. The impurities in penicillin G sulfoxide can not be removed by microwave-enhanced dehydration process. Except

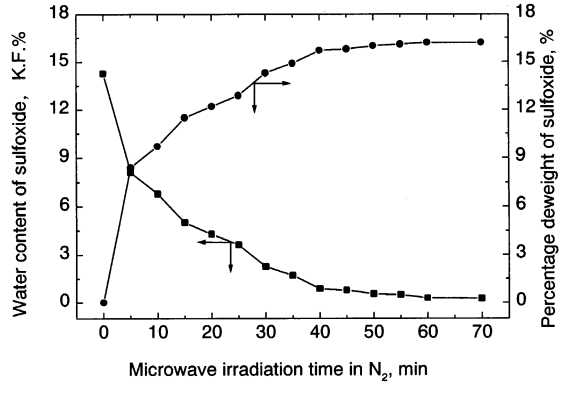


Fig. 4. The effect of microwave irradiation time on water content and percentage deweight of sulfoxide in $N_2$ (water content of sulfoxide: K.F. = 14.26%, sulfoxide: 10 g).

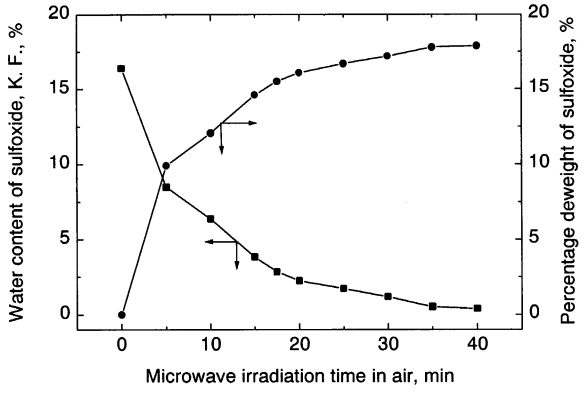


Fig. 5. The effect of microwave irradiation time on water content and percentage deweight of sulfoxide in air (water content of sulfoxide: K.F. = 15.65%, sulfoxide: 10 g).

water content, the content of impurities was another important factor influencing the recovery and the purity of cephalosporanic acid. If penicillin G sulfoxide was washed with ethyl acetate after microwave irradiation, most of penicillin sulfones and other impurities can be removed, increasing the purity of penicillin G sulfoxide.

After microwave irradiation, the penicillin G sulfoxide was mixed with ethyl acetate in low liquid/solid ratio and then re-crystallized in ethyl acetate. The residual water can be removed. The water content of the penicillin G sulfoxide can be decreased.

Fig. 6a and b, shows that water content in the sulfoxide after washing with ethyl acetate can be decreased from 0.6 or 1.0 to below 0.2% when the

Table 1
The results of microwave-enhanced dehydration from sulfoxide (sulfoxide: 10 g, microwave irradiation for 60 min in $N_2$)

| Before microwave irradiation | After microwave irradiation | |
|---|---|---|
| Water content, K.F. (%) | Water content, K.F. (%) | The purity of penicillin G sulfoxide (%) |
| 25.85 | 0.37 | 97.88 |
| 19.65 | 0.28 | 98.02 |
| 15.65 | 0.22 | 98.16 |
| 14.26 | 0.33 | 97.95 |

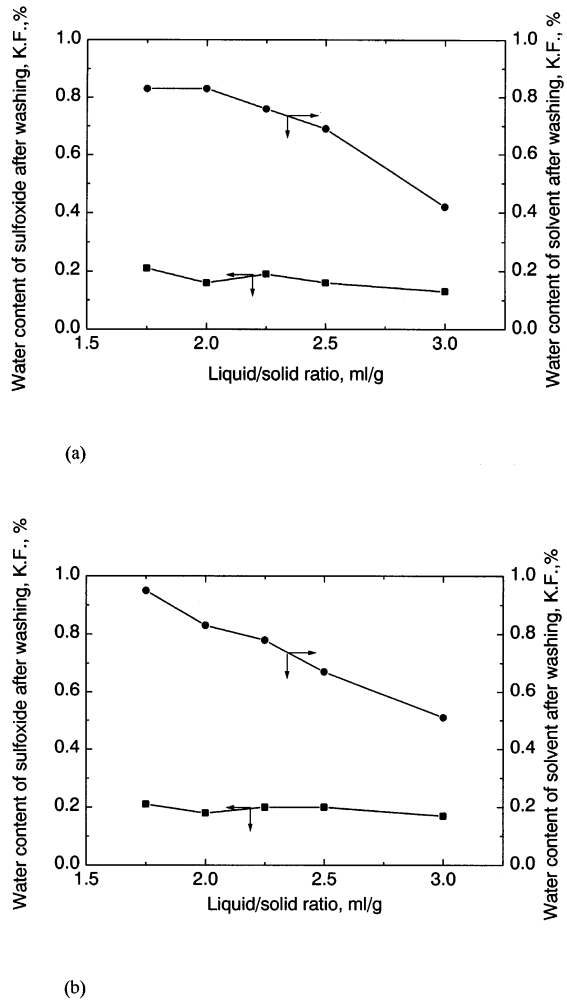


Fig. 6. The effect of washing liquid/solid ratio on water content of penicillin G sulfoxide and ethyl acetate after washing with solvent (sulfoxide: 20 g, agitation for 30 min and cooling for 15 min below 0°C). (a) Water content of sulfoxide before washing is 0.6% (K.F.). (b) Water content of sulfoxide before washing is 1.0% (K.F.).

washing liquid/solid ratios were 1.75:1–3:1. If the liquid/solid ratios were lower than 1.75:1, it was very difficult to be stirred and re-crystallized. If the liquid/solid ratios were higher than 3:1, the recovery of penicillin G sulfoxide was decreased. So the optimum liquid/solid ratio was 2:1.

Fig. 7 shows that when the water content of penicillin G sulfoxide before washing ranged from 0.38 to 4.79%, the remaining water content of penicillin G sulfoxide after washing with solvent

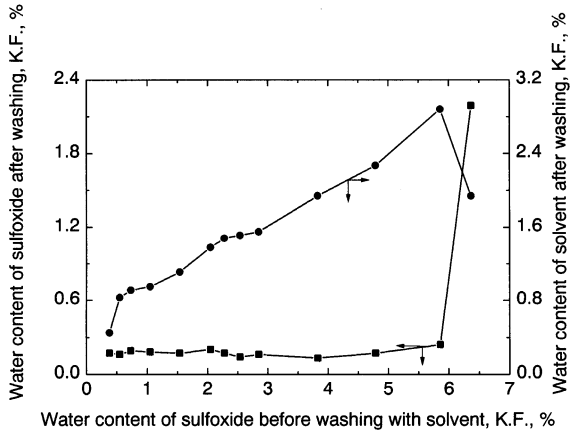


Fig. 7. The effect of water content of penicillin G sulfoxide before washing on water content of penicillin G sulfoxide and ethyl acetate after washing with solvent (sulfoxide: 20 g, liquid/solid: 2:1 ml/g, agitation for 30 min and cooling for 15 min below 0°C).

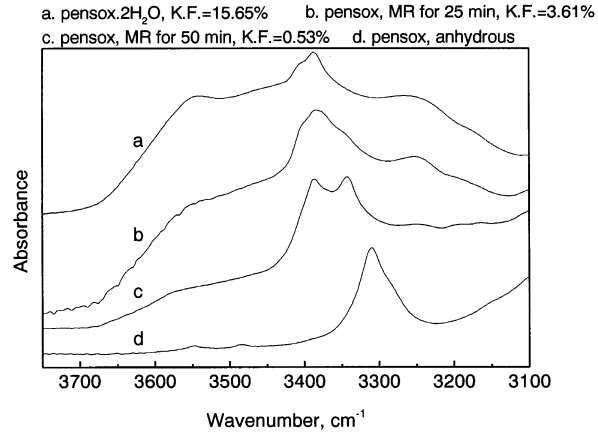


Fig. 8. FTIR spectra of penicillin G sulfoxide in 3750–3100 $cm^{-1}$ region before and after microwave irradiation.

was below 0.2%, and the water content of ethyl acetate increased from 0.47 to 2.27%. This confirmed that the residual water could be dissolved in ethyl acetate. When the water content of penicillin G sulfoxide before washing was higher than 5.86%, the water content of penicillin G sulfoxide after washing with solvent was obviously increased, the water content of ethyl acetate was decreased. The results show that water could not be completely removed from penicillin G sulfoxide when water content of penicillin G sulfoxide was higher than 5.86% before washing. The other experiments results show that the water content of penicillin G sulfoxide could not be dehydrated to below 0.2% if microwave irradiation was not used.

Table 2 shows that the water content of Penicillin G sulfoxide was decreased to below 0.2% and the purity of penicillin G sulfoxide can be increased about 1% and reached about 99% after washing with ethyl acetate.

Penicillin G sulfoxide, washed with ethyl acetate after microwave irradiation for 60 min (K.F. = 0.15%, the purity of penicillin G sulfoxide: 99.12%), was used to synthesize cephalosporanic acid. The recovery and purity of cephalosporanic acid were 84.23% (the current dehydration methodology: $>84\%$) and 96.17% (the current dehydration methodology: $>94\%$), respectively. The recovery of cephalosporanic acid was equal to and the purity of cephalosporanic

Table 2
The results of washing with ethyl acetate (sulfoxide: 20 g, liquid/solid: 2:1, agitation for 30 min and cooling for 15 min below 0°C)

| Before washing | | After washing | | The recovery of penicillin G sulfoxide (%) |
|---|---|---|---|---|
| Water content, K.F. (%) | Purity of penicillin G sulfoxide (%) | Water content K.F. (%) | Purity of penicillin G sulfoxide (%) | |
| 0.33 | 97.95 | 0.15 | 99.12 | 98.25 |
| 0.76 | 98.24 | 0.15 | 98.89 | 98.32 |
| 1.69 | 98.16 | 0.17 | 99.08 | 98.35 |
| 2.85 | 97.89 | 0.18 | 98.87 | 98.06 |
| 3.73 | 98.19 | 0.16 | 99.05 | 98.26 |

acid was higher by 2% than that of the current dehydration technique.

### 3.3. Study the process of microwave-enhanced dehydration and solvent washing purification of penicillin G sulfoxide by FTIR spectroscopy

Fig. 8 shows that absorption of water in infrared region was very strong, the absorption peaks of water were very clear in 3750–3100 $cm^{-1}$ region. These were obviously decreased with the increase of microwave irradiation time.

Fig. 9 shows that there was three absorption peaks in 3750–3100 $cm^{-1}$ region. One was in about 3550 $cm^{-1}$, presumably the free water absorption peak. The other was in about 3250 $cm^{-1}$, presumably the crystalline-bound water absorption peak. Those two peaks were all decreased with the increase of microwave irradiation time. When water content of sulfoxide was 15.65%, the ratio of peak area of free water and crystalline-bound water was 1.564. When the sulfoxide was irradiated under microwaves for 50 min, the water content of sulfoxide was 0.53%, the ratio of peak area of free water and crystalline-bound water was 0.436. It shows that the rate of dehydration of free water was faster than that of dehydration of crystalline-bound water. It also

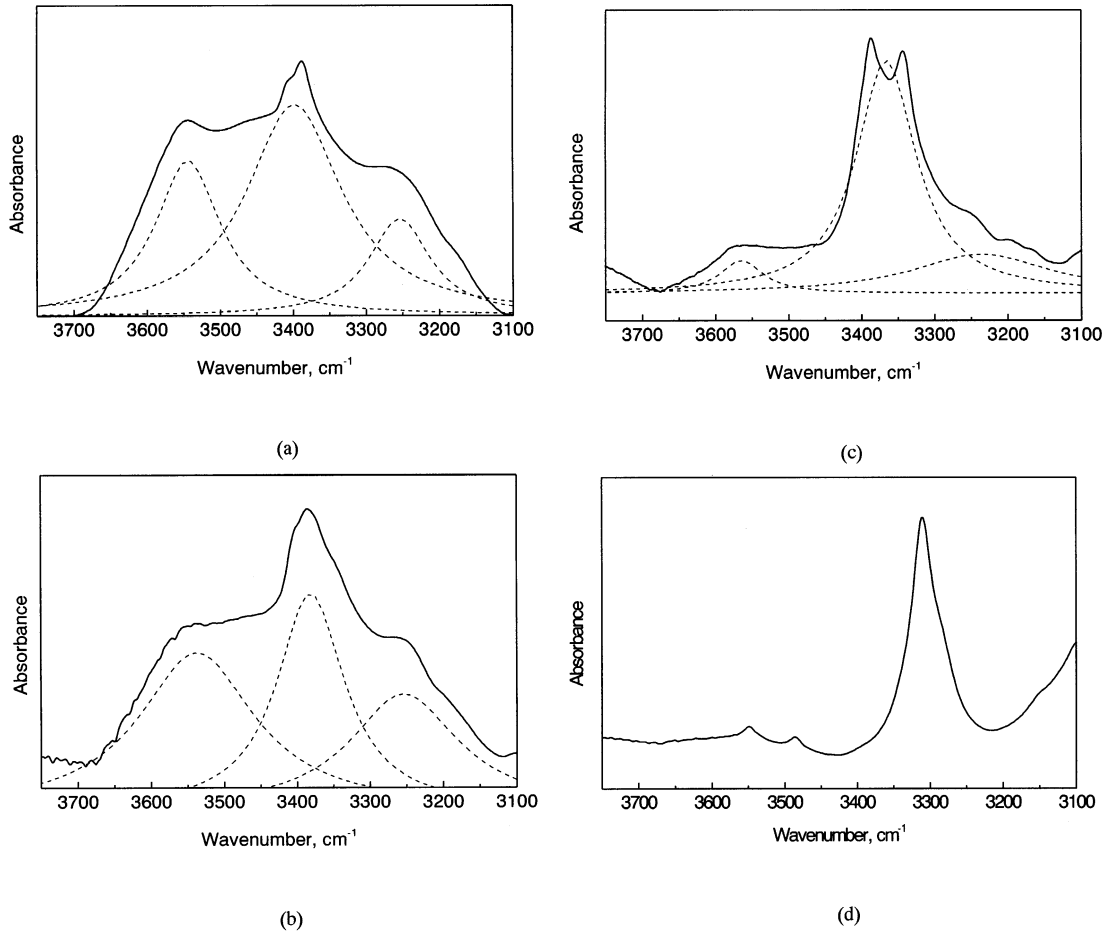


Fig. 9. FTIR spectra of sulfoxide in 3750–3100 $cm^{-1}$ region fitting multi-peaks with Lorentzian method. (a) Pensox · $2H_2O$, K.F. = 15.65%; (b) penicillin G sulfoxide, K.F. = 3.61%, microwave irradiation for 25 min; (c) penicillin G sulfoxide, K.F. = 0.53%, microwave irradiation for 50 min; (d) penicillin G sulfoxide, anhydrous.

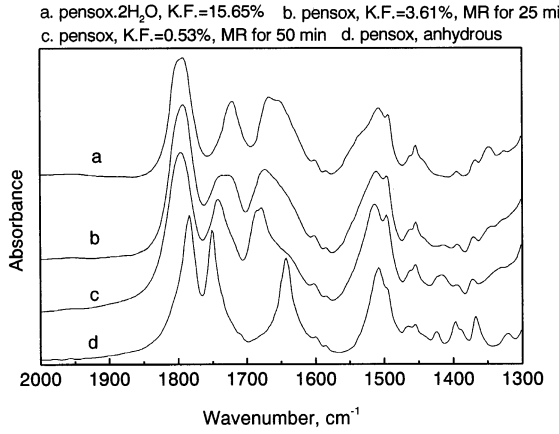


Fig. 10. FTIR spectra of penicillin G sulfoxide in 2000–1300 $cm^{-1}$ region before and after microwave irradiation.

shows that there was more crystalline-bound water was residual than free water at last. Fig. 9 also shows that absorption peak of acylamide I ( ) was shifted from a higher to lower wave number with the decrease of water content (K.F. = 15.65%, 3398.3 $cm^{-1}$; K.F. = 3.61%, 3380.5 $cm^{-1}$, K.F. = 0.53%, 3366.7 $cm^{-1}$; anhydrous, 3310 $cm^{-1}$).

Fig. 10 (line d) shows that absorption peak of the acylamide carbonyl ( ) was about 1643 $cm^{-1}$. The peak for the acid carbonyl ( ) was about 1747 $cm^{-1}$ and the peak for the lactum carbonyl ( ) was increased from 1775 to 1784 $cm^{-1}$ because of 4-member ring-strain. Due to hydrogen bonding with water, the stretching vibration frequency decreased and the bending vibration frequency increased. The characteristic peaks of penicillin G sulfoxide were all shifted in different degrees when dehydrated. When water content of penicillin G sulfoxide was 15.65%, three characteristic peaks of carbonyl were in 1667, 1727, 1793 $cm^{-1}$, respectively (Fig. 10, line a).

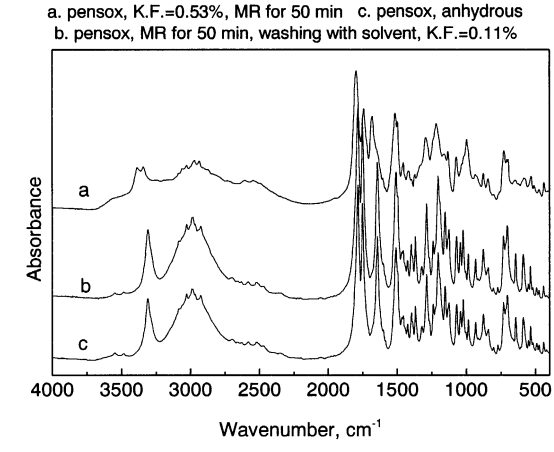


Fig. 11. FTIR spectra of sulfoxide in 4000–400 $cm^{-1}$ region before and after washing with ethyl acetate.

Fig. 11 (line a) shows that the spectra of penicillin G sulfoxide (K.F. = 0.53%) after microwave irradiation for 50 min were similar with that of anhydrous penicillin G sulfoxide. The spectra of penicillin G sulfoxide (K.F. = 0.11%) washing with solvent after microwave irradiation for 50 min were same as that of anhydrous penicillin G sulfoxide. It shows that penicillin G sulfoxide was not decomposed after microwave irradiation. This also had been demonstrated by synthetic conversion to cephalosporanic acid.

## 4. Conclusions

A new microwave-enhanced dehydration and solvent washing purification technique has been developed for purification of penicillin G sulfoxide. Microwave irradiation can reduce penicillin G sulfoxide water content from 14–26 to below 0.5% after 40–60 min in $N_2$ or air. After washing with ethyl acetate to remove impurities and residual water, the penicillin G sulfoxide could be used to synthesize cephalosporanic acid. The recovery of cephalosporanic acid was equal to and the purity of cephalosporanic acid was higher by 2% than that of the current dehydration technique. The new technique can reduce treating time (from 18–20 to 1–2 h), reduce solvent consumption (from liquid/solid 7:1–10:1 to 2:1), and little increase the recovery of penicillin G sulfoxide (from 97 to more than 98%).

FTIR spectroscopy studies confirmed water dehydration with the increase of microwave irradiation time. Absorption peaks of polar bond were shifted from high to low wave number with the decrease of water content. The peaks of penicillin G sulfoxide washing with solvent after microwave

irradiation for 50 min were same as that of anhydrous penicillin G sulfoxide, indicating that decomposition of penicillin G sulfoxide did not occur.

## Acknowledgements

This work was supported by National Natural Science Foundation of China (No. 2983 6130). The authors wish to thank Zhiyong Peng for analysis of purity of penicillin G sulfoxide and cephalosporanic acid by HPLC and synthesis of cephalosporanic acid.

## References

Chauvette, R.R., Pennington, P.A., Ryan, C.W., et al., 1971. Chemistry of cephalosporin antibiotics. XXI. Conversion of penicillin to cephalexin. J. Org. Chem. 36 (9), 1259–1267.

Chow, A.W., Hall, N.M., Hoover, J.R.E., 1962. Penicillin sulfoxides and sulfones. J. Org. Chem. 27 (9), 1381–1383.

Harrison, C.R., Hodge, P., 1976. Oxidation of some penicillin and other sulphides by use of a polymer-supported peroxy-acid. J. Chem. Soc. Perkin. Trans. I 21, 2252–2254.

Kingston, H.M., Jassie, L.B., 1988. Introduction to Microwave Sample Preparation: Theory and Practice. American Chemical Society, Washington, DC.

Koning, J.J.D., Kooreman, H.J., Tan, H.S., et al., 1975. One-step high yield conversion of penicillin sulfoxides to deacetoxycephalosporins. J. Org. Chem. 40 (9), 1346–1347.

Meek, T.T., 1987. Proposed model for the sintering of a dielectric in a microwave field. J. Mat. Sci. Lett. 6, 638–640.

Morin, R.B., Jackson, B.G., Mueller, R.A., et al., 1969. Chemistry of cephalosporin antibiotics. XV. Transformations of penicillin sulfoxide. A synthesis of cephalosporin compounds. J. Am. Chem. Soc. 91 (6), 1401–1407.

Pande, A., 1974. Handbook of Moisture Determination and Control, vol. 1. Marcel Dekker, p. 199.