REACTIVE EXTRACTION OF CEPHALOSPORIN C

TADASHI HANO, MICHIAKI MATSUMOTO, TAKAAKI OHTAKE AND FUMIAKI HORI

Department of Environmental Chemistry and Engineering, Oita University, Oita 870-11

Key Words: Extraction, Cephalosporin, Antibiotics, Quaternary Ammonium Salt, Equilibrium

The decomposition rate of cephalosporin C was measured in order to determine the stable operating conditions for extraction, and the same pH dependence as those reported in the past works was obtained. The physical extraction of cephalosporin C with butyl acetate did not occur since it was amphoteric and the fraction of undissociated form was very low.

The reactive extraction and strippting of cephalosporin C were studied by using various extractants and buffer solutions. The reactive extraction occurred when tri-*n*-octylmethylammonium chloride and carbonate buffer were used as an extractant and buffer, respectively. This was the first example of cephalosporin extraction. Cephalosporin C extracted into organic phase was stripped by acetate buffer solution. In the stripping, the anion exchange reaction between cephalosporin C and acetate occurred.

Introduction

Along with penicillins, cephalosporin derivatives are representative and important β-lactam antibiotics. Cephalosporin C, produced by *Cephalosporium acremonium*, has been recovered from fermentation broth by chromatographic or chemical processes[14]. The former process requires rather cumbersome and expensive plants. Therefore, it is meaningful to develop a simple technique of separation. Solvent extraction based on the distribution between aqueous and organic phases (henceforth physical extraction), which has been widely employed for the purification of lipophilic antibiotics such as penicillins, is regarded as one of the most promising alternatives. However, physical extraction is not used for the purification of cephalosporin because cephalosporin is amphoteric and is barely soluble in organic solvents. To establish a recovery system by extraction, Andrisano *et al.* proposed to render cephalosporin C more lipophilic by a chemical reaction in the broth and then to use physical extraction[1]. Such a chemical reaction method, however, has been applied only to a few cephalosporines because of the complicated processes involved.

Extraction accompanied with the reaction of antibiotics and extractant (henceforth reactive extraction) has been studied for penicillin G recovery[10,11,13]. This process is preferable since extraction is carried out at middle-range pH, where penicillin is relatively stable. In those studies, secondary long-chain alkyl amines were recommended as extractant due to appropriate extraction-stripping ability and no formation of a third phase. However, cephalosporin C cannot be extracted by secondary amines because it is amphoteric and the formation of an ionpair complex with amines is difficult. It was reported that quaternary ammonium salts could extract amino acids, which are representative amphoteric compounds, by anion-exchange reaction[2,6]. Therefore, the reactive extraction of amphoteric cephalosporin C is thought to occur in the same way by using quaternary ammonium salts. If a reactive extraction is established, it will become possible to apply liquid-membrane processes, which are considered to be well-suited for separation of unstable antibiotics like cephalosporins. Recovery of various bioproducts

* Received August 26, 1991. Correspondence concerning this article should be addressed to T. Hano. T. Ohtake is now at Kagoshima National College of Technology, Hayato, Kagoshima 899-51.

such as organic acids[3,7,12], amino acids[4,15] and penicillin G[5,8] from fermented broths by liquid-membrane processes have already been reported. This paper discusses the recovery of cephalosporin C by solvent extraction to obtain the basic information necessary for liquid-membrane separation.

1. Experimental

1.1 Reagents

Figure 1 shows the molecular structure of cephalosporin C (henceforth abbreviated as P). The values shown in the figure are the p*K*a values of carboxyl and amino groups. Tri-*n*-octylmethylammonium chloride (henceforth TOMAC and abbreviated as QC1) was mainly employed as extractant. Di-2-ethylhexylphosphoric acid (henceforth D2EHPA) and tri-*n*-octylamine (henceforth TOA) were commercial extractants used for comparison. Butyl acetate was used as organic solvent since it has been used for penicillin extraction.

1.2 Decomposition rate

The decomposition rate of cephalosporin C was measured in 2 mol/m^3 aqueous solution at 298 K. The pH was changed from 1 to 12 by acetate and carbonate buffers. Aqueous solution was sampled at proper time intervals. Cephalosporin C concentration in the sample was determined by HPLC using a C-18 column (Biofine RPCSC18, Japan Spectroscopic Co. Ltd.) and a mixture of 5 mol/m^3 aqueous ammonium carbonate solution and methanol as eluent.

1.3 Extraction equilibria

In the study of reactive extraction equilibrium, an organic solution was prepared by dissolving the extractant in butyl acetate. In physical extraction, the extractant was not added. Aqueous solution was prepared according to the same procedure as described for the measurement of decomposition rate. When TOMAC was used as extractant, a large excess of sodium chloride compared with cephalosporin was added to the aqueous solution to keep Cl^- concentration constant during the extraction. Aqueous and organic solutions of equal volumes (25 cm^3) were shaken in a flask at constant temperature (298 K) to attain equilibrium. After two hours the concentration of cephalosporin C in the aqueous solution was measured by HPLC. Stripping from organic to aqueous solutions was carried out with the aqueous acetate buffer solution containing sodium chloride. Concentrations of cephalosporin C and acetate in the stripping solution were measured by HPLC.

2. Results and Discussion

2.1 Decomposition rate

In previous studies[9,16] the decomposition rates of cephalosporin C in aqueous buffers were found to

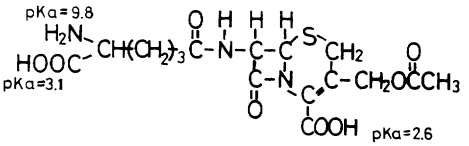

Fig. 1. Chemical formula of cephalosporin C

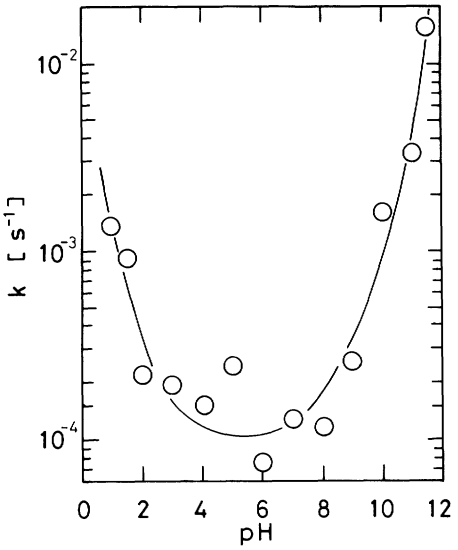

Fig. 2. Effect of pH on decomposition rate constant

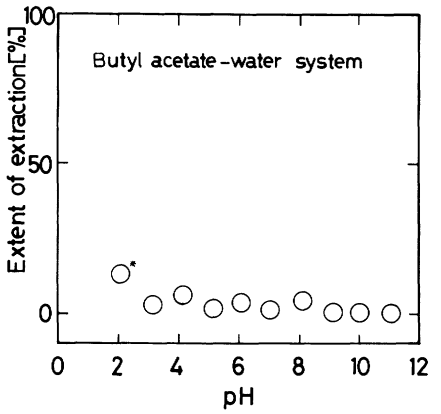

Fig. 3. Effect of pH on extent of extraction without extractant

obey first-order kinetics. **Figure 2** shows the effect of pH on the decomposition rate constant, *k*. In basic and acidic solutions the rate was relatively fast due to hydrolysis of the acetyl group. Such behavior agreed with that in previous works[9,16]. From Fig. 2, recovery in the middle pH range is desirable to reduce activity loss by decomposition.

2.2 Extraction

First, the physical extraction of cephalosporin C was examined using butyl acetate as a solvent. **Figure 3** shows the effect of pH on the extent of extraction. In this figure, the asterisk indicates that cephalosporin C decomposed after equilibration. Extraction did not occur because the fraction of

undissociated form of cephalosporin C was very low over the whole pH range due to its amphoteric properties.

Therefore, the possibility of reactive extraction was examined. TOMAC was employed as extractant since it could extract amino acids at high pH[2,6]. As a result of preliminary experiments, extraction was found to be possible when carbonate buffer (pH = 10) was used in aqueous cephalosporin solution. This is the first report of reactive extraction of cephalosporin C. The combination of TOMAC and phosphate buffer did not give a satisfactory result since phosphate is significantly extracted with TOMAC compared to carbonate[6]. Thereafter, extraction was performed by using TOMAC and carbonate buffer. **Figures 4** and **5** show the effects of extractant (QCl) and Cl^- concentrations on the distribution ratio, D, which is defined as the ratio of total concentration of cephalosporin C in the organic phase to that in the aqueous phase. During these experiments, cephalosporin C did not decompose despite the high pH (pH = 10). This finding indicates that the anion exchange reaction was relatively fast compared with decomposition. Under the conditions in Figs. 4 and 5, the maximum extent of extraction was about 80%. In both figures, the slopes of logarithmic plots of D versus [QCl] and [Cl^-] were 1 and -1, respectively. From *pKa* values, cephalosporin C exists mainly in the monoanionic form at pH = 10. Therefore, the extraction reaction is expressed as follows:

$$P^- + QCl \rightleftharpoons QP + Cl^- \quad : K \tag{1}$$

where K is the extraction equilibrium constant defined by Eq. (2).

$$K = \frac{[QP][Cl^-]}{[P^-][QCl]} \tag{2}$$

By rearranging Eq. (2), Eq. (3) is obtained.

$$\log D = \log K + \log[QCl]/[Cl^-] \tag{3}$$

where D ($=[QP]/[P^-]$) is the distribution ratio.

From the plots based on Eq. (3) shown in **Fig. 6**, the extraction equilibrium constant, K, was found to be 0.25. This value was higher than those for inorganic anions like phosphate, sulfate and carbonate which coexisted in the fermented broth[6]. Therefore, the selective extraction of cephalosporin C is possible.

2.3 Stripping

The stripping of cephalosporin C from organic to aqueous phases was carried out. The pH of the aqueous solution was adjusted by 100 mol/m^3 acetate buffer to pH = 4, where cephalosporin C is relatively stable as is shown in Fig. 2. **Figure 7** shows the effect of sodium chloride concentration on the extent of stripping, which was 60–70%. During the experiment, cephalosporin C could be recovered in strip-

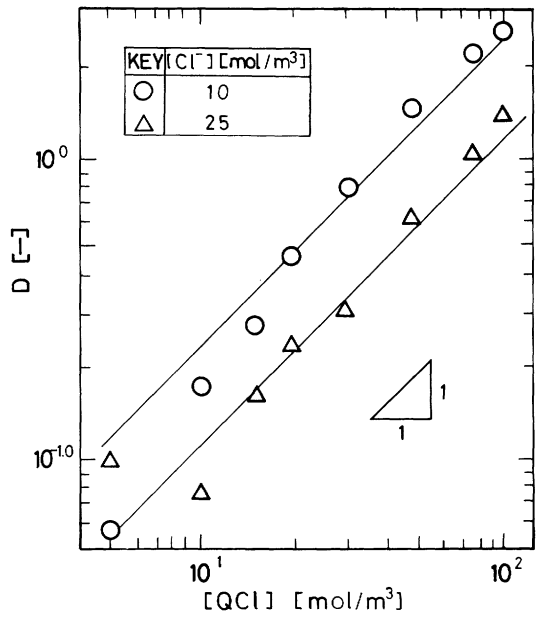

Fig. 4. Relationship between TOMAC concentration and distribution ratio

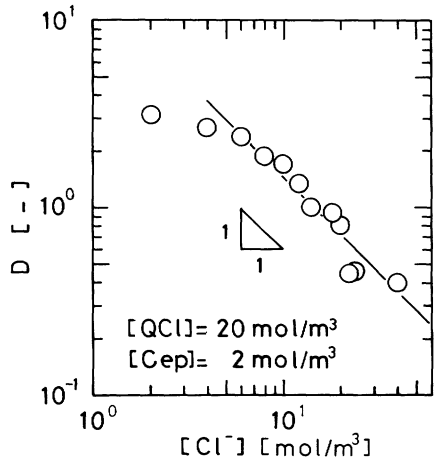

Fig. 5. Relationship between sodium chloride concentration and distribution ratio

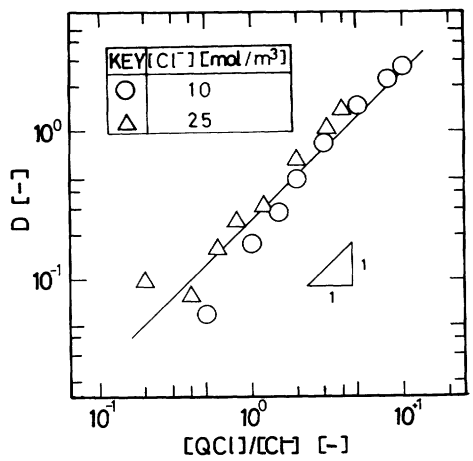

Fig. 6. Determination of extraction equilibrium constant

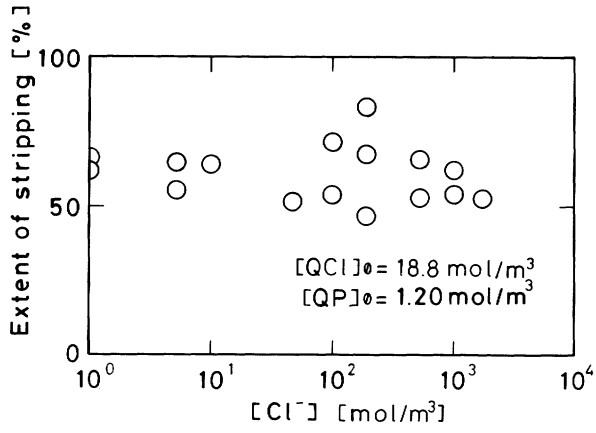

Fig. 7. Effect of sodium chloride concentration on extent of stripping

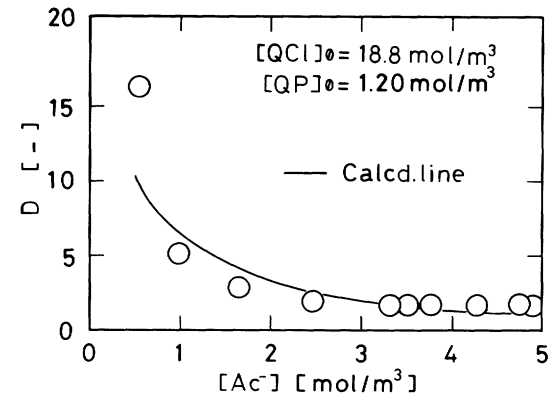

Fig. 8. Effect of acetate concentration on distribution ratio in stripping

ping solution without decomposition. This indicates that cephalosporin in the organic solution was more stable than that in the aqueous solution. According to Eq. (1), the extent of stripping should increase with sodium chloride concentration. Figure 7, however, indicates that stripping was independent of sodium chloride concentration. This discrepancy was thought to be caused by reaction between TOMAC and acetate anion used as a buffer. **Figure 8** shows the effect of acetate anion concentration on distribution ratio, D. As expected, D decreased with increasing acetate anion concentration.

To simulate the results shown in Fig. 8, the following anion exchange reaction was considered.

$$Ac^- + QCl \rightleftharpoons QAc + Cl^- \quad : K_A \tag{4}$$

where K_A is the extraction equilibrium constant of Eq. (4). From Eqs. (1) and (4) and the mass balance of acetate, the distribution ratio of cephalosporin C was derived as follows.

$$D = \frac{K([Ac]_0 - ((1+K_d)[H^+]/K_a + 1)[Ac^-])}{K_A[Ac^-]} \tag{5}$$

where K_d and K_a are the distribution equailibrium constant and dissociation constant of acetic acid, respectively. The value of K_d was determined by another experiment to be 0.3. From the experimental results shown in Fig. 8 and Eq. (5), the value of K_A was determined to be 0.045. The solid line in Fig. 8 is the calculated result using these values. The experimental data agreed well with calculated lines.

Conclusion

Extraction and stripping of cephalosporin C was carried out by using various extractants and buffer solutions. Reactive extraction occurred with a combination of TOMAC and carbonate buffer, and was the first example of reactive extraction of cephalosporin. Cephalosporin C in organic phase could be stripped in acetate buffer solution. In stripping, anion exchange reaction between cephalosporin C and acetate occurred. In neither extraction nor stripping did cephalosporin C decompose.

Acknowledgment

The authors express their thanks to Mr. H. Moriyama and Mr. K. Yamamoto for their valuable experimental assistance. The present work was supported by a Grant-in-Aid for General Scientific Research (03650787) from the Ministry of Education, Science and Culture of Japan.

Nomenclature

D	=	distribution ratio	[—]
K	=	extraction equilibrium constant of cephalosporin C defined by Eq. (2)	[—]
K_A	=	extraction equilibrium constant of acetate defined by Eq. (4)	[—]
K_a	=	acid dissociation constant	[mol/dm³]
K_d	=	distribution equilibrium constant	[—]
k	=	decomposition rate constant	[s⁻¹]
[ ]	=	concentration	[mol/m³]

〈Subscript〉

0	=	initial state

Literature Cited

1) Andrisano, R., G. Guerra and G. Mascellani: *J. Appl. Chem. Biotechnol.*, **26**, 459 (1976).
2) Behr, J. P. and J. M. Lehn: *J. Am. Chem. Soc.*, **95**, 6108 (1975).
3) Friesen, D. T., W. C. Babcock, D. J. Brose and A. R. Chambers: *J. Membr. Sci.*, **56**, 127 (1991).
4) Hano, T., T. Ohtake, M. Matsumoto, F. Hori and F. Nakashio, Proc. Asia-Pacific Biochem. Eng. Conf. 1990, p. 428 (1990).
5) Hano, T., T. Ohtake, M. Matsumoto, S. Ogawa and F. Hori: *J. Chem. Eng. Japan*, **23**, 772 (1990).
6) Hano, T., T. Ohtake, M. Matsumoto, D. Kitayama, F. Hori and F. Nakashio: *J. Chem. Eng. Japan*, **24**, 20 (1991).
7) Hano, T., M. Matsumoto, T. Ohtake, K. Sasaki, F. Hori and Y. Kawano, Proc. Int. Solv. Extr. Conf. 1990 in press.
8) Hano, T., M. Matsumoto, T. Ohtake and F. Hori, Proc. China-Japan Chem. Eng. Conf. 1991, p. 529 (1991).
9) Konecny, J., E. Felber and J. Gruner: *J. Antibiotics*, **26**, 135

(1973).
10) Likidis, Z., E. Schlichting, L. Bishoff and K. Schügerl: *Biotechnol. Bioeng.*, **33**, 1385 (1989).
11) Modin, R. and M. Schroder-Nielsen, *Acta Pharm. Suec.*, **8**, 573 (1971).
12) Nuchonoi, P., I. Izawa, N. Nishio and S. Nagai: *J. Ferment. Technol.*, **65**, 669 (1987).
13) Reschke, M. and K. Schügerl: *Chem. Eng. J.*, **28B**, 11 (1984).
14) Ridgway, K. and E. E. Thorpe, "Handbook of Solvent Extraction", Chap. 19, p. 583, John Wiley and Sons, New York, 1983.
15) Thien, M. P. and T. A. Hatton: *Separ. Sci. Technol.*, **23**, 819 (1988).
16) Yamana, T. and A. Tsuji: *J. Pharm. Sci.*, **65**, 1563 (1976).

(Presented at 56th Annual Meeting of The Society of Chemical Engineers, Japan, at Tokyo, March 1991)