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SEPARATION OF HYDROCORTISONE AND ITS STEROISOMER FROM FERMENTATION LIQUOR BY CHLOROFORM EXTRACTION PROCESS

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ABSTRACT

The distribution of hydrocortisone and its steroisomer, epi-hydrocortisone, in chloroform/fermentation liquor and chloroform/water systems was studied experimentally. The distribution ratio of hydrocortisone was 6.2 and that of epi-hydrocortisone was 2.0 at 25°C; the separation factor was 3.1. The distribution ratios of hydrocortisone and epi-hydrocortisone increased with increasing temperature, but the separation factor was almost unchanged. A chloroform extraction process for downstream separation of hydrocortisone and epi-hydrocortisone was then developed using these results. The hydrocortisone and epi-hydrocortisone in the fermentation liquor were extracted into chloroform, then both were separated by a fractional scrubbing process. The recovery of hydrocortisone and epi-hydrocortisone through the extraction process was 95% with a flow ratio of 1.1 and 6 stages. The hydrocortisone content after scrubbing was over 98% and the hydrocortisone recovery in the scrubbing process was over 95%

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with a feed:water:chloroform flow ratio of 1:5:1.1 using 10–stage scrubbing and 10–stage re-extraction processes.

Key Words: Hydrocortisone; Epi-hydrocortisone; Steroisomer; Solvent extraction.

INTRODUCTION

Hydrocortisone ($C_{21}H_{30}O_5$) is an important hormone drug usually used in the therapy for Addison's disease or chronic adrenocortical insufficiency and in treatment of various skin disorders. The annual output of hydrocortisone in China is much higher than other hormone drugs. In pharmaceutical engineering, a microbiological fermentation process is used to produce hydrocortisone from the Reichstein's compound S ($C_{21}H_{29}O_4$), 11-desoxy-17-hydroxy-corticosterone, which is hydroxylated at carbon 11 of the steroid nucleus by enzymic synthesis of *Absidia orchidis* (1). Because the reaction selectivity is not very high, epi-hydrocortisone, a steroisomer of hydrocortisone, and other by-products also appear in the process. The only difference between hydrocortisone and epi-hydrocortisone is that the hydroxyl group at carbon 11 has the opposite orientation. The hydroxyl group of hydrocortisone is located above the steroid nucleus plane, but that of epi-hydrocortisone is below. Hydrocortisone and epi-hydrocortisone are optical isomers with different specific rotatory powers. Epi-hydrocortisone can be used for synthesis of cortisone and other steroid hormones, but it is less pharmaceutically active. The commercially used separation process is as follows (2):

1. The hydrocortisone and epi-hydrocortisone are extracted by butyl acetate from the fermentation liquor, then the loaded organic phase is evaporated to obtain the crude product containing hydrocortisone, epi-hydrocortisone, and other impurities.
2. The hydrocortisone in the crude product is recrystallized with 1,2-dichloroethane/ethanol, while the epi-hydrocortisone is retained in the mother solution.

Because the recrystallization is a heating–cooling process and the solid–liquid separation has to be repeated many times, the process has some drawbacks: lower yield, higher energy consumption, and batch operation.

The recent work of Tang et al. showed that the solubility of hydrocortisone in chloroform is higher than that of epi-hydrocortisone and their distributions in chloroform/water can be predicted from the solubility data (3). Preliminary tests showed that hydrocortisone and epi-hydrocortisone extracted by chloroform can be separated by multistage cross–flow scrubbing (4).

The aim of this work is to develop a chloroform extraction process for the downstream separation of hydrocortisone and epi-hydrocortisone to be used in place of recrystallization to improve the downstream separation process.

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EXPERIMENTAL

Materials

The fermentation liquor was supplied by the Shandong Xinhua Pharmaceutical Company, Ltd., China. The total content of hydrocortisone, epi-hydrocortisone, and other steroid by-products was 1.7–2.8 g/L. The ratio of hydrocortisone to epi-hydrocortisone was about 3.5:1. The composition of the fermentation liquor is given in Table 1.

The chloroform and butyl acetate were AR-grade reagents produced by the Beijing Chemical Corp. Deionized water was used for scrubbing the loaded chloroform.

Apparatus

The phase equilibrium experiment for the solvent extraction was conducted in a 50-mL test tube with magnetic stirring in a thermostat bath having a temperature fluctuation of ±0.5° C. A Shimadzu–RC 3A HPLC chromatograph was used for the composition analysis of each phase.

Procedure

In the phase equilibrium test, the two phases with a specified volume ratio were stirred for 10 min to completely mix them; they were then separated by centrifugation. The hydrocortisone and epi-hydrocortisone contents in each phase were determined using HPLC with an ODS Hypersil–C18 reversed phase column using the following conditions: mobile phase water/methanol (35:65 v/v), pressure 166 bar, flow rate 0.7 mL/min, a UV detector set at 254 nm.

Table 1. The Fermentation Liquor Composition (wt%)

Hydrocortisone	0.13–0.20
Epi-hydrocortisone	0.37–0.57
Glucose	1.05
Corn syrup	1.25
Yeast	0.23
Ethanol	4.5–5.0 vol%
Ammonium sulfate	0.50
pH	6.4–6.7

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A batch stagewise test was used to simulate the continuous countercurrent process. Before the test, a simulation calculation of the separation process was conducted to determine the optimum flow ratio and equilibrium stage number based on the data obtained in the distribution equilibrium test for the hydrocortisone and epi-hydrocortisone in the chloroform/water system. The optimum results were then evaluated by the batch stagewise test. Each test tube simulated a shake-out unit in the stagewise phase equilibrium.

The Janecke diagram triangular series was used for simulating the countercurrent extraction for simple-solvent extraction or dual-solvent extraction using a series of batch equilibrium tests. For a sufficient number of transfer rows, the phase equilibrium in each test tube asymptotically approached the continuous countercurrent extraction process (5,6). If the concentration of each stage remained unchanged with further transfer operations, the simulation test was terminated.

RESULTS AND DISCUSSION

Extraction of Steroids in a Chloroform/Fermentation Liquor System

Hydrocortisone, epi-hydrocortisone, and other steroid by-products in the fermentation liquor can be extracted simultaneously by chloroform in order to separate them from other impurity components retained in the raffinate. In the distribution equilibrium diagram, Fig. 1, x and y are the total steroid concentrations including hydrocortisone, epi-hydrocortisone, and by-products in the aqueous phase and the organic phase, respectively. The apparent distribution ratio decreased sharply at lower concentrations, which meant that more stages were needed for recovery of hydrocortisone and epi-hydrocortisone. The apparent distribution equilibrium in the chloroform/fermentation liquor system at 25°C can be described by Eq. (1).

$$y = 0.0045x^2 + 0.20x \tag{1}$$

The mean deviation between the curve–fitting and the experimental concentration is 7.3%.

Besides the apparent distribution ratio of the total steroid concentration in the fermentation liquor, the distribution ratios of hydrocortisone, epi-hydrocortisone, and steroid by-products in the fermentation liquor were also determined; they are shown in Fig. 2. The hydrocortisone distribution ratio was higher than that of epi-hydrocortisone and the steroid by-products. The hydrocortisone distribution ratio decreased when the hydrocortisone concentration in the aqueous phase was less than 0.4 g/L, while the epi-hydrocortisone and steroid by-product distribution ratios were essentially unchanged. The decrease of the hydrocortisone distribution ratio probably resulted from the presence of 5 vol% ethanol in the fer-

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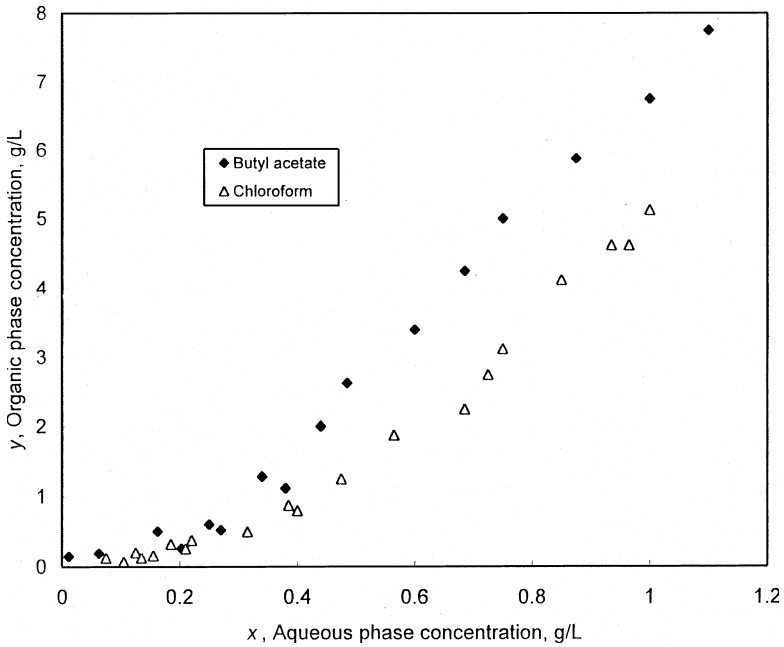

Figure 1. Distribution of steroids in chloroform/fermentation liquor and butyl acetate/fermentation liquor systems at 25°C.

mentation liquor. In the chloroform extraction process, the 5 vol% ethanol was mainly retained in the aqueous phase based on the phase equilibrium data for the ethanol/chloroform/water system (7). The solubility of hydrocortisone in ethanol is much higher than that in water. The effect of the 5 vol % ethanol on the hydrocortisone distribution ratio would increase significantly at lower hydrocortisone concentrations.

The apparent distributions at 25 and 35°C for the chloroform/fermentation liquor system are given in Fig. 3. The apparent distribution increased at the higher temperature, but increasing the distribution ratio by increasing the temperature would not be suitable, because chloroform, with its lower boiling point, is more volatile.

Distribution of Hydrocortisone and Epi-hydrocortisone in the Chloroform/Water System

The distributions of hydrocortisone and epi-hydrocortisone in the chloroform/water system were determined in two ways. One method was to extract the aqueous solution of hydrocortisone or epi-hydrocortisone by chloroform without

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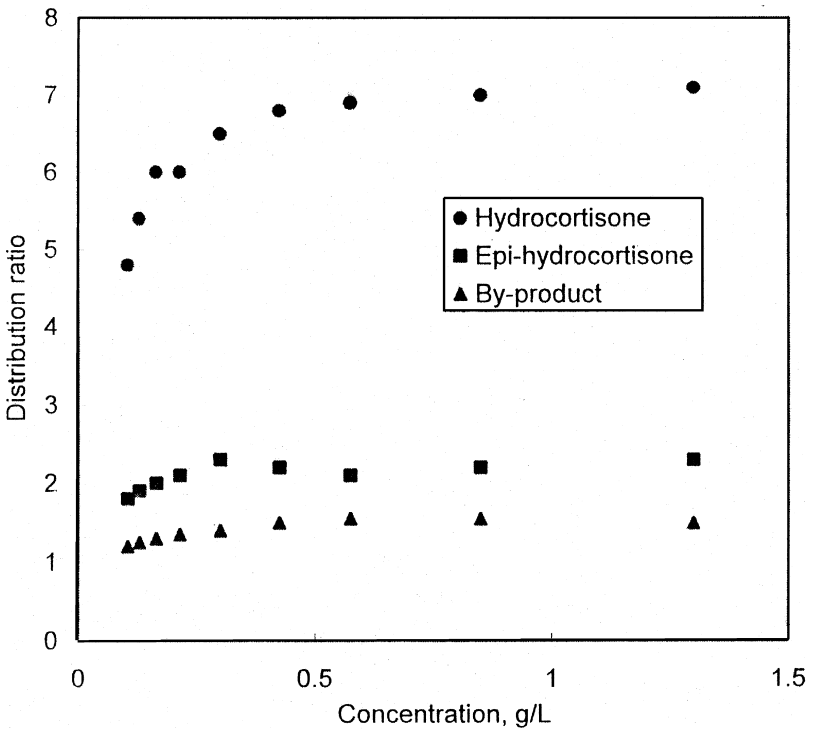

Figure 2. The distribution of hydrocortisone, epi-hydrocortisone, and by-products in a chloroform/fermentation liquor system at 25°C.

any solute. The other method was to strip the chloroform previously loaded with hydrocortisone or epi-hydrocortisone by water. The results for the two methods, seen in Fig.4, show that all the data fell on the same straight line. The distribution ratios for hydrocortisone and epi-hydrocortisone at 25°C were 6.2 and 2.0, respectively, so the separation factor was 3.1. Experimental results also showed that the distribution ratios of hydrocortisone and epi-hydrocortisone increased with increasing temperature, but the separation factor was almost unchanged, as shown in Table 2. The results revealed that the steroisomers could be separated by scrubbing the chloroform loaded with hydrocortisone and epi-hydrocortisone with water. The distribution behavior in the chloroform/water system could be used to design a scrubbing process for the downstream separation of hydrocortisone and epi-hydrocortisone.

The addition of some ammonium sulfate into the aqueous phase significantly increased the distribution ratio, seen in Table 3. The hydrocortisone distribution ratio with 2.5% ammonium sulfate in the aqueous phase was 48% higher than that with no ammonium sulfate in the aqueous phase. In general, the salt-

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ing–out effect resulted from the reduced water activity due to the salt addition. In addition, the strong electrolyte reduced the extractant solubility in the aqueous phase, which decreased solvent losses. But the salt is difficult to recover and reuse, so commercial application of the salting–out effect would be limited (8).

Comparison with Butyl Acetate Extraction

The apparent distributions of hydrocortisone, epi-hydrocortisone, and other steroid by-products were also measured in butyl acetate/fermentation liquor; the results are given in Fig. 1. The distributions were slightly higher than that for extraction with chloroform. This drawback can be reduced by adjusting the flow ratio and the number of stages. The lower chloroform viscosity and the higher density difference between chloroform and water will favor the throughput and mass transfer in an extraction column (9). Because hydrocortisone is thermosensitive, evaporation of the loaded butyl acetate would require a vacuum system to lower the boiling point, which would be unnecessary with chloroform because it has a lower boiling point. Therefore, the energy consumption would be less with chlo-

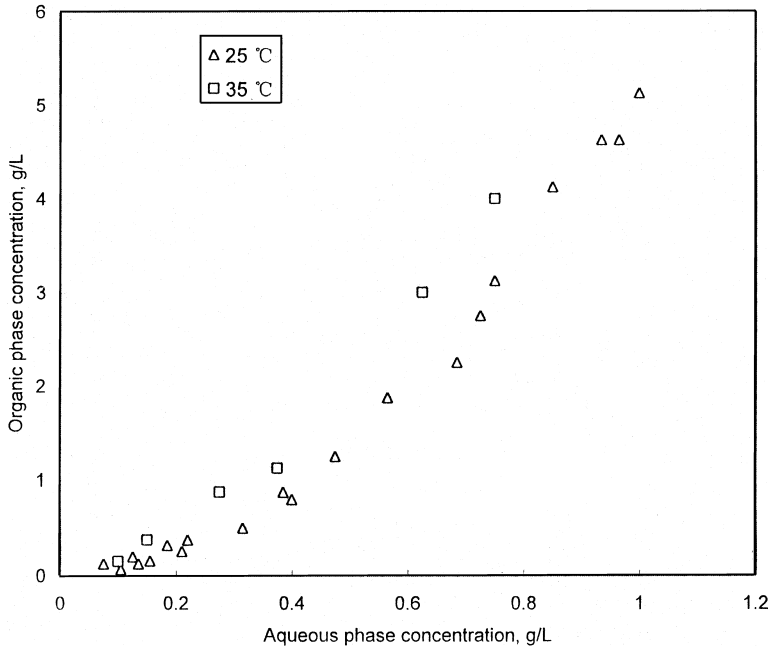

Figure 3. The effect of temperature on the distribution of steroids in a chloroform/fermentation liquor system.

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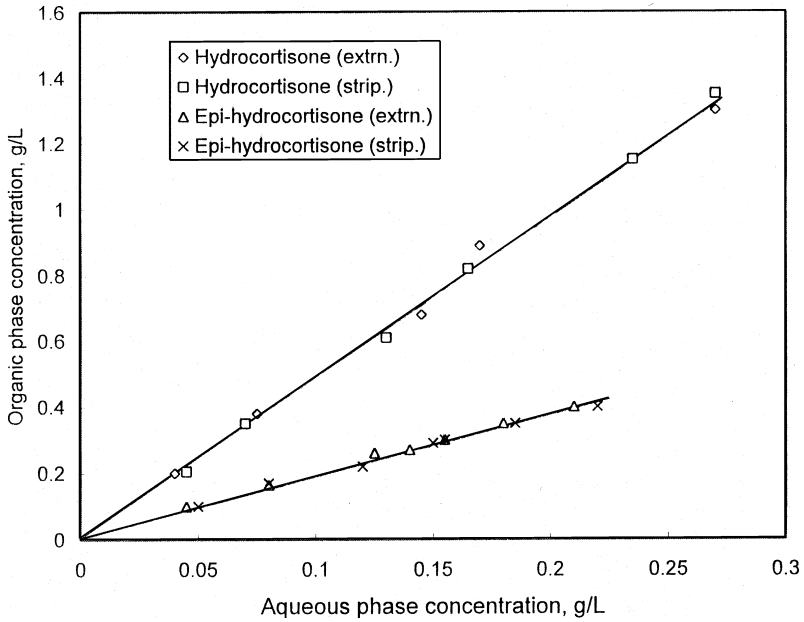

Figure 4. The distribution of hydrocortisone and epi-hydrocortisone in a chloroform/water system.

Table 2. The Effect of Temperature on the Distribution of Hydrocortisone and Epi-hydrocortisone in a Chloroform/Water System

Temperature (°C)	Distribution Ratio of Hydrocortisone	Distribution Ratio of Epi-hydrocortisone	Separation Factor
17	5.7	1.9	3.0
25	6.2	2.0	3.1
35	10.0	3.2	3.1

Table 3. The Effect of Ammonium Sulfate on the Distribution of Hydrocortisone and Epi-hydrocortisone in a Chloroform/Water System at 25°C

Ammonium Sulfate Content in Water (wt%)	Distribution Ratio of Hydrocortisone	Distribution Ratio of Epi-hydrocortisone	Separation Factor
0	6.2	2.0	3.1
0.8	7.3	2.2	3.2
2.5	9.3	2.9	3.2

roform extraction. The chloroform, with a higher density than water, can be covered with water in an extraction column to decrease the volatile losses and improve the worksite atmosphere without the fumes resulting from butyl acetate. Furthermore, the chloroform recovery process in raffinate presented by Hu could be used to further decrease the solvent loss in the extraction process (10). Therefore, chloroform is suitable for commercial application.

Separation Process Design

The separation process was designed with extraction and scrubbing steps, as shown in Fig. 5. In the extraction step, the fermentation liquor was contacted with chloroform, so that the hydrocortisone and epi-hydrocortisone entered the organic phase and separated from the other impurities in the fermentation liquor.

In the scrubbing step, the loaded organic phase was contacted with deionized water, so that hydrocortisone was retained in the organic phase and epi-hydrocortisone was scrubbed to the aqueous phase using a suitable solvent-to-water flow ratio. The hydrocortisone was recovered from the scrubbing water with a scrubbing process designed as a fractional extraction process with organic phase feeding. At the purification section outlet, the purified hydrocortisone was obtained by countercurrent scrubbing. In the recovery section, the hydrocortisone scrubbed into the aqueous phase was re-extracted into the organic phase with fresh chloroform.

The separation process was optimized based on the following assumptions:

1. The hydrocortisone and epi-hydrocortisone recovery from the fermentation liquor for extraction was over 95%.
2. The hydrocortisone content in the organic phase at the purification section outlet was over 98%.
3. The hydrocortisone content in the aqueous phase at the recovery section outlet was less than 5%.

Using a complex algorithm, the optimization balanced the number of stages and the flow ratios of feed, scrubbing water, and solvent used for re-extraction. The optimized system included extraction, scrubbing, solvent regeneration, and solvent recovery processes. Minimizing the cost of the equipment and the operation was the functional objective. The boundary condition was the limit flow ratio for the desired recovery and purification. The optimization procedure was as follows:

1. Input a set of initial data for output, feed composition, specified separation and recovery, and equilibrium relations, then select a flow ratio to iteratively calculate the number of stages.
2. For this flow ratio and number of stages, calculate the corresponding cost function including extractor investment and operating cost, especially the cost of the solvent recovery and losses.

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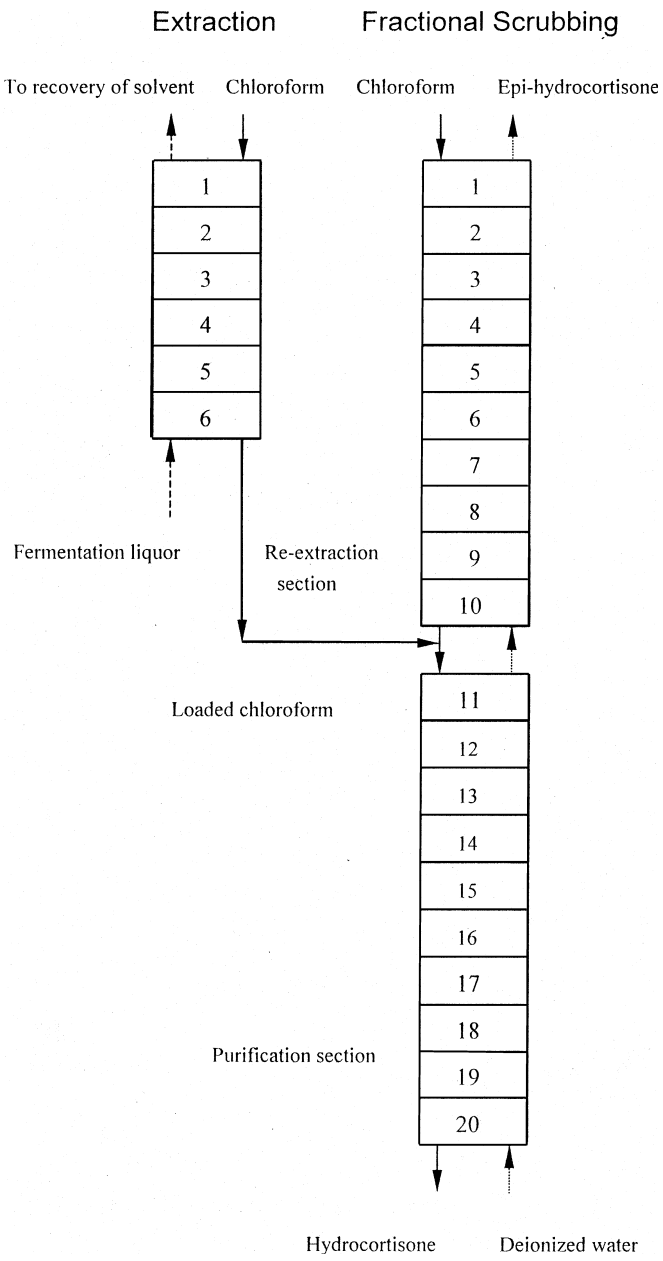

Figure 5. Schematic diagram of the downstream separation of hydrocortisone and epi-hydrocortisone by the chloroform extraction process.

3. Select another flow ratio and repeat steps (1) and (2) using a multivariable, nonlinear system to search for the optimum flow ratio and number of stages to minimize the total cost. The optimum results are given in Table 4 (11).

Stagewise Test

The Janecke diagram mode shows that the extraction of hydrocortisone and epi-hydrocortisone from the fermentation liquor was a single–solvent extraction process with 6 stages. A 27–row transfer process was needed for a flow ratio of 1.1 to recover 95%. The scrubbing process for the separation of hydrocortisone and epi-hydrocortisone was a chloroform–water, dual-solvent, fractional extraction process with 10–stage purification and 10-stage recovery sections. A 90–row transfer process was needed to satisfy the purification and recovery requirement for the optimum flow ratio.

Extraction

The concentration profiles in the extraction process are plotted in Fig. 6 after the 27–row transfer for 6 stages and O/A = 1.1, with an initial concentration of 2.77 g/L. The profiles are the total concentrations of hydrocortisone, epi-hydrocortisone, and the steroid by-products. If the output concentration of the fermentation liquor was decreased to 0.10 g/L, the corresponding recovery was 96%. The determined concentration profiles were in good agreement with the data predicted by Eq. (1).

Because the hydrocortisone distribution ratio was higher than that of epi-hydrocortisone and the by-products, the hydrocortisone concentration sharply de-

Table 4. Optimum Flow Ratio and Number of Stages

Extraction	
Chloroform: Fermentation liquor	1.1
Number of stages	6
Recovery	95%
Scrubbing	
Feed:water:fresh chloroform flow ratio	1:5:1.1
Number of stages	
Purification section	10
Re-extraction section	10
Purification of hydrocortisone	98%
Recovery of hydrocortisone	95%

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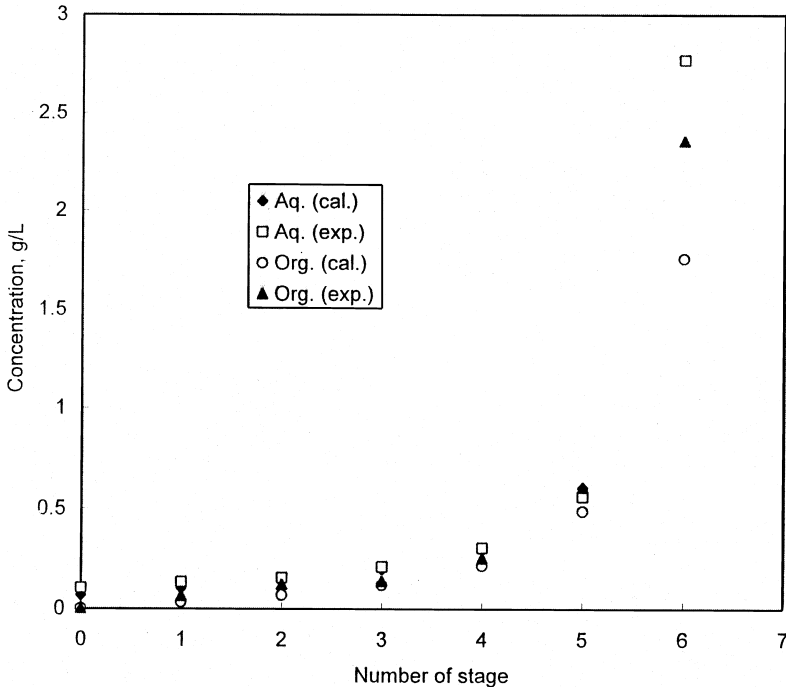

Figure 6. Concentration profiles of steroids for the extraction process.

creased along the direction of the aqueous phase. More stages would be needed to increase the hydrocortisone recovery, but the by-product impurities would also be extracted simultaneously into the organic phase in the stages near the outlet. If a much higher recovery was desired, more by-product impurities would be extracted into the organic phase, which would make the process design very difficult.

Scrubbing

The hydrocortisone and epi-hydrocortisone concentration profiles in the organic and aqueous phases after the 90–row transfer are given in Figs. 7 and 8. The hydrocortisone concentration in the loaded organic phase as feed for the scrubbing process was 2 g/L and the feed, scrubbing water, and fresh chloroform flow ratio was 1:5:1.1. The hydrocortisone to epi-hydrocortisone concentration ratio at the purification section outlet was 99, which was higher than the design value. The hydrocortisone concentration at the recovery section outlet was 25 mg/L for a recovery of 99%. Therefore, the hydrocortisone and epi-hydrocortisone separation was completed using the scrubbing process.

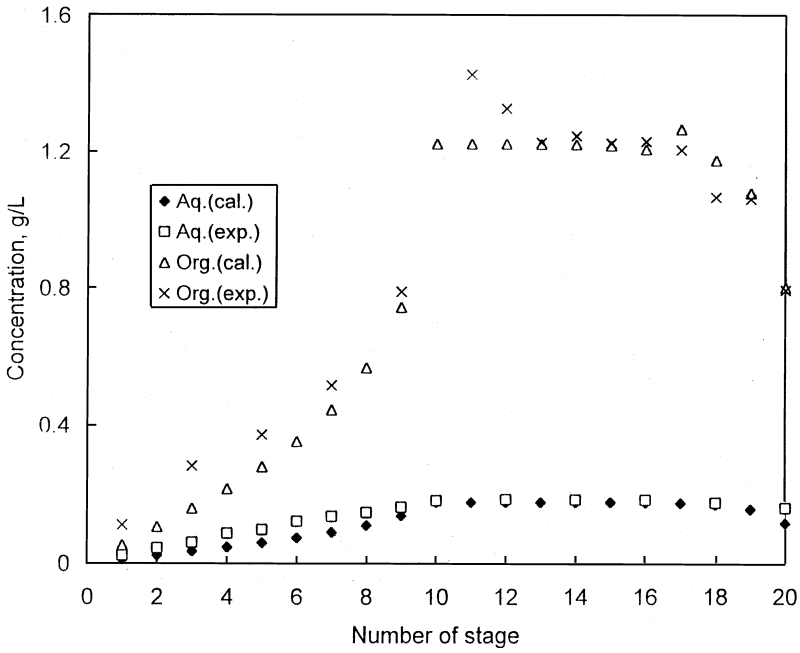

Figure 7. Concentration profiles of hydrocortisone in the scrubbing process (1–10 stages for the re-extraction section; 11–20 stages for the purification section).

The concentration profiles also show that the hydrocortisone and epi-hydrocortisone distribution ratio in the recovery section was almost unchanged, and the hydrocortisone distribution ratio in the purification section decreased with decreasing concentration.

CONCLUSIONS

The distribution behaviors of hydrocortisone and epi-hydrocortisone in a chloroform extraction process were measured experimentally. The data showed that hydrocortisone and epi-hydrocortisone had different distribution ratios in the chloroform/water system, with the distribution ratio of hydrocortisone equal to 6.2 and that of epi-hydrocortisone equal to 2.0 at 25°C, so that the corresponding separation factor was 3.1. The distribution ratios of hydrocortisone and epi-hydrocortisone increased with increasing temperature, but the separation factor was almost unchanged. The distribution was also affected by the ammonium sulfide content, but it had little effect on the separation factor.

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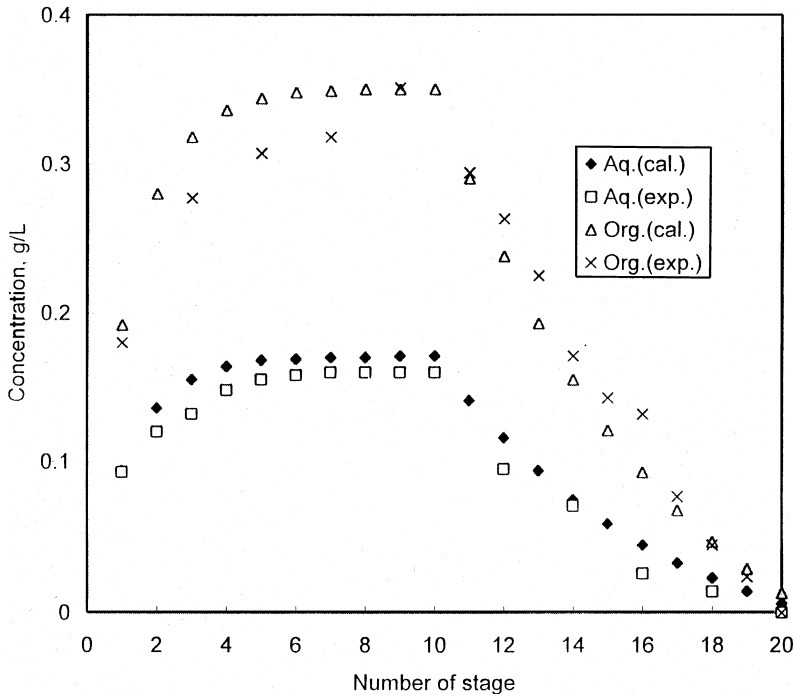

Figure 8. Concentration profiles of epi-hydrocortisone in the scrubbing process (1–10 stages for the re-extraction section; 11–20 stages for the purification section).

To replace the recrystallization process, a chloroform solvent extraction process was developed to separate hydrocortisone and epi-hydrocortisone. The hydrocortisone and epi-hydrocortisone in the fermentation liquor were first extracted into chloroform, then both were separated by a fractional scrubbing process. The total steroids recovery was 95% with a flow ratio of 1.1 and 6 stages. In the subsequent scrubbing process with deionized water, the hydrocortisone content in the purified organic phase was over 98% and the recovery in the scrubbing process was over 95% with a feed/water/chloroform flow ratio of 1:5:1.1, 10-stage scrubbing, and 10-stage re-extraction.

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