J Chem Technol Biotechnol 80:772–781 (2005)
DOI: 10.1002/jctb.1234

Study on new solvent extraction systems for erythromycin

Zhou Li,* Feng Qin, Hao Bao and Xueqiang Gu
Department of Chemical Engineering, Tsinghua University, Beijing 100084, China

Abstract: New solvent systems for the extraction of erythromycin were studied in which octanol was used as the extractant instead of butyl acetate. The mechanism for these new extraction systems is not simple physical distribution but the formation of a neutral complex of erythromycin. A neutral extraction complex formed between the neutral molecules of erythromycin and extractant by hydrogen bonding, and the formed neutral extraction complex moves into the organic phase. Extraction reaction equations and mathematical models of extraction equilibrium and re-extraction equilibrium are proposed for these new extraction systems. Experimental results show that the new extraction systems offer technological and economic advantages owing to the low solubility of the extraction solvents in the aqueous phase. The solvent consumption of the new extraction process was less than 3kg per billion active units compared with 9–10 kg per billion active units for a butyl acetate extraction system. In addition, the recovery of solvent from raffinate may be eliminated, thus reducing energy consumption.

Keywords: erythromycin; complex extraction; synergistic extraction; octanol

NOTATION

A Aqueous phase
B Extractant
c Constant
C Concentration of erythromycin (extraction equilibrium) ($\mu\,cm^{-3}$)
C' Concentration of erythromycin (re-extraction equilibrium) ($\mu\,cm^{-3}$)
D Extraction distribution coefficient
D' Re-extraction distribution coefficient
ΔH_m Enthalpy change ($kJ\,mol^{-1}$)
K_a Dissociation constant
K_{agg} Aggregate coefficient
K_s Thermodynamic equilibrium constant
M Molecular form of erythromycin
n Aggregated number of extractant
O Organic phase
pH_e Equilibrium pH value in aqueous phase
R Gas constant ($8.3143\,J\,mol^{-1}K^{-1}$)
R_{syn} Synergistic extraction coefficient
t Temperature (°C)
T Temperature (K)
V Volume (cm^3)
w Weight (g)
X Molar fraction
γ Related coefficient

Superscript
0 Initial value

Subscripts
add Additive
B Extractant
o Organic phase
S Polar solvent
syn Synergistic
w Aqueous phase

INTRODUCTION

Butyl acetate is widely used as the extraction solvent for the industrial extraction of erythromycin.[1,2] During the extraction process, consumption of butyl acetate is high (9–10 kg per billion active units of erythromycin) during the extraction process owing to its solubility in aqueous media. This consumption is costly, being about one-third of the total cost for the downstream process of erythromycin.[3] To overcome this disadvantage, new extraction systems have been studied, in which octanol was used as the extractant instead of butyl acetate. The extraction mechanism of erythromycin by octanol differs from the physical distribution of erythromycin between two phases by neutral complex extraction.

Experimental results show that the neutral complex extraction system possesses a higher extraction efficiency and consumes less solvent. Also, the cost of the new extraction system is cheaper than that using butyl acetate.

In addition, a synergistic extraction effect is shown when a polar solvent is used as the diluent or additive

* Correspondence to: Zhou Li, Department of Chemical Engineering, Tsinghua University, Beijing 100084, China
E-mail: Lizhou@mail.tsinghua.edu.cn
(*Received 28 January 2004; revised version received 7 September 2004; accepted 14 September 2004*)
Published online 31 March 2005

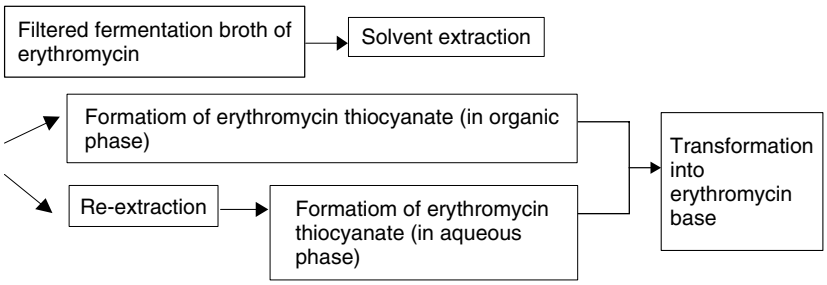

Figure 1. Process flows for erythromycin extraction.

solvent, ie a system is synergistic if the system is more effective using the combination of extractant and additive solvent than any of the individual components. Defining the synergistic coefficient $R = D_{syn}/D_{add}$, where D_{syn} is the extraction distribution coefficient of synergistic extraction system, and D_{add} is the extraction distribution coefficient of non-synergistic extraction system, for $R_{syn} > 1$, there is a synergistic effect (positive synergistic effect).[4]

Based on our research, two process flows for erythromycin extraction were developed (see Fig 1), The difference between these two flow sheets is the medium in which the erythromycin thiocyanate is formed.

EXPERIMENTAL

Technological conditions and experimental methods

Extraction

A known volume of filtered fermentation broth (25 L) of erythromycin (or simulated feed of erythromycin) was adjusted to pH 10.5 with 10% (w/w) NaOH solution, and mixed with the extraction solvent to give 1:6–1:8 of o/a (v/v) phase ratios in a separating funnel under a determined temperature (30–35 °C). These two phases were then separated, and the potency of erythromycin in both phases and the equilibrium pH value in the aqueous phase were measured.

The experimental conditions for extraction equilibrium studies are noted in the relevant tables and figures.

Re-extraction

Glacial acetic acid was used for re-extraction of erythromycin from the organic phase. The loaded organic phase and acetic acid solution were mixed to give 1:1–2:1 of o/a (v/v) phase ratios in a separating funnel under the ambient temperature, and the final pH was controlled at 4.5–5.0. After separating the two phases, the potency of erythromycin in both phases and the equilibrium pH value in the aqueous phase were measured.

The experimental conditions for re-extraction equilibrium studies are noted in the relevant tables and figures.

Formation of erythromycin thiocyanate

After regulating the pH value to 6.0 in the re-extracted solution of erythromycin by using 10% (w/w) NaOH solution, the residual solvent was eliminated from the re-extracted solution by bubbling air through the re-extraction solution. The pH value was adjusted to 6.8, the solution was placed in a water bath at 25–35 °C, and then 1.2–2.0 times (mol potassium thiocynate/mol erythromycin) of 50% potassium thiocyanate solution was added slowly. For the growth of erythromycin thiocyanate crystals, it was necessary to keep the temperature of the water bath constant for 0.5–1.0 h. The wet crystals of erythromycin thiocyanate were separated by filtration, rinsed three times with deionized water, and vacuum-dried at 40 °C for 12h. A sample of dried crystals of erythromycin thiocyanate was taken to analyze for potency, water content, and other properties.

Transformation of erythromycin thiocyanate into erythromycin base

A known amount of erythromycin thiocyanate (100 g) was first dissolved in acetone, the ratio between the volume of acetone and the weight of erythromycin thiocyanate being 4.0–4.5 times.

According to stoichiometric proportions, 10–20% (w/w) NaOH solution was added to the acetone solution of erythromycin while stirring. After settling, a little liquor could be separated. Then, 3.0 times of deionized water were added to the acetone solution to initiate crystallization of the erythromycin base. After standing overnight, the crystals of erythromycin base were filtered, rinsed by deionized water and vacuum-dried at 40 °C for 12 h to give the final product of erythromycin.

Erythromycin feed and chemicals

Simulated feed and filtered fermentation erythromycin broth were used in the experiments. The simulated feed was made using an erythromycin standard sample provided by the Chinese Medicine & Bioproducts Inspecting Institute. The filtered erythromycin fermentation broth was obtained from the Ping-Yuan Pharmaceutical Factory in China. Erythromycin is a metabolite of *Streptomyces erythreus* grown in a culture medium, in which the main carbon sources were glucose (80–85%) and starch (15–20%), and the main nitrogen source was soybean powder, followed by corn slurry and ammonium sulfate; calcium

carbonate and potassium dihydrogen phosphate were also constituents. The fermentation period was about 6–7 days.

The extractant and other chemical reagents were all AR grade except for kerosene. Deionized water was used throughout.

Analytical methods

Spectrophotometry was used to determine the erythromycin concentration.[5] A brownish yellow color is produced when sulfuric acid reacts with erythromycin, having a maximum absorption peak at 485 nm, then the concentration of erythromycin was measured by using the spectrophotometer.

The examinations of erythromycin thiocyanate and erythromycin base were carried out using a bioassay, as reported elsewhere.[6]

EXPERIMENTAL RESULTS

Neutral complex extraction by octanol–nonpolar diluent system

Choice of extraction systems

New extraction systems were studied on the basis of the principles of extraction chemistry. As known, there is a tertiary amine group in the chemical structure of erythromycin, with $pK_a = 8.6$,[7] so erythromycin may be present either in molecular form or in ionic form in the aqueous phase. The following equilibrium exists:

$$MH^+_{(w)} \xleftrightarrow{K_a} M_{(w)} + H^+_{(w)} \qquad (1)$$

where $M_{(w)}$ and $MH^+_{(w)}$ represent the molecular and ionic states of erythromycin in the aqueous phase. This equilibrium and its transition depend on the pH value in the aqueous phase.

If erythromycin is maintained in the molecular state, a neutral complex extractant may be adopted for the extraction of erythromycin, and the re-extraction of erythromycin may be carried out by decreasing the pH value owing to the transition of the molecular state of erythromycin to its ionic state.

In neutral complex extraction systems neutral phosphorated extractants, neutral oxygen-bearing extractants, neutral sulfur-bearing extractants, neutral amide extractants and neutral aromatic extractants may all be suitable. However, when considering the limitations associated with these extractants in pharmaceutical processes, the choice was reduced considerably.

There are relatively few reported studies dealing with the extraction of erythromycin by new extractants.[8–11]

In our experiments the following indices were considered for the choice of an extractant;

(1) high extractability and easy to re-extract;
(2) appropriate physical properties, especially low solubility in the aqueous phase;
(3) easy to regenerate and good reusability;
(4) good anti-emulsifying performance;
(5) non-toxicity or very low toxicity;
(6) price comparable to or lower than that of butyl acetate.

On the basis of the above, octanols were chosen as extractants. First, the extraction kinetics were examined. Experimental results showed that 3 min was sufficient to reach extraction equilibrium.

The extraction performances of some alternative extractants are listed in Table 1. Table 1 shows that the extraction performance of octanols was superior to that of butyl acetate, and 2-ethyl hexanol was chosen as the extractant. To improve the physical properties of the extraction system, diluent was added into the system, and the effects of various diluents on the extraction performance were studied. The results are presented in Table 2, of these diluents hydrogenated kerosene was the first choice because of its low price.

Extraction conditions

The effects of the concentration of erythromycin and pH value of the aqueous phase, the concentration of

Table 2. Effect of diluent on extraction performance of 2-ethyl hexanol

Diluent	C^0_w ($\mu g\,cm^{-3}$)	C_w ($\mu g\,cm^{-3}$)	C_o ($\mu g\,cm^{-3}$)	D
Cyclohexane	1000	32.0	978	30.56
n-Heptane	1000	21.0	966	46.00
Hydrogenated kerosene	1000	22.2	958	43.15

Experimental conditions: aqueous phase: simulated solution of erythromycin, equilibrium pH value: 10.6; organic phase: 60% (v/v) 2-ethyl hexanol–40% (v/v) diluent; phase ratio (O/A): 1:1($100\,cm^3/100\,cm^3$); extraction temperature: 22 °C; mixing time for the two phases: 3 min.

Table 1. Extraction of erythromycin by various extractants

Extractant	Initial concentration of erythromycin in aqueous phase, C^0_w ($\mu g\,cm^{-3}$)	Concentration of erythromycin in raffinate, C_w ($\mu g\,cm^{-3}$)	Concentration of erythromycin in extract, C_0 ($\mu g\,cm^{-3}$)	Extraction distribution coefficient, D
Butyl acetate	960	20.0	934	46.7
2-Ethyl hexanol	616	16.0	3000	187.5
n-Octanol	616	19.8	3200	161.7
2-Octanol	616	20.0	3400	170.0

Experimental conditions: aqueous phase: simulated solution of erythromycin, equilibrium pH value: 10.7; phase ratio (O/A): 1:5($100\,cm^3/500\,cm^3$); extraction temperature: 23 °C; mixing time for the two phases: 3 min.

extractant in the organic phase and temperature on the extraction distribution coefficient of erythromycin were studied.

Effect of the concentration of erythromycin in the aqueous phase. The experimental results shown in Fig 2 indicate that the curve can be expressed by a binominal expansion as follows:

$$C_o = 3.21C_w^2 + 54.17C_w + 83.65 \qquad (2)$$

Effect of equilibrium pH value in aqueous phase. The pH value is an important parameter for the extraction equilibrium of erythromycin since it is the determining factor for the equilibrium between the molecular and the ionic state of erythromycin.

This effect is shown in Fig 3, from which it can be seen that the extraction distribution coefficient D was very low when the pH value was less than 8.0, and D increased along with increasing pH value in the range of pH 8–10. When pH > 10, D was constant, thus, the best pH range for extraction of erythromycin should be in the range of 9.8–10.3.

Effect of extractant concentration. The experimental results given in Table 3 show that D increases as the concentration of 2-ethyl hexanol in the organic phase is increased.

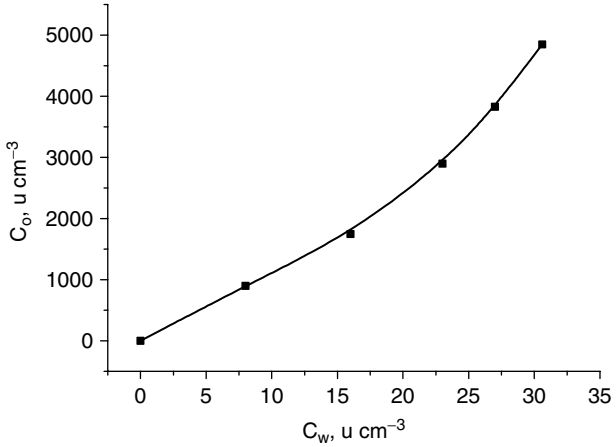

Figure 2. Extraction equilibrium isotherm of erythromycin. Experimental conditions: aqueous phase: simulated solution of erythromycin, equilibrium pH value: 10.6; organic phase: 2-ethyl hexanol; phase ratio(O/A):1:1(100 cm^3/100 cm^3); extraction temperature: 20 °C; mixing time for the two phases:3 min.

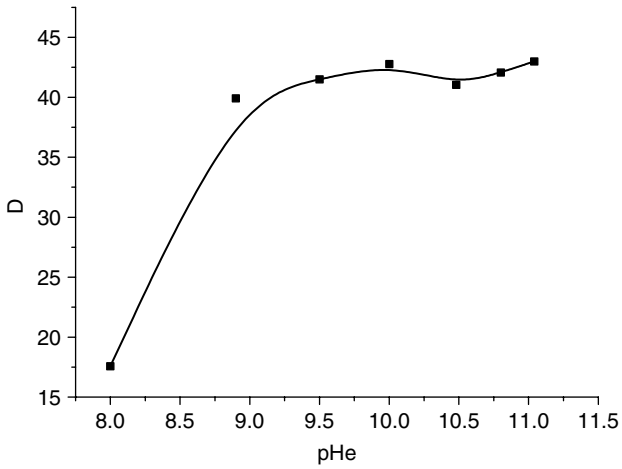

Figure 3. Effect of equilibrium pH on extraction equilibrium of erythromycin. Experimental conditions: aqueous phase: simulated solution of erythromycin: 1000 μ cm^{-3}; organic phase: 60% (v/v) 2-ethyl hexanol–40% (v/v) hydrogenated kerosene; phase ratio (O/A): 1:1(100 cm^3/100 cm^3); extraction temperature: 23 °C; mixing time for the two phases: 3 min.

Effect of temperature. Temperature not only affects extraction equilibrium, but also the physical properties of the two phases. The effect of temperature on the extraction distribution coefficient is presented in Table 4.

A plot of $\ln D - 1/T$ from the data in Table 4 may yield a straight line with a negative slope, ie D increases as the temperature increases and, thus, it may be judged that the extraction reaction of erythromycin is an endothermic reaction. Further, the enthalpy change of the extraction process, $\Delta H_m \cong 41.8\,\text{kJ mol}^{-1}$, is obtained by calculation using the Gibbs–Helmholtz equation.

Research on re-extraction conditions

Glacial acetic acid solution was used as the re-extraction solvent because of the high solubility of erythromycin in this solution.[7]

Determination of re-extraction equilibrium isotherm. The study of re-extraction kinetics showed that the

Table 3. Effect of extractant concentration

Concentration of extractant in organic phase, % (v/v)	C_w (μ cm^{-3})	C_o (μ cm^{-3})	D
20	36.0	960	26.67
40	25.8	930	36.04
60	21.4	900	42.06
80	10	990	99
100	5.5	1000	181.8

Experimental conditions: aqueous phase: simulated solution of erythromycin: 1000 μ cm^{-3}, equilibrium pH value: 10.6; organic phase: 2-ethyl hexanol–hydrogenated kerosene; phase ratio (O/A): 1:1(100 cm^3/100 cm^3); extraction temperature: 23 °C; mixing time for the two phases: 3 min.

Table 4. Effect of temperature on extraction distribution of erythromycin

No	t (°C)	C_w (μ cm^{-3})	C_o (μ cm^{-3})	D
1	14	122.0	800	6.56
2	20	34.0	912	26.82
3	24	20.6	880	42.72
4	30	20.0	1000	50.0
5	35	12.4	960	77.42

Experimental conditions: aqueous phase: simulated solution of erythromycin: 960 μ cm^{-3}, equilibrium pH value: 10.6; organic phase: 60% (v/v) 2-ethyl hexanol–40% (v/v) hydrogenated kerosene; phase ratio (O/A): 1:1(100 cm^3/100 cm^3); mixing time for the two phases: 3 min.

necessary mixing duration for reaching re-extraction equilibrium was only about 100 s.

The re-extraction equilibrium isotherm is illustrated in Fig 4, which shows that the re-extraction isotherm is almost a straight line, and the following equation is deduced by regression:

$$C'_o = 70.85C'_w - 662.61 \quad (3)$$

Relationship of the re-extraction distribution coefficient D′ with the pH value. It can be seen from Fig 5 that there was almost a linear relationship between the re-extraction distribution coefficient of erythromycin, D', and the equilibrium pH in the aqueous phase. The following equation was obtained by regression:

$$D' = -35.01 \times \text{pH} + 202.27 \quad (4)$$
$$r = 0.97$$

Thus, the pH value is an important factor for the re-extraction of erythromycin, a low pH value is of benefit to the re-extraction of erythromycin, but it should be noted that erythromycin becomes unstable when the pH value decreases below 6.0. Thus, a suitable pH value should be determined by consideration of both the re-extraction and stability of erythromycin. In practice, a pH value of 4.5–5.0 was found to be suitable. For minimizing the decomposition of erythromycin, the pH value in the re-extracted solution should immediately be adjusted to 6.0.

Relationship of the re-extraction distribution coefficient with temperature. Figure 6 shows that the re-extraction distribution coefficient decreased with increasing temperature, ie the re-extraction process is an exothermic reaction process.

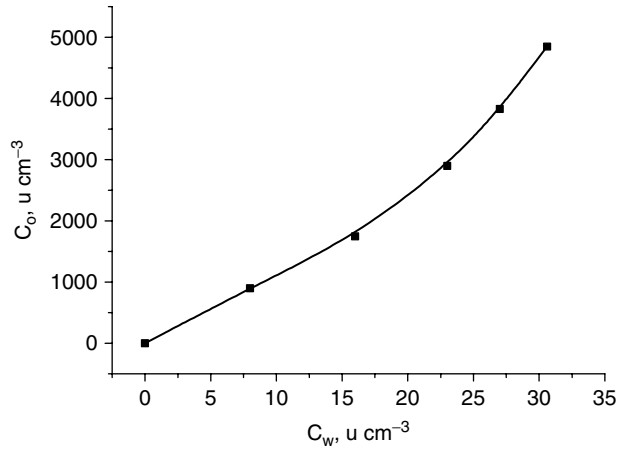

Figure 4. Re-extraction equilibrium isotherm of erythromycin. Experimental conditions: organic phase: 60% (v/v) 2-ethyl hexanol–40% (v/v) hydrogenated kerosene loaded with erythromycin; aqueous phase: acetic acid buffer solution pH — 4.1, and pH: 4.4 after re-extraction; phase ratio (O/A): 1:1(100 cm^3/100 cm^3); temperature: 17 °C; mixing time for the two phases: 3 min.

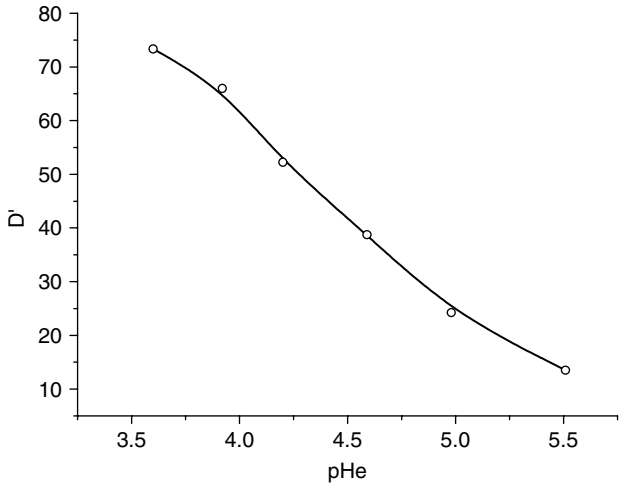

Figure 5. Effect of pH on re-extraction equilibrium of erythromycin. Experimental conditions: organic phase: 60% (v/v) 2-ethyl hexanol–40% (v/v) hydrogenated kerosene loaded with 10 000 μ cm^{-3} of erythromycin; aqueous phase: buffer solution of acetic acid as re-extraction solution; phase ratio (A/O): 1:1(100 cm^3/100 cm^3); temperature: 17 °C; mixing time for the two phases: 3 min.

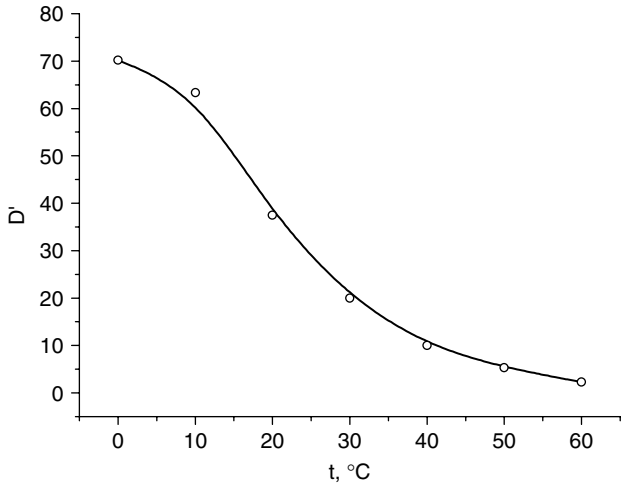

Figure 6. Effect of temperature on re-extraction equilibrium of erythromycin. Experimental conditions: organic phase: 60% (v/v) 2-ethyl hexanol–40% (v/v) hydrogenated kerosene loaded with 10 000 μ cm^{-3} of erythromycin; aqueous phase: buffer solution of acetic acid as the re-extraction solution, equilibrium pH value: 4.3; phase ratio (A/O): 1:1(100 cm^3/100 cm^3); mixing time for the two phases: 3 min.

Effect of the extractant concentration on the re-extraction distribution coefficient. The experimental results are listed in Table 5, from which it can be concluded that there is an inverse relationship between the concentration of extractant and the re-extraction distribution coefficient of erythromycin.

Determination of technological conditions for extraction, re-extraction and formation of erythromycin thiocyanate

The data given above indicate the optimum parameters for extraction and re-extraction.

Extraction:

aqueous phase: filtered fermentation broth, equilibrium pH value: 9.8–10.3

Table 5. Effect of the concentration of extractant on re-extraction distribution coefficient D'

Concentration of extractant in organic phase, % (v/v)	C'_o (μ cm^{-3})	C'_w (μ cm^{-3})	D'
40	134.28	10 259.72	76.41
60	197.13	9794.15	49.68
80	469.49	10 445.94	22.25
100	817.5	9607.93	11.75

Experimental conditions: loaded organic phase: 2-ethyl hexanol–hydrogenated kerosene with 10 000 μ cm^{-3} erythromycin; re-extraction liquor: acetic acid buffer solution, equilibrium pH value: 4.2–4.3; phase ratio(A/O):1:1(100 cm^3/100 cm^3); temperature: 20 °C; mixing time for the two phases: 3 min.

organic phase: 60% (v/v) 2-ethyl hexanol-40% (v/v) hydrogenated kerosene
phase ratio (a/o): 6–8:1
temperature: ambient

Re-extraction:
re-extraction reagent: acetic acid buffer solution
equilibrium pH value in aqueous phase: 4.5–5.0
phase ratio (o/a): 1–2
temperature: ambient

Formation of erythromycin thiocyanate:
potassium thiocyanate: 50%(w/w) solution
crystallization process: pH value: 6.8–7.0,
temperature: 25–30 °C
crystallization duration: 3 h.

According to the data in Table 6 the average extraction yield was 96.08%, the average re-extraction yield was 94.96%, and the average yield of erythromycin thiocyanate formation was 82.10%. Thus, the average total yield (extraction, re-extraction and formation of erythromycin thiocyanate included) was 78.62%.

The main advantage of the new extraction system is the low consumption of extraction solvent, only about 1.72 kg per billion active units of erythromycin, this consumption amount of solvent is far less than that for extraction with butyl acetate.

Synergistic extraction by an octanol–polar diluent system

Studies on the synergistic extraction system were carried out by using simulated and filtered fermentation broth.

In the binary synergistic extraction system B is an aliphatic alcohol with low solubility in the aqueous phase, for example, *n*-octanol, 2-octanol or 2-ethyl hexanol. 2-Ethyl hexanol was adopted in this research. S is a polar solvent of which there are three, pentyl formate, pentyl acetate and ethyl butyrate, employed in these studies and shown as S_1, S_2 and S_2.

The extraction distribution coefficients of erythromycin were examined under the conditions of various combinations of B and S.

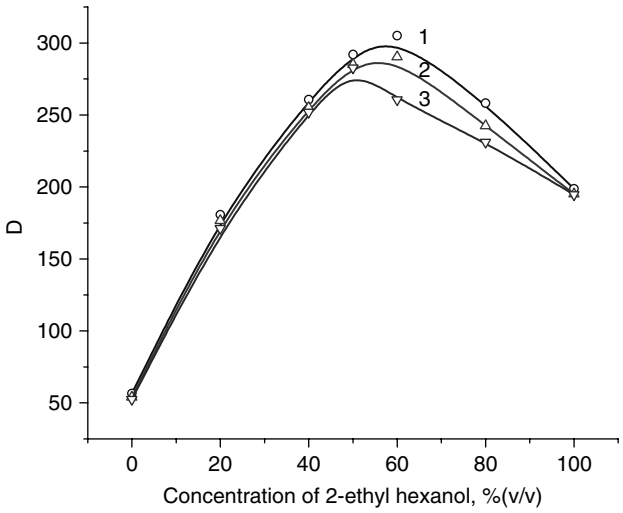

Figure 7. Synergistic extraction of erythromycin from filtered fermentation broth. 1 — B + S_1, 2 — B + S_2, 3 — B + S_3. Experimental conditions: aqueous phase: filtered fermentation solution of erythromycin: 4667 μ cm^{-3} (B + S_1, B + S_2), 4313 μ cm^{-3} (B + S_3); equilibrium pH value: 10.6–10.8; organic phase: 2-ethyl hexanol–polar solvent; phase ratio (O/A): 1:6(100 cm^3/600 cm^3); temperature: 28 °C; mixing time for the two phases: 3 min.

The experimental results using simulated feed are tabulated in Table 7. The experimental results using filtered fermentation broth are shown in Fig 7.

From the data listed in Tables 7 and shown in Fig 7 it can be seen that similar trends with peaks were obtained with the B + S_1, B + S_2 and B + S_3 extraction systems. The extraction distribution coefficients for these extraction systems are larger than that in the 2-ethyl hexanol–hydrogenated kerosene extraction system, ie there is a synergistic effect for the extraction of erythromycin. Comparing the experimental results of B + S_1, B + S_2 and B + S_3 shows a trend of $D_{B+S_1} > D_{B+S_2} > D_{B+S_3}$ owing to the polarity of $S_1 > S_2 > S_3$.

From the experimental data listed in Table 7 for the B + S_3 extraction system the distribution coefficients were calculated as $D_B = 179.5$ and $D_{S_3} = 17.2$.

When the concentration of 2-ethyl hexanol in the organic phase was 54% (v/v), with molar fraction of $X_B = 0.48$:

$$D_{syn,max} = 234.1 \quad (5)$$

$$D_{add} = D_B X_B + D_{S_3}(1 - X_B) = 90.1 \quad (6)$$

The synergistic extraction coefficient is:

$$R_{syn} = \frac{D_{syn}}{D_{add}} = 2.6 \quad (7)$$

Since $R_{syn} > 1$, there is a synergistic effect.

Considering various factors such as the extraction performance, physical properties, toxicity and economy of extraction system, the B + S_3 extraction system was chosen for further research.

The practical filtered fermentation broth is adopted for extraction of erythromycin. The step of erythromycin thiocyanate formation was carried out in

Table 6. Experimental results by using filtered fermentation broth

Sample no	Aqueous phase (filtered fermentation broth)		Organic phase (extraction solvent)		Phase ratio of extraction (A/O)	Raffinate		Extract		Extraction yield (%)	Re-extracted aqueous phase		Organic phase after re-extraction		Phase ratio of re-extraction (A/O)	Re-extraction yield (%)	Mother solution after crystallization		Erythromycin thiocyanate (after drying)		Yield of erythromycin thiocyanate (%)	Total yield (%)	Consumption of organic solvent (dm^3 per billion active units of erythromycin thiocyanate)
	Volume (cm^3)	Potency ($u\,mL^{-1}$)	Volume (cm^3)	Potency ($u\,mL^{-1}$)		Potency ($u\,mL^{-1}$)	Equilibrium pH	Volume (cm^3)	Potency ($u\,mL^{-1}$)		Volume (cm^3)	Potency ($u\,mL^{-1}$)	Volume (cm^3)	Potency ($u\,mL^{-1}$)			Volume (cm^3)	Potency ($u\,mL^{-1}$)	Weight (g)	Potency ($u\,mL^{-1}$)			
1	8584	2624	1430	0	6.0	87	10.0	1440	15100	96.68	800	—	1194	890	2:3	94.35	800	4960	20.0	717	97.34	74.20	—
2	8616	2464	1370	0	6.3	87	10.0	1360	13650	96.47	923	—	1340	460	2:3	96.59	910	4190	22.4	717	—	75.65	1.87
3	10148	1578	1526	0	6.7	60	10.0	1510	9250	96.20	807	16000	1490	500	1:2	94.59	780	3070	16.8	718	80.81	75.33	2.98
4	9920	2032	4150	0	6.8	87	10.0	1467	13150	95.75	900	22600	1435	1085	2:3	91.75	900	2861	20.4	722	87.34	73.07	1.05
5	7953	2344	1452	1190	6.9	93	10.2	1168	17050	96.03	900	—	1145	740	1:1.3	95.66	900	3540	23.9	715	—	8531	0.44
6	4212	2464	635	0	6.6	0	10.3	641	15400	95.10	600	18560	620	435	1:1	97.18	600	3730	12.5	716	79.90	86.23	1.62
7	4212	2464	635	0	6.6	0	10.3	641	15400	95.10	600	21680	622	565	1:1	96.33	600	3340	12.1	716	84.59	83.47	1.57

Extraction yield, re-extraction yield and total yield are calculated on potency (u = active unit of erythromycin).
Consumption of organic solvents is calculated by the difference between the initial volume and the volume after re-extraction of solvent.

Table 7. Extraction distribution coefficients of erythromycin in synergistic extraction systems (simulated feed)

Synergistic system	Concentration of 2-ethyl hexanol in organic phase, % (v/v)						
	0	20	40	50	60	80	100
$B + S_1$	24.3	147.5	212.8	261.3	254.9	232.8	179.5
$B + S_2$	20.6	132.8	207.9	230.0	234.3	221.7	180.1
$B + S_3$	17.2	133.2	202.7	234.1	229.4	207.6	179.5

Experimental conditions: aqueous phase: simulated solution of erythromycin: 1380 μ cm^{-3}, equilibrium pH value: 10.6–10.8; organic phase: 2-ethyl hexanol–polar solvent; phase ratio (O/A): 1:8(100 cm^3/800 cm^3); temperature: 19 °C; mixing time for the two phases: 3 min.

the organic phase and the aqueous phase after re-extraction, and the experimental results are listed in Tables 8 and 9. From the data listed in Table 8 and Table 9 it can be seen that the amount of consumption of the new extraction solvent was much less than that of butyl acetate.

EXTRACTION MECHANISM

Development of mathematical models for extraction and re-extraction equilibrium for neutral complex extraction

There are three equilibrium relationships in the extraction process:

(1) dissociation equilibrium of erythromycin molecule in the aqueous phase expressed in eqn (1);
(2) aggregate equilibrium of 2-ethyl hexanol in the organic phase:

$$nB \xleftrightarrow{K_{agg}} Bn \tag{8}$$

(3) extraction reaction equilibrium.

The analytical results of infra-red absorption spectrometry showed that there is a hydrogen bond formed between the erythromycin molecule and a single molecule of 2-ethyl hexanol,[5] so the extraction reaction is as follows:

$$M_{(w)} + B_{(o)} \xleftrightarrow{K_s} MB_{(o)} \tag{9}$$

$$K_s = \frac{[MB]_{(o)}}{[M]_{(w)}[B]_{(o)}}$$

and

$$[MB]_{(o)} = K_s[M]_{(w)}[B]_{(o)} \tag{10}$$

As known,

$$D = \frac{[MB]_{(o)}}{[M]_{(w)} + [MH^+]_{(w)}}. \tag{11}$$

Substitution of eqn (9) into eqn (10), and from eqn (1):

$$K_a = \frac{[M]_{(w)}[H^+]_{(w)}}{[MH^+]_{(w)}} \tag{12}$$

Table 8. Experimental results of erythromycin thiocyanate formation in aqueous phase[a]

Sample no	Extractant	Filtered fermentation broth of erythromycin		Organic solvent		Phase ratio of extraction (O/A)	Potency in raffinate ($u\,mL^{-1}$)	Potency in extract ($u\,mL^{-1}$)	Extraction yield in single equilibrium (%)	Consumption of organic solvent (cm^3)	Extract volume for erythromycin thiocyanate formation[b] (cm^3)	Consumption of organic solvent in erythromycin thiocyanate formation (cm^3)	Total consumption of organic solvent, (%)
		Volume, (cm^3)	Potency, ($u\,mL^{-1}$)	Volume (cm^3)	Potency, ($u\,mL^{-1}$)								
1	Butyl acetate	1000	2940	200	0	1:5	190	13 700	93.5	45	155	7	27
2	$B + S_3$	1500	2940	300	0	1:5	82	14 000	97.2	20	150	10	13.3
3	$B + S_3$	3000	3400	600	0	1:5	102	20 800	97.0	15	300	20	9.1
4	$B + S_3$	3000	3400	600	1090	1:5	12	21 200	97.1	12	300	30	12
5	$B + S_3$	3000	3000	500	1090	1:6	20	17 520	97.3	20	200	25	14.5
6	$B + S_3$	3000	3870	500	1090	1:6	12	22 000	94.7	12	200	17	12.9

[a] Experimental conditions: the synergistic extraction system is composed of 50%B and 50%S_3, the other experimental conditions are described in the section 'Determination of technological conditions for extraction, re-extraction and formation of erythromycin thiocyanate'.
[b] Part of organic solvent is taken for erythromycin thiocyarate formation.

Table 9. Experimental results of erythromycin thiocyanate formation in organic phase[a]

Sample no	Filtered fermentation broth of erythromycin		Organic solvent (50%B–50%S_3)		Phase ratio of extraction (O/A)	Potency in raffinate ($u\,mL^{-1}$)	Potency in extract ($u\,mL^{-1}$)	Extraction yield in single equilibrium (%)	Consumption of organic solvent (cm^3)	Volume of organic solvent for re-extraction[b] (cm^3)	Phase ratio of re-extraction (A/O)	Re-extracted acidic liquor			Potency in organic raffinate ($u\,mL^{-1}$)	Re-extraction yield (%)	Consumption of organic solvent in re-extraction (cm^3)	Total consumption of organic solvent (%)
	Volume (cm^3)	Potency ($u\,mL^{-1}$)	Volume (cm^3)	Potency ($u\,mL^{-1}$)								Volume (cm^3)	Potency ($u\,mL^{-1}$)	pH				
1	3000	3400	600	0	1:5	129	20 200	96.2	0	300	1:2	150	34 000	4.0	6400	84.1	0	0
2	3000	3870	500	950	1:6	206	21 000	94.7	10	290	1:2	145	38 500	4.5	3800	91.7	5	3.7
3	3000	4540	500	950	1:6	190	26 000	95.8	5	295	1:2	147	48 300	4.0	2317	92.9	0	1.0
4	3000	3750	500	0	1:6	129	21 800	96.6	5	295	1:2	147	43 100	4.0	1270	98.9	0	1.0
5	3000	4438	500	0	1:6	205	25 200	95.4	10	290	1:2	145	48 700	4.5	1700	96.6	5	3.7

[a] Experimental conditions are as described in the section 'Determination of technological conditions for extraction, re-extraction and formation of erythromycin thiocyanate'.
[b] Part of organic solvent is taken for re-extraction.

and

$$[MH^+]_{(w)} = \frac{[M]_{(w)}[H^+]_{(w)}}{K_a} \quad (13)$$

the following model is deduced:

$$D = \frac{K_s[B]_{(o)}}{1 + \frac{[H^+]_{(w)}}{K_a}}. \quad (14)$$

Substitution of

$$K_s = \exp\left(\frac{-\Delta H_m}{RT} + c\right)$$

into eqn (13) yields

$$D \approx \exp\left(\frac{-\Delta H_m}{RT} + c\right)[B]_{(o)}(1 + 10^{pK_a - pH})^{-1} \quad (15)$$

Regression was performed on 16 groups of experimental data to yield the following equation:

$$D \approx \exp(-36.33 \times 10^3/RT + 18.77)[B]_{(o)} (1 + 10^{8.60-pH})^{-1} \quad (16)$$

for which a value of ΔH_m of 36.33 kJ mol^{-1} is obtained.

Thus, D is a function of the concentration of erythromycin, the pH value in the aqueous phase, the extractant concentration in the organic phase and the extraction temperature. In addition, D is related to the pK_a of erythromycin.

For the re-extraction equilibrium regression analysis on 18 groups of experimental data in a similar fashion yields the following model:

$$D' \approx \exp(42.41 \times 10^3/RT - 24.88)[B]_{(o)}{}^{-1.0} (1 + 10^{8.60-pH}) \quad (17)$$

where $\Delta H_m \approx -42.41$ kJ mol^{-1}.

Synergistic extraction mechanism

In the research of synergistic extraction systems B is the main extractant, the extraction reaction was described above, S is a polar solvent, its extraction for erythromycin belongs in the physical distribution, and its extractability is far less than that of B.

From eqn (14):

$$D = \frac{K_s[B]_{(o)}}{1 + \frac{[H^+]_{(w)}}{K_a}}$$

and $pK_a = 8.60$

Owing to $[H^+]_{(w)} < 10^{-10}$:

$$\ln\left(1 + \frac{[H^+]_{(w)}}{K_a}\right) \approx 0 \quad (18)$$

and:

$$\ln D = \ln K_s + \ln[B]_{(o)} \quad (19)$$

where $\ln K_s$ is a constant.

Thus, D depends mainly on $\ln[B]_{(o)}$, the concentration of 2-ethyl hexanol in monomolecular form.

As it is known that there is an equilibrium between the aggregate and the monomolecular states of 2-ethyl hexanol, the peak curve shown in Fig 7 implies that the concentration of the monomolecular state of 2-ethyl hexanol changes from low to high, and then to low again. This phenomenon is different from that in the 2-ethyl hexanol–kerosene system owing to the different diluents used in these two extraction systems. One was the nonpolar solvent kerosene (dipolar moment = 0), and the other a polar solvent (dipolar moment >0). The polar solvent inhibits the aggregation of 2-ethyl hexanol molecules, ie it causes an increase of the monomolecular state ratio of 2-ethyl hexanol. Therefore the extraction distribution coefficient of erythromycin increases. In addition, there is the 'diluent effect', which as Taube[12,13] pointed out, depends on the interaction between the dipoles of the extracted complex and the diluent molecule. This interaction makes the extraction complexes easier to enter into the organic phase. In fact, either the effect of polar solvent on the aggregate equilibrium of 2-ethyl hexanol or the interaction between the extracted complex and diluent molecules is the 'effect of diluent'.

There are two factors which affect the extraction of erythromycin. One factor is the increase of extraction ability for erythromycin with the increase of B concentration. The other is the decrease in the inhibiting effect on the aggregation of 2-ethyl hexanol as a result of the interaction between the extracted complex and the diluent molecules with decreasing concentration of S. The latter hinders the extraction of erythromycin. For the first half of the synergistic extraction curve, ie the rising section of extraction distribution coefficient, the first factor dominates. For the second half of the synergistic extraction curve, ie the falling section of extraction distribution coefficient, the second factor dominates.

CONCLUSIONS

1. A new neutral complex extraction system, 2-ethyl hexanol–kerosene system was researched and adopted for the extraction of erythromycin.
2. A new synergistic extraction system using octanol and polar system is proposed for the extraction of erythromycin from fermentation broth. This new synergistic extraction system possesses advantages of higher extractability, lower consumption and cheaper price than that of butyl acetate extraction, and higher extractability, cheaper price than that of 2-octanol–kerosene extraction.

ACKNOWLEDGEMENTS

The authors thank Professor LL Tavlarides and Professor Jing-xu Zhu for their assistance in revising this paper and improving the English.

REFERENCES

1 Jining Antibiotics Factory (in chief), *Industrial Production of Antibiotics*, Chinese Medical Industrial Company, Beijing. pp 285–298 (1981). (in Chinese).
2 Biedrzycki M, Chmielnicki P, Grochalski B, Szymanski J, Zulowski E, Dahlig H, Drzewinski W, Orlowski A, Kubinska T, Glabski T and Czyzyk E, Method of isolating erythromycin from fermentation medium. Patent pol PL 142175 (1987). (in Polish).
3 Wang Yue and Feng Jinrui, *Antibiotics*, Science Press, Beijing. pp 337 (1988). (in Chinese).
4 Xu Guangxian, Wang Wenqing, Wu Jinguang, *et al*, *Principles of Extraction Chemistry*, Science and Technology Press, Shanghai. pp 138–183 (1984). (in Chinese).
5 Chen Lingjun and Zhang Ziliang, Measurement of erythromycin content by absorption spectrometry. *Physical Testing and Chemical Analysis, part B: Chemical Analysis* **29**:293–294 (1993). (in Chinese).
6 National Pharmaceutical Industrial Council, *Pharmacopoeia of People's Republic of China*, Detection method of antibiotics microorganism. (2000. edition.) 2nd division, Chemical Industry Press, Beijing. Appendix XIA, pp 81–84 (2000) (in Chinese).
7 Liu Changsheng and Wu Xingyan, Solubility of erythromycin in some buffer solutions. *Chinese Journal of Antibiotics* **20**:17–19 (1995). (in Chinese).
8 Li Zhou and Lei Wen, Process for extracting macrolide antibiotics. CN1125230A, 26 Jun, (1996). (in Chinese).
9 Pursell MR, Mendes Tatsis MA and Stuckey DC, The effect of surfactants during solvent extraction of erythromycin A from buffer and filtered fermentation broth, in *Proc ISEC'99, Barcelona, Spain, July 11–16, 1999*, ed by Cox M, Hidalgo M and Valiente M. vol 1, pp 155–160 (1999).
10 Lye GJ and Stuckey DC, Extraction of erythromycin-A using colloidal liquid aphrons:1. Equilibrium partitioning. *J Chem Technol Biotechnol* **75**:339–347 (2000).
11 Oniscu C, Cascaval D, Galaction AI and Dumitru IF, Study on reactive extraction of erythromycin. *Roumanian Biotechnological Letters* **5**:439–447 (2000).
12 Taube M, The influence of diluent polarity on the extraction of plutonium complexer to organic media. *J Inorg Nucl Chem* **12**:174–180 (1959).
13 Taube M, The influence of diluent polarity on the extraction of neptunium and uranium compounds to organic media. *J Inorg Nucl Chem* **15**:171–176 (1960).