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# Solvent Extraction of Lincomycin with Neutral Donor Extractants

ZHICHUN WU,* XIA ZHANG, RONGFANG XU,
and JIAYONG CHEN
INSTITUTE OF CHEMICAL METALLURGY
CHINESE ACADEMY OF SCIENCES
P.O. BOX 353, BEIJING 100080, PEOPLE'S REPUBLIC OF CHINA



## ABSTRACT

The extraction of lincomycin was carried out with different types of neutral donor extractants, and the stripping of extracted lincomycin was performed with hydrochloric acid. The effects of such factors as concentrations of solvent and lincomycin, pH of solution, temperature, and additives on the extraction of lincomycin was investigated. The relationships between the electron-donating ability of extractant and extraction selectivity plus the ease of stripping with hydrochloric acid are discussed. A new solvent system is suggested for the extraction of lincomycin from aqueous solutions.

## INTRODUCTION

Lincomycin, with the structure formula shown schematically in Fig. 1, is an important antibiotic widely used in medicine (1, 2). The recovery of lincomycin from fermentation filtrate was carried out either by solvent extraction with *n*-butyl alcohol or methylene dichloride or by adsorption with activated carbon (3, 4). In a typical process (5) the whole broth is adjusted to pH 3–3.5 with hydrochloric acid and filtered at about 50°C. The filtrate is adjusted to pH 9.5–10.5 with sodium hydroxide and extracted twice with *n*-butyl alcohol. The combined extracts are concentrated through a thin film evaporator under reduced pressure. The concentrated extract is contacted four times with hydrochloric acid of pH 2.5–3

* To whom correspondence should be addressed.

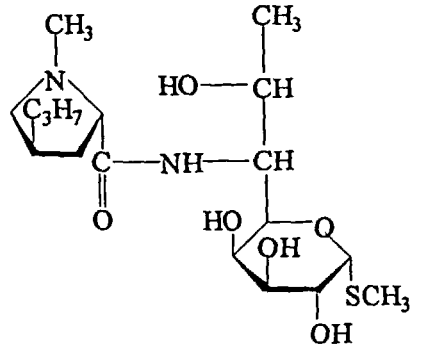

FIG. 1 Schematic structure of lincomycin.



after washing with deionized water. The aqueous extract is washed with *n*-butyl alcohol and concentrated under vacuum at 65°C. The concentrated aqueous extract is decolorized with activated carbon and then lincomycin hydrochloride is crystallized by the addition of acetone and cooling. The final product is filtered and washed twice with acetone and dried under vacuum. An azeotropic distillation process was also developed to recover lincomycin from fermentation beer (6). The problems associated with these processes are high solvent loss, high energy consumption, and low recovery.

To improve the solvent extraction of lincomycin, new processes have been developed (7, 8). We feel it is necessary to investigate the extraction chemistry in detail. The extraction of lincomycin with chloroform and *n*-butanol (9) and their mixture (10) as the solvent has been reported. Extractions with oximes (11) and with long-chain alcohols (12) as the solvent were studied and discussed. Neutral phosphorus esters and amine oxides have been widely used as extractants. This work reports the extraction of lincomycin with these extractants.

## EXPERIMENTAL

The extraction experiments were carried out in separatory funnels at room temperature or in a 0.1-$dm^3$ mechanically agitated cylindrical glass reactor placed in a thermostat. Trialkyl amine oxide with the formula $R_3N{=}O$ (TRAO, R = $C_7$–$C_9$, $d_4^{25}$ 0.875), trialkyl phosphine oxide with the formula $R_3P{=}O$ (TRPO, R = $C_6$–$C_9$, average molecular weight

345), and dibutyl isoamyl phosphonate with the formula iso-$C_5H_{11}P(O)(OC_4H_9)_2$ (DBIAP, $d_4^{25}$ 0.935) were synthesized and purified by the methods described in References 13–15. TRAO and TRPO have purities ≥ 95%, and DBIAP ≥ 98%. The other solvents and reagents used were all of analytical grade. Lincomycin hydrochloride was supplied by Northern China Pharmaceutical Factory, Shijiazhuang, People's Republic of China. Aqueous solutions of lincomycin were prepared from lincomycin hydrochloride with deionized water. The pH of the solutions was adjusted with hydrochloric acid or sodium hydroxide and measured by a PH3-S acidity meter. Analysis of lincomycin in the initial aqueous solution and the extraction raffinate was conducted as follows. The aqueous solution containing lincomycin was adjusted with sodium hydroxide to pH 10.5–11.5 and then contacted with an equal volume of *n*-butanol. The optical rotation of the *n*-butanol extracts was measured with a WZZ-1 automatic polarimeter (16, 17). The amount of lincomycin extracted into the solvent was obtained by difference.



## RESULTS AND DISCUSSION

### Extraction of Lincomycin with Different Neutral Extractants

The results of the extraction of lincomycin (*E*) with different neutral extractants are shown in Fig. 2. It can be seen that among the neutral extractants studied, TRAO has the highest power of extraction and long-chain alcohols the lowest. This is consistent with the strength of their donor properties (18). Obviously, the oxygen atom attached to the nitrogen in an amine oxide has a higher donating ability than ones attached to the phosphorus atoms in esters and to the carbon atoms in alcohols. Among neutral phosphorus esters, the donating ability of the oxygen atom in phosphonyl group increases with an increase in the number of alkyl groups directly attached to the phosphorus atom. The percentage of extraction of lincomycin increases with the increasing donating ability of a neutral extractant as shown below:

TRAO > TRPO > DBIAP > TBP (tributyl phosphate) > alcohols

### Effect of Equilibrium pH on the Extraction of Lincomycin with Neutral Extractants

Lincomycin is a basic antibiotic with a $pK_a$ of 7.6 (3) and exists mainly as a free base in solutions with a pH higher than 8. Therefore, neutral phosphorus esters extract lincomycin as a neutral molecule by the mechanism of solvation extraction from the aqueous solutions with a pH higher

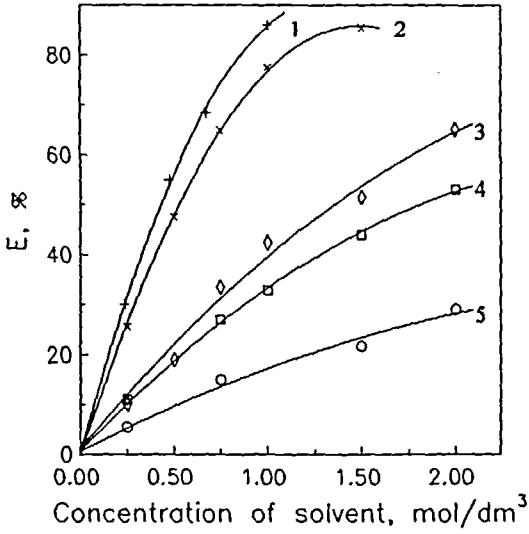


FIG. 2 A comparison of the extraction of lincomycin with different neutral extractants. Equilibrium pH 10–11, ratio of organic to aqueous phase (O/A) = 1, 3 minutes. Curve 1 = TRAO, 25°C; 2 = TRPO, 60°C; 3 = DBIAP, 60°C; 4 = tributyl phosphate (TBP), 60°C; 5 = *n*-hexyl alcohol, 25°C. Diluent: kerosene.



than 8. As shown in Fig. 3, the optimal extraction is consistent with the pH range in which lincomycin exists as a free base. As the pH of a solution decreases, the trend of association of free lincomycin with $H^+$ ion increases, and the percentage of extraction decreases rapidly. With TRAO as solvent, the extraction of lincomycin is different from that with neutral phosphorus esters and decreases only slowly with a decrease in pH. It has been shown that TRAO has a higher power for extraction than neutral phosphorus esters and can still extract inorganic acids from a solution with a pH lower than 7 (18). Accordingly, it may extract lincomycin by the ion association mechanism. This feature makes stripping of lincomycin back to the aqueous phase with hydrochloric acid rather difficult.

## Effect of the Initial Concentration of Lincomycin in Aqueous Solutions on the Distribution Ratio of Lincomycin

Figure 4 shows that the distribution ratio ($D$) increases with an increase of the initial lincomycin concentration in the aqueous phase. This indicates that the composition of the extracted species depends on the initial con-

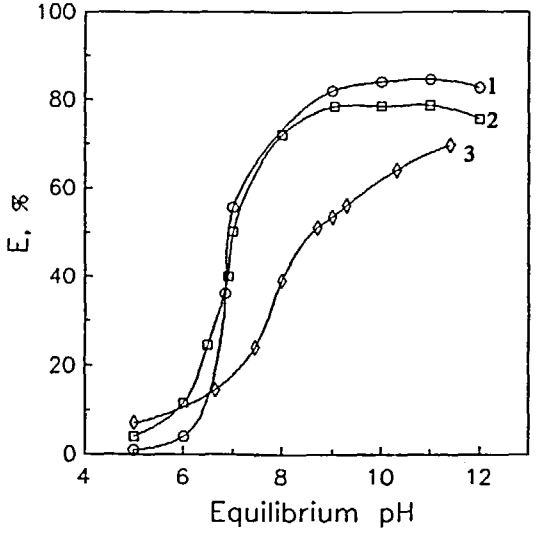


FIG. 3 The effect of equilibrium pH on the extraction of lincomycin with different donor extractants. O/A = 1, 25–30°C, 3 minutes. Curve 1 = 60% TRPO in kerosene; 2 = pure TBP; 3 = 30% TRAO and 10% *n*-hexyl alcohol in kerosene.

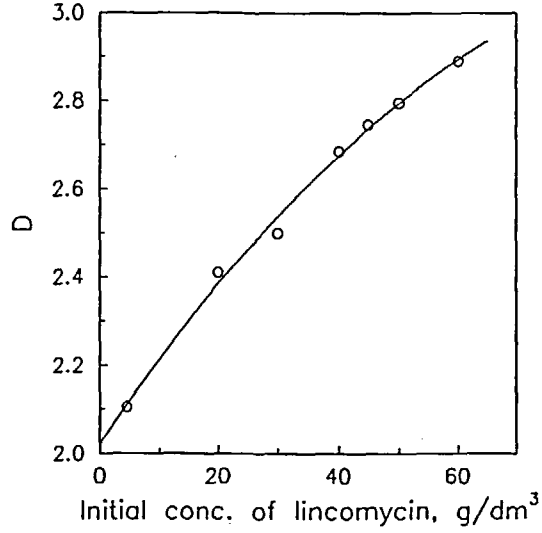


FIG. 4 The effect of initial concentration of lincomycin on the distribution ratio. Pure TBP as solvent, equilibrium pH 10–11, O/A = 0.5, 27°C, 3 minutes.

centration of lincomycin in the aqueous phase. It has been reported that lincomycin can self-associate at high concentrations (9). The species with a higher degree of association can be extracted predominantly by neutral extractants due to its high hydrophobicity. This has been verified for many extraction systems. For example, the dimer of butyric acid increases with an increase of the initial concentration in the aqueous phase, and hence the extraction with chloroform or nitrobenzene as the solvent also increases with an increase of its initial concentration in the aqueous phase (19).

## Effect of Temperature on the Extraction of Lincomycin with Neutral Phosphorus Esters

The effects of temperature are shown in Fig. 5. It can be seen that the extraction of lincomycin with TBP or TRPO as the solvent increases with an increase in temperature. Temperature has a stronger influence on TBP than on TRPO as solvent. According to the following equation,

$$\log D = -\frac{\Delta H}{2.303RT} + C$$

the heat effect of the extraction process, $\Delta H$, is 14.0 kJ/mol for TRPO

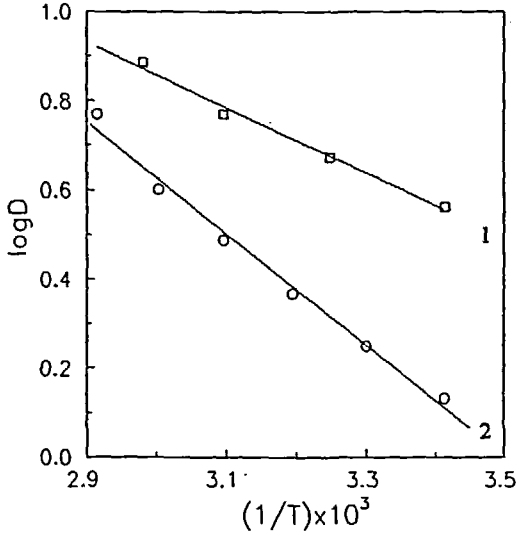


FIG. 5 Dependence of the distribution ratio on temperature. Curve 1 = 60% TRPO in kerosene, 2 = pure TBP; O/A = 1, 3 minutes.

and 23.9 kJ/mol for TBP. It can be seen that the extraction of lincomycin with neutral phosphorus esters is an endothermal process. Therefore, an increase in temperature in the production process favors the extraction of lincomycin.

## Effect of Additives on the Extraction of Lincomycin with Neutral Donor Extractants

Our previous work (20) showed that extraction can be depressed by using a mixture of solvents as extractants in some cases. In this work it has been found that the addition of long-chain alcohol is unfavorable for the extraction of lincomycin with neutral donor solvents. As shown in Fig. 6, *n*-hexyl alcohol has a seriously antagonism to TRAO for the extraction of lincomycin. This is due to the interaction between *n*-hexyl alcohol and TRAO as shown below:

$$R_3N{=}O\cdots\cdots HOC_6H_{13}$$

Accordingly, the effective concentration of extractant is greatly decreased. The stability of this kind of molecular association species increases with an increasing donating ability of the oxygen atom. It can be



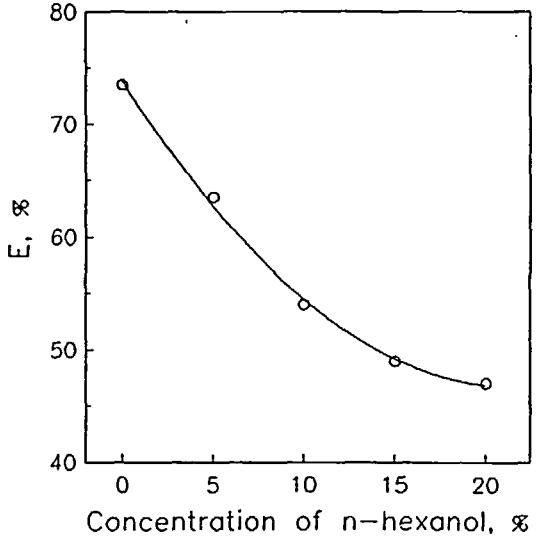


FIG. 6 Effect of addition of *n*-hexanol on the extraction of lincomycin with TRAO. 30% TRAO and *n*-hexanol in kerosene; equilibrium pH 9–10; O/A = 1, 27°C, 3 minutes.

deduced that the effect of interaction between *n*-hexyl alcohol and TBP should be less because TBP has a lower donating ability than TRAO. This has been verified by the results shown in Fig. 7.

## Stripping of Lincomycin in the Organic Phase with Hydrochloric Acid

As mentioned above, lincomycin is extracted as a free base (or in polymeric form) from weakly alkaline solutions by neutral donor reagents. The percentage of extraction of lincomycin decreases sharply with a decrease in pH, as shown in Fig. 3. Therefore, the stripping of lincomycin ($E_s$) in the organic phase back to the aqueous phase could be carried out with inorganic acids as stripping agent. In the practical process of production, hydrochloric acid is used and the equilibrium pH of the solution is controlled to 2–3. The stripping process of lincomycin with hydrochloric acid is carried out through the formation of lincomycin hydrochloride.

The stripping of lincomycin extracted with neutral donor extractants follows the common rule of solvent extraction, that is, the easier the extraction, the more difficult is the stripping. The stripping of lincomycin



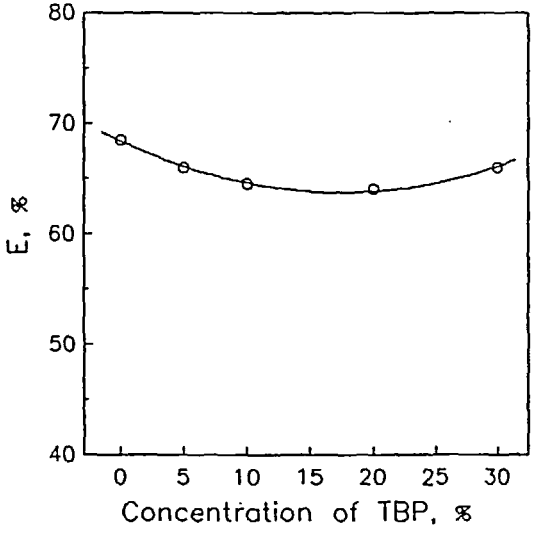


FIG. 7 Effect of addition of *n*-hexanol on the extraction of lincomycin with TBP. TBP and 50% *n*-hexanol in kerosene; equilibrium pH ~11; O/A = 1, 30°C, 5 minutes.

extracted with TRPO or TBP as solvent is shown in Fig. 8. For TBP as the solvent, the percentage of stripping is independent of the ratio of organic phase to aqueous phase used in the experiments. This indicates that lincomycin can be concentrated through the stripping process. For TRPO as the solvent, the percentage of stripping decreases with an increase in the phase ratio. The percentage of stripping can only reach 80% when TRAO is used as the extractant.

The extraction of lincomycin with neutral donor extractants is different from that with alcohols in which the hydroxyl group has either a donor or an acceptor property. For neutral donor reagents, the power for extraction of lincomycin increases with an increase of the donating ability of the active oxygen atom. For long-chain alcohols, extraction is not greatly influenced by structure and carbon-chain length, but depends mainly on the molarity of the alcohols used (12). The difference in the two types of extractants also can be explained by the donor and acceptor properties of functional groups in lincomycin molecules. It can be seen from Fig. 1 that oxygen atoms in the hydroxyl and carbonyl groups have electron donor properties, and that hydrogen atoms in the amide and hydroxyl groups have electron acceptor properties. Neutral donor reagents have



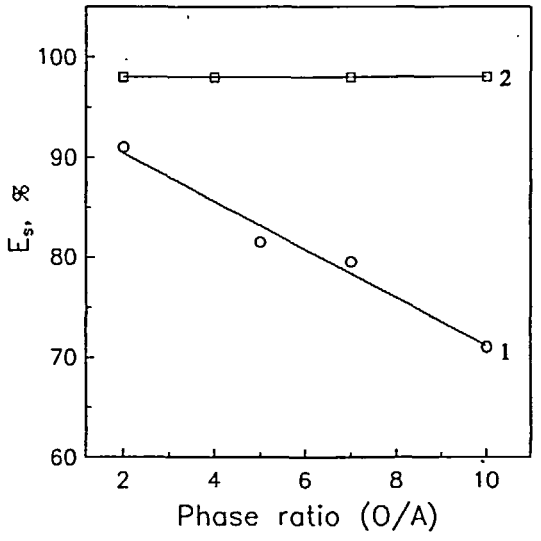


FIG. 8 Effect of phase ratio on the stripping of lincomycin with hydrochloric acid. Curve 1 = 60% TRPO and 4 g/dm$^3$ lincomycin in kerosene; 2 = 4 g/dm$^3$ lincomycin in TBP; equilibrium pH 2–3, 20°C, 5 minutes.

only an electron donor group and can only extract lincomycin through interaction with the acceptors in lincomycin. Obviously, an extractant with a higher donor power can form more stable association species with lincomycin.

Research on any new solvent system applicable to the extraction of lincomycin should consider such factors as extraction, stripping, and selectivity. Amine oxides can extract impurities such as protein, amino acids, and coloring matter in the fermentation broth filtrate due to its high donor power. It has been found that when TRAO is used to extract lincomycin from real fermentation filtrate, the stripping solutions contain some impurities which can be precipitated with acetone, resulting in a lower recovery of lincomycin of bad quality. Therefore, by comprehensively considering various factors, it is estimated that TBP or TRPO as extractant has good prospects for the recovery of lincomycin from fermentation filtrate. The experimental results to date have shown that a high-grade lincomycin product can be prepared from a process with TBP or TRPO used as the solvent (8).



## CONCLUSIONS

1. The extraction of lincomycin with neutral donor extractants has been studied, and the powers of neutral donor reagents for the extraction of lincomycin are in accord with the strength of their donor property.
2. The extraction of lincomycin with neutral phosphorus esters is an endothermal process, and the derived values of $\Delta H$ are 14.0 kJ/mol for TRPO and 23.9 kJ/mol for TBP.
3. The addition of a long-chain alcohol to the neutral donor reagents is unfavorable for the extraction of lincomycin because interaction between them through hydrogen bonding results in a decrease of the effective concentration of the reagents.
4. Amine oxides are not suitable as extractants for lincomycin because their higher extraction power results in low selectivity and difficulty in stripping.

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