

## DETERMINATION OF EXTRACTION CONSTANT, TRUE PARTITION COEFFICIENT AND FORMATION CONSTANT OF ION-PAIR COMPLEXES OF QUATERNARY AMMONIUM SALTS, TETRABUTYLAMMONIUM BROMIDE AND ISOPROPAMIDE IODIDE, WITH SOME ORGANIC ANIONS BY A SOLVENT EXTRACTION TECHNIQUE

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### SUMMARY

A new method of determining the extraction constant ( $K_e$ ), the true partition coefficient (TPC) and the formation constant ( $K_f$ ) of ion-pairs, was developed by the solvent extraction technique.  $K_e$  and TPC were estimated from the reciprocals of the intercept and the slope of the regression line obtained by plotting

$$\frac{[B_w^T]}{APC - dA} \text{ vs } \left( \frac{[B_w^T] dA}{APC - dA} + [A_w^T] + [B_w^T] \right)$$

in the following equation.

$$\frac{[B_w^T]}{APC - dA} = \frac{1}{K_e} + \left( \frac{[B_w^T] dA}{APC - dA} + [A_w^T] + [B_w^T] \right) \times \frac{1}{TPC}$$

where  $[A_w^T]$  and  $[B_w^T]$  are the total concentrations of the cationic compound A and that of the anionic compound B in the aqueous phase respectively, APC is the apparent partition coefficient of A, dA is the partition coefficient of cation  $A^+$ .  $K_f$ , which is expressed by  $K_e/TPC$ , was then calculated. These constants were determined for the ion-pair extraction of tetrabutylammonium bromide and isopropamide iodide with 4 organic anions, i.e. benzoic acid, *p*-toluenesulfonic acid, salicylic acid and taurodeoxycholic acid. This new method might be applicable to other ion-pairs without further assumptions except that the molar ratio of the ion-pair formation be 1 : 1.

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## INTRODUCTION

Although the extraction of pharmaceutical cations into organic solvents as ion-pairs with organic anions has received a great deal of attention, few investigations into the true partition coefficient (TPC) of the ion-pairs and into the formation constant ( $K_f$ ) of the ion-pairs in the aqueous phase have been reported.

The extraction constant ( $K_e$ ), the ionization constant and TPC were determined for the ion-pair of di-iso-amylamine with tropaeolin 00, of which moieties, i.e. di-iso-amylamine (cation) and tropaeolin 00 (anion), were not extracted at all into the methylene chloride phase (organic phase) from the aqueous phase (Divita and Biles, 1961). Since both cation and anion of an ion-pair are often extracted to some extent into the organic phase, a more generally acceptable method would be necessary.

Many investigators have determined  $K_e$  of several ion-pairs by assuming the TPC to be large enough to neglect the existence of the ion-pairs in the aqueous phase (Schill, 1965; Persson and Schill, 1966; Modin and Schill, 1967; Fung and Ow, 1972; Lee et al., 1978; Kitakawa et al., 1980). However, this assumption has not been confirmed yet.

The purpose of the present study was to develop a method of determining  $K_e$ , TPC and  $K_f$  without any limitations on either TPC or extraction of the cation and anion into the organic phase.

## THEORY

The extraction of a cation into the organic phase as an ion-pair with an anion may be represented by Scheme 1 when the molar ratio of the ion-pair formation is 1 : 1.



where  $A_w^+$  represents the cation in the aqueous phase,  $B_w^-$  is the anion in the aqueous phase,  $AB_w$  is the ion-pair in the aqueous phase,  $AB_o$  is the ion-pair in the organic phase,  $K_f$  is the formation constant of the ion-pair, and TPC is the true partition coefficient of the ion-pair.

$K_f$  and TPC are defined as:

$$K_f = \frac{[AB_w]}{[A_w^+][B_w^-]} \quad (1)$$

$$TPC = \frac{[AB_o]}{[AB_w]} \quad (2)$$

The extraction constant of the ion-pair,  $K_e$ , is defined as:

$$K_e = \frac{[AB_o]}{[A_w^+][B_w^-]} = K_f \times TPC \quad (3)$$

The partition coefficient of cation  $A^+$ ,  $dA$  and the apparent partition coefficient of  $A$ ,  $APC$ , are defined as:

$$dA = \frac{[A_0^+]}{[A_w^+]} \quad (4)$$

$$APC = \frac{[A_0^T]}{[A_w^T]} \quad (5)$$

where  $A_0^+$  is the cation in the organic phase and

$$[A_0^T] = [AB_0] + [A_0^+] \quad (6)$$

$$[A_w^T] = [AB_w] + [A_w^+] \quad (7)$$

Combining and rearranging Eqns. 1–7 give Eqn. 8 or Eqn. 10 through the intermediate steps included in the Appendix.

$$\frac{[B_w^T]}{APC - dA} = \frac{1}{K_e} + \left( \frac{[B_w^T] dA}{APC - dA} + [A_w^T] + [B_w^T] \right) \times \frac{1}{TPC} - \frac{[AB_w]}{TPC} \quad (8)$$

$$\text{where } [B_w^T] = [AB_w] + [B_w^-] \quad (9)$$

$$\frac{APC - dA}{[B_w^-]} = K_e - K_f \times APC \quad (10)$$

Eqn. 10 indicates that the  $TPC (= K_e/K_f)$  of an ion-pair is always larger than  $APC$  inasmuch as  $APC$  increases with the addition of an anion. Therefore,  $[AB_w]/TPC$  may be neglected when compared to  $[B_w^T]/APC - dA$  in Eqn. 8, and  $[B_w^-]$  would approximate to  $[B_w^T]$  in Eqn. 10 assuming  $[B_w^T] \gg [AB_w]$ . This requirement,  $[B_w^T] \gg [AB_w]$ , may be satisfied either when  $TPC$  is large enough to neglect  $[AB_w]$ , or when  $[B_i] \gg [A_i]$  holds, where  $[A_i]$  and  $[B_i]$  mean the concentrations of  $A$  and  $B$  added initially to the aqueous phase respectively.

Thus, Eqns. 8 and 10 yield Eqns. 11 and 12 under this condition, respectively.

$$\frac{[B_w^T]}{APC - dA} = \frac{1}{K_e} + \left( \frac{[B_w^T] dA}{APC - dA} + [A_w^T] + [B_w^T] \right) \times \frac{1}{TPC} \quad (11)$$

$$\frac{APC - dA}{[B_w^T]} = K_e - K_f \times APC \quad (12)$$

Values for  $K_e$ ,  $TPC$  and  $K_f$  may be estimated from the slope and intercept of the respective lines obtained by plotting Eqns. 11 and 12. However, Eqn. 11 has an advantage over Eqn. 12 in the reproducibility of the final data because small errors in  $APC$  affect

both the slope and the intercept of the regression line of Eqn. 12 more greatly than those of Eqn. 11, when the line is determined by the method of linear least-squares. Therefore, Eqn. 11 was employed in this study for the estimation of  $K_e$ , TPC and  $K_f$ .

## MATERIALS AND METHODS

### Materials

Tetrabutylammonium bromide (I)<sup>1</sup> and isopropamide iodide (II)<sup>2</sup> were selected as cationic compounds. Benzoic acid<sup>3</sup>, *p*-toluenesulfonic acid<sup>4</sup>, sodium salicylate<sup>5</sup> and sodium taurodeoxycholate<sup>6</sup> were selected as anionic compounds. The anionic solution of benzoic acid or *p*-toluenesulfonic acid was prepared with each equivalent of sodium hydroxide. All other chemicals used were commercially available and of analytical grade.

### Partition studies

*n*-Octanol saturated with 0.1 M phosphate buffer (pH 7.4) and 0.1 M phosphate buffer (pH 7.4) saturated with *n*-octanol were used. The anionic compounds and I or II were dissolved in 0.1 M phosphate buffer (pH 7.4) saturated with *n*-octanol. The concentration of I or II was kept constant at  $1 \times 10^{-4}$  M, with only the concentration of the anionic compound being varied. The concentration of sodium taurodeoxycholate was kept far below the critical micelle concentration to avoid the micelle formation. A 10-ml aliquot of each aqueous buffer solution containing I or II and an anionic compound was mixed vigorously<sup>7</sup> with 10 ml of *n*-octanol in a screw-capped test tube for about 5 min and was shaken for 2 h at 25°C in a temperature-controlled water bath. After standing 30 min at 25°C in the water bath, two phases were separated by centrifugation at 3000 rpm for 10 min and the organic phase was discarded by aspiration. The aqueous phase was assayed for  $[A_w^T]$  or  $[B_w^T]$ .

For the analysis of I or II in the aqueous phase, a modification of Santoro (1960) was used. A 2-ml aliquot of the aqueous phase was added to a screw-capped test tube containing 5 ml of methyl orange buffer (pH 10.2)<sup>8</sup> and 10 ml of chloroform. After vigorous agitation for about 5 min, two phases were separated by centrifugation at 3000 rpm for about 5 min and a 5-ml aliquot of the chloroform layer was added to 2 ml of 0.5 N HCl in ethanol. Then the absorbance was measured spectrophotometrically at 525 nm.

The  $[B_w^T]$  of the aqueous phases containing I were measured spectrophotometrically after appropriate dilution of each aqueous phase with 0.1 M phosphate buffer (non-saturated with *n*-octanol) at 224 nm for benzoic acid, 222 nm for *p*-toluenesulfonic acid and 295 nm for salicylic acid. The  $[B_w^T]$  of salicylic acid in the aqueous phase containing II was also determined at 295 nm.

On the other hand, the absorbance of benzoic acid or *p*-toluenesulfonic acid was inter-

<sup>1,4</sup> Tokyo Kasei Kogyo, Tokyo, Japan.

<sup>2</sup> Sumitomo Chemicals, Osaka, Japan.

<sup>3</sup> Kanto Chemicals, Tokyo, Japan.

<sup>5</sup> Koso Chemicals, Tokyo, Japan.

<sup>6</sup> Sigma Chemicals, St. Louis, U.S.A.

<sup>7</sup> Vortex Mixer, Scientific Industries, New York, U.S.A.

<sup>8</sup> Prepared fresh on the day of use according to Gaginella et al. (1973).

ferred with by the co-existence of II. In these cases and in the assay of taurodeoxycholic acid,  $[B_w^T]$  were calculated as follows:

$$[B_w^T] = [B_i] - ([A_i] - [A_w^T]) + [A_w^+] dA - [B_w^-] dB \quad (13)$$

The dB can be measured independently and defined as:

$$dB = \frac{[B_0^-]}{[B_w^-]} \quad (14)$$

where  $B_0^-$  is the anion in the organic phase.

When Eqn. 11 or 12 holds,  $[AB_w]$  may be neglected and Eqn. 13 will be modified to Eqn. 15 and the  $[B_w^T]$  of the anions mentioned above can be calculated.

$$[B_w^T] = \frac{([B_i] - [A_i]) + (1 + dA)[A_w^T]}{1 + dB} \quad (15)$$

dB of taurodeoxycholic acid was assumed to be zero in this study.

## RESULTS AND DISCUSSION

TPC of ion-pairs could be predicted approximately from APC of the ion-pairs, since APC approached TPC at  $[B_i]$  infinity, as suggested by Eqns. 3 and 12. When TPC of ion-pairs was predicted to be relatively small, the anions were added to hold the conditions of  $[B_i] \gg [A_i]$ ; thus, the requirement for Eqns. 11 and 12 could be fulfilled.

TPC and  $K_e$  were estimated from the reciprocals of the slope and the intercept of the regression line obtained by plotting

$$\frac{[B_w^T]}{APC - dA} \text{ vs } \left( \frac{[B_w^T] dA}{APC - dA} + [A_w^T] + [B_w^T] \right)$$

of Eqn. 11. The representative plots are shown in Figs. 1 and 2, and the calculated values for  $K_e$ , TPC and  $K_f (= K_e/TPC)$  are listed in Table 1. All systems followed a good linear relationship, as defined by Eqn. 11.

When the slope of the line approached zero, TPC could not be estimated reproducibly, and this suggested that TPC of the ion-pair was very large. Therefore, it was impossible to calculate TPC and  $K_f$  for the ion-pair of taurodeoxycholic acid with I or II, but  $K_e$  could be estimated reproducibly even when the slope approached zero.

The values for  $K_e$  and the molar ratio of an anion against a cation,  $n$ , were also calculated according to the definition by Lee et al. (1978) and are listed in Table 1 for the comparison with the present study.

A small error in TPC may greatly affect  $K_e$  when  $K_f$  is small, as is clear from Eqn. 3. The  $K_e$  value calculated by the method of Lee et al. (1978) was very different from the  $K_e$  obtained from Eqn. 11 in the case of the II-benzoate ion-pair, probably due to the small TPC and small  $K_f$  of the ion-pair. Consequently, the 'n' of the II-benzoate ion-pair

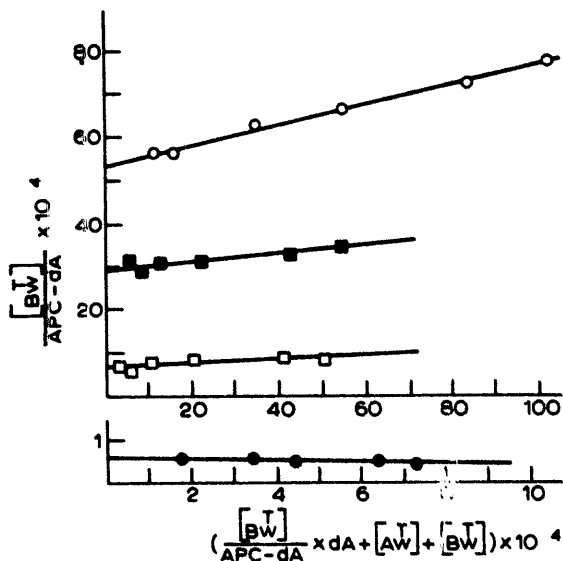


Fig. 1. Plots of  $[B_W^T]/APC - dA$  vs  $([B_W^T]dA/APC - dA + [A_W^T] + [B_W^T])$  for the ion-pairs of tetra-butylammonium bromide with various anions. Representative data from single experiment are shown. Tetrabutylammonium bromide ( $1.0 \times 10^{-4}$  M) was equilibrated with various concentrations of anions at  $25^\circ\text{C}$ . Symbols and the initial concentrations of each anion ( $[B_i]$ ) are:  $\bullet$ , taurodeoxycholate ( $2-8 \times 10^{-4}$  M);  $\square$ , salicylate ( $10-50 \times 10^{-4}$  M);  $\blacksquare$ , *p*-toluenesulfonate ( $10-50 \times 10^{-4}$  M);  $\circ$ , benzoate ( $30-80 \times 10^{-4}$  M).

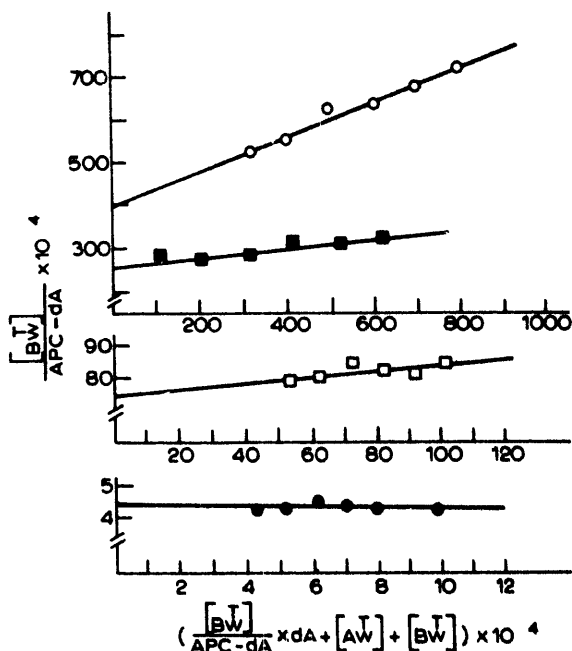


Fig. 2. Plots of  $[B_W^T]/APC - dA$  vs  $([B_W^T]dA/APC - dA + [A_W^T] + [B_W^T])$  for the ion-pairs of isopropamide iodide with various anions. Representative data from single experiment are shown. Isopropamide iodide ( $1.0 \times 10^{-4}$  M) was equilibrated with various concentrations of anions at  $25^\circ\text{C}$ . Symbols and the initial concentrations of each anion ( $[B_i]$ ) are:  $\bullet$ , taurodeoxycholate ( $4-10 \times 10^{-4}$  M);  $\square$ , salicylate ( $50-100 \times 10^{-4}$  M);  $\blacksquare$ , *p*-toluenesulfonate ( $100-600 \times 10^{-4}$  M);  $\circ$ , benzoate ( $300-800 \times 10^{-4}$  M).

TABLE 1  
 VALUES<sup>a</sup> FOR EXTRACTION CONSTANT ( $K_e$ ), TRUE PARTITION COEFFICIENT (TPC), FORMATION CONSTANT ( $K_f$ ) AND MOLAR RATIO (n) OF TETRABUTYLAMMONIUM ION-PAIRS AND ISOPROPAMIDE ION-PAIRS

Ion-pair	This study <sup>b</sup>			Lee's method <sup>c</sup>		
	Anion	$K_e$ ( $M^{-1}$ )	TPC	$K_f$ ( $M^{-1}$ )	$K_e$ ( $M^{-1}$ )	n
Tetrabutylammonium	Benzoate	$1.75(0.07) \times 10^2$	5.3(0.2)	$3.29(0.28) \times 10$	$2.24(0.11) \times 10^2$	0.89(0.02)
	<i>p</i> -Toluenesulfonate	$3.33(0.03) \times 10^2$	10.6(1.2)	$3.14(0.38) \times 10$	$3.35(0.20) \times 10^2$	0.97(0.02)
	Salicylate	$1.37(0.07) \times 10^3$	27.2(6.4)	$5.04(0.97) \times 10$	$1.56(0.18) \times 10^3$	0.92(0.02)
	Taurodeoxycholate	$1.55(0.05) \times 10^4$	—	—	$1.45(0.12) \times 10^4$	1.13(0.05)
Isopropamide	Benzoate	$2.81(0.19) \times 10$	2.6(0.1)	$1.08(0.04) \times 10$	$1.61(0.45) \times 10^2$	0.61(0.01)
	<i>p</i> -Toluenesulfonate	$4.33(0.27) \times 10$	8.7(0.7)	$0.50(0.01) \times 10$	$0.99(0.27) \times 10^2$	0.83(0.04)
	Salicylate	$1.37(0.05) \times 10^2$	11.7(1.1)	$1.17(0.14) \times 10$	$1.69(0.10) \times 10^2$	0.93(0.01)
	Taurodeoxycholate	$2.44(0.32) \times 10^3$	—	—	$2.41(0.37) \times 10^3$	1.04(0.06)

<sup>a</sup> Shown by the mean of 3 different experiments at 25°C with S.D. in the parentheses.

<sup>b</sup> Calculated by Eqn. 11 of this study.

<sup>c</sup> Calculated by the method of Lee et al. (1978). The n is the molar ratio of the anion against the cation.

was far from unity. These results suggested that the assumption of Lee et al. (1978), that TPC are large enough to neglect  $[AB_w]$  compared to  $[A_w^+]$ , might not generally hold.

Since the  $K_f$  of I or II ion-pairs were not affected greatly by the kinds of anion, it seemed that TPC might be the primary factor in the magnitude of the  $K_e$ .

In summary, a new method proposed in this paper might be applicable to other ion-pairs without limitations either on TPC or on extraction of cation and anion into the organic phase inasmuch as the molar ratio of the ion-pair formation is 1 : 1.  $K_e$ , TPC and  $K_f$  could be estimated simultaneously by this method.

#### APPENDIX

Eqn. 8 was derived as follows:

$$K_e = \frac{[AB_0]}{[A_w^+][B_w^-]} = \frac{[AB_0]}{([A_w^T] - [AB_w])([B_w^T] - [AB_w])} \quad (16)$$

Substituting Eqn. 2 into Eqn. 16 and rearranging it yields:

$$\frac{1}{K_e} = \frac{[A_w^T][B_w^T]}{[AB_0]} - \frac{[A_w^T] + [B_w^T]}{TPC} + \frac{[AB_w]}{TPC} \quad (17)$$

$[AB_0]$  may be replaced by Eqn. 18 through the following intermediates:

$$\begin{aligned} [AB_0] &= [A_0^T] - [A_0^+] = [A_0^T] - [A_w^+]dA = [A_0^T] - ([A_w^T] - [AB_w]) \\ &= [A_0^T] - \left( [A_w^T] - \frac{[AB_0]}{TPC} \right) dA \end{aligned}$$

Then,

$$[AB_0] = \frac{[A_0^T] - [A_w^T] dA}{1 - \frac{dA}{TPC}} \quad (18)$$

Substituting Eqns. 15 and 18 into Eqn. 17 and rearranging it gives Eqn. 8.

Eqn. 10 was derived from the rearrangement of Eqn. 19.

$$APC = \frac{[A_0^T]}{[A_w^T]} = \frac{[A_0^+] + [AB_0]}{[A_w^+] + [AB_w]} = \frac{[A_w^+] dA + [A_w^+][B_w^-] K_e}{[A_w^+] + [A_w^+][B_w^-] K_f} = \frac{dA + [B_w^-] K_e}{1 + [B_w^-] K_f} \quad (19)$$

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