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A Rapid Method for the Determination of Distribution Coefficient of Bases for Biological Purposes

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A rapid method is described for the determination of the distribution coefficient of bases between an organic solvent and water using an automatic titrator. The errors involved in the method are discussed and the reproducibility of the method is demonstrated on the system lidocain, cod liver oil, water.

The distribution of amino compounds between water and lipoids is of interest in studying pharmacological properties. The traditional method of determining the distribution coefficient is to mix the hydrochloride of the compound with a buffer solution and an organic solvent and then analyse one or both layers [1]. This type of investigation is rather time consuming and unsuitable for screening new compounds. The following method which utilizes an automatic titrator is rapid and gives reproducible results.

PRINCIPLE

To an aqueous solution of the pure hydrochloride, NaOH is added by an automatic titrator operating as a pH-stat until the pH at which approximately 1/5 of the hydrochloride is converted to the free base is obtained. When the pH is between 6 and 10 the quantity of alkali added is equivalent to the amount of free base liberated. An organic solvent, immiscible with water, is then added. As some of the base dissolves in the organic layer, more alkali is required to maintain the pH at the original value. This amount is therefore added by the instrument. When equilibrium is obtained the quantity of alkali added is equivalent to the total quantity of free base present in both the aqueous and the organic layers. It is possible to calculate the distribution coefficient from the amount of alkali added before and after the addition of the organic solvent.

Calculations

The following symbols are used:

W_1	ml of water before the addition of the organic layer
W_2	» » » after » » » » » »
L_o	milliequivalents of hydrochloride used
L_1	milliequivalents of base present before the addition of the organic layer
L_2	milliequivalents of base present after the addition of the organic layer
S	weight in grams of organic solvent
C_b^S	molal concentration of base in the organic layer
C_b^W	molar concentration of base in the aqueous layer
C_a^+	molar concentration of acid in the aqueous layer
$a_{H_3O^+}$	activity of the hydrogen ion
γ	activity coefficient of the acid
K_a	dissociation constant of the acid
F	distribution coefficient of the base
*	is used to indicate concentrations in the water layer after the addition of the organic layer

Since the base is uncharged its activity can be taken as C_b^W. The dissociation equations (1) before and (2) after the addition of the organic solvent are

$$K_a = \frac{C_b^W a_{H_3O^+}}{\gamma C_a^+} \qquad (1) \qquad\qquad K_a = \frac{{}^*C_b^W a_{H_3O^+}}{\gamma\, {}^*C_a^+} \qquad (2)$$

$a_{H_3O^+}$ is the same in both cases since the pH is constant and similarly for γ as the ionic strength is constant (a^+ is replaced by Na^+). From (1) and (2) it follows that

$${}^*C_b^W = C_b^W \frac{{}^*C_a^+}{C_a^+} \qquad (3)$$

but

$$C_b^W = \frac{L_1}{W_1} \quad (4); \qquad C_a^+ = \frac{L_o - L_1}{W_1} \quad (5); \qquad {}^*C_a^+ = \frac{L_o - L_2}{W_2} \quad (6)$$

If (4), (5), and (6) are substituted in eqn. (3) it follows that

$${}^*C_b^W = \frac{L_1(L_o - L_2)}{W_2(L_o - L_1)} \qquad (7)$$

Since the total quantity of base after the addition of the organic solvent is L_2 the equation becomes

$$L_2 = S\ C_b^S + W_2 {}^*C_b^W \qquad (8)$$

If (7) is combined with (8)

$$C_b^S = \frac{L_o(L_2 - L_1)}{S(L_o - L_1)} \qquad (9)$$

and since $F = C_b^S / {}^*C_b^W$ eqn. (10) is obtained

$$F = \frac{L_o(L_2 - L_1)W_2}{L_1(L_o - L_2)S} \qquad (10)$$

DISCUSSION

The distribution coefficient F obtained from eqn. (10) refers to concentrations expressed in molality in the organic layer. It is noteworthy that neither the pH nor the activity coefficients are used in the calculation of F. The only important factors are constant temperature and constant pH during the determination of L_1 and L_2. The experimental errors can be calculated in the following way.

By partial differentiation of eqn. (10) it can be shown that an error δL in one of the parameters L_o, L_1 or L_2 results in an error δF in F, which may be obtained from the equations

$$\frac{\delta F}{\delta L_o} = F\frac{L_2}{L_o(L_o-L_2)} \quad (11); \qquad \frac{\delta F}{\delta L_1} = F\frac{L_2}{L_1(L_2-L_1)} \quad (12)$$

$$\frac{\delta F}{\delta L_2} = F\frac{L_o-L_1}{(L_o-L_2)(L_2-L_1)} \quad (13)$$

Since the instrument is adjusted to give a predetermined pH and L_1 is read after the solution has reached equilibrium the pH instability of the instrument will only effect the L_2 readings. If the pH instability is dpH the error dL_2 in L_2 can be calculated as follows:

The total quantity of the amino compound in the aqueous layer can be expressed in two ways giving

(*1*) $$W_2(*C_b^W + *C_a{}^+) = L_o - S\ C_b^S \quad (14)$$

but since $C_b^S = F*C_b^W$ and $*C_a{}^+ = \frac{L_o-L_2}{W_2}$ the equation

becomes $W_2*C_b^W + L_o - L_2 = L_o - S\ F*C_b^W$ or

(*2*) $$*C_b^W = \frac{L_2}{W_2 + SF} \quad (15)$$

Substituting the values for C_a and C_b^W in (2) gives

$$K_a = \frac{L_2\ W_2\ a_{H_3O^+}}{\gamma(W_2 + SF)\ (L_o-L_2)} \quad (16)$$

Differentiating with respect to pH gives

$$-K_a\gamma(W_2 + SF)\frac{dL_2}{dpH} = W_2 a_{H_3O^+}\frac{dL_2}{dpH} - \frac{L_2W_2}{\log e}a_{H_3O^+} \quad (17)$$

Considering eqn. (16) and rearranging

$$\frac{dL_2}{dpH} = \frac{L_2(L_o-L_2)}{L_o \log e} \quad (18)$$

If this is substituted in (13) it follows that

$$\frac{dF}{dpH} = F\frac{L_2(L_o-L_1)}{L_o(L_2-L_1)\log e} \quad (19)$$

Table 1. $\frac{L_2(L_o-L_1)}{L_o(L_2-L_1)} \cdot \frac{1}{\log e}$

L_1/L_o \ L_2/L_o	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
0.1	4.14	3.11	2.76	2.59	2.49	2.42	2.37	2.33
0.2		5.53	3.68	3.07	2.76	2.58	2.46	2.37
0.3			6.45	4.03	3.22	2.82	2.58	2.42
0.4				6.91	4.15	3.22	2.76	2.49
0.5					6.91	4.03	3.07	2.59
0.6						6.45	3.68	2.76
0.7							5.53	3.11
0.8								4.14

The standard deviation σ pH in pH can be determined if the test solution is replaced by a buffer with known capacity. From this value the relative standard deviation $\sigma F/F$ resulting from the pH instability is calculated. To simplify the calculation the values of

$$\frac{L_2(L_o-L_1)}{L_o(L_2-L_1)\ \log\ e}$$

for different L_1/L_o and L_2/L_o are given in Table 1. It is readily seen that if σ pH is 0.003 the standard deviation of F is about 1 % for most experimental conditions.

The values L_1, L_2 and L_o are obtained from the recording paper. L_1 and L_2 are obtained from assymptotic curves and L_o from the point of inflexion. The assymptotic values can be extrapolated by any standard method, *e.g.* by plotting the values of L_1 or L_2 at different times t, after an arbitrarily chosen zero time, against $1/t$ and extrapolating to $1/t = 0$.

In the determination of L_o the endpoint can be considerably sharpened by adding a few ml of a solvent immiscible with water.

Even with these precautions the values of L_o, L_1 and L_2 include small evaluation errors. By means of eqns. (11)—(13) it is possible to calculate the error in F resulting from the evaluation errors. If the standard deviations in the L values are σL_o, σL_1 and σL_2 the corresponding standard deviations in F, σF_o, σF_1 and σF_2 are thus obtained from

$$\frac{\sigma F_o}{F} = \frac{\sigma L_o}{L_o} \frac{L_2}{L_o-L_2} \tag{20}$$

$$\frac{\sigma F_1}{F} = \frac{\sigma L_1}{L_o} \frac{L_o\ L_2}{L_1(L_2-L_1)} \tag{21}$$

$$\frac{\sigma F_2}{F} = \frac{\sigma L_2}{L_o} \frac{L_o(L_o-L_1)}{(L_o-L_2)(L_2-L_1)} \tag{22}$$

Table 2. The value of $L_2/(L_0-L_2)$ for different values of L_2/L_0.

L_2/L_0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
$\frac{L_2}{L_0-L_2}$	0.11	0.25	0.43	0.67	1.00	1.50	2.33	4.00	9.00

Table 3. The values of $L_0L_2/[L_1(L_2-L_1)]$ for different values of L_1/L_0 and L_2/L_0.

L_2/L_0 \ L_1/L_0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8
0.2	20.0	—	—	—	—	—	—	—
0.3	15.0	15.0	—	—	—	—	—	—
0.4	13.3	10.0	13.3	—	—	—	—	—
0.5	12.5	8.3	8.3	12.5	—	—	—	—
0.6	12.0	7.5	6.7	7.5	12.0	—	—	—
0.7	11.7	7.0	5.8	5.8	7.0	11.7	—	—
0.8	11.4	6.7	5.3	5.0	5.3	6.7	11.4	
0.9	11.3	6.4	5.0	4.5	4.5	5.0	6.4	11.3

Table 4. The values of $\frac{L_0(L_0-L_1)}{(L_0-L_2)(L_2-L_1)}$ for different values of L_1/L_0 and L_2/L_0.

L_2/L_0 \ L_1/L_0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8
0.2	11.3	—	—	—	—	—	—	—
0.3	6.4	11.4	—	—	—	—	—	—
0.4	5.0	6.7	11.7	—	—	—	—	—
0.5	4.5	5.3	7.0	12.0	—	—	—	—
0.6	4.5	5.0	5.8	7.5	12.5	—	—	—
0.7	5.0	5.3	5.8	6.7	8.3	13.3	—	—
0.8	6.4	6.7	7.0	7.5	8.3	10.0	15.0	—
0.9	11.3	11.4	11.7	12.0	12.5	13.3	15.0	20.0

The total standard deviation σF in F due to evaluation errors is obtained from

$$\frac{\sigma F}{F} = \frac{1}{F}\sqrt{(\sigma F_0)^2 + (\sigma F_1)^2 + (\sigma F_2)^2} \qquad (23)$$

To facilitate the calculations the values of

$$\frac{L_2}{L_0-L_2}\,, \qquad \frac{L_0L_2}{L_1(L_2-L_1)} \quad \text{and} \quad \frac{L_0(L_0-L_1)}{(L_0-L_2)(L_2-L_1)}$$

for different values of L_1/L_0 and L_2/L_0 are given in Tables 2—4.

The L_o value can usually be estimated with a relative standard deviation of 0.1—0.5 % by direct or indirect methods. The $\sigma L_1/L_o$ and $\sigma L_2/L_o$ values can in each case be approximated from the shape of the curves. With good stirring of the solution they are very small, probably about 0.002.

If these values of σL_o, σL_1 and σL_2 are inserted in eqns. (20)—(23) it is found that $\sigma F/F$ is 0.02—0.03 provided that L_1 is not too small and the values L_1, L_2 and L_o are not too close together but evenly distributed on the recording paper.

Taking both the pH instability and the evaluation difficulties into consideration it is thus possible to obtain values of F with a relative standard deviation of 2—3 % when the following procedure is carried out.

PROCEDURE

4—5 milliequivalents of a salt of the base are accurately weighed, dissolved in water containing 0.1 % Bruj 35, and made up to the mark in a 50 ml volumetric flask. For each run, 5.00 ml of this solution is removed and kept well stirred during the experiment.

As a preliminary study an ordinary titration curve is run with the automatic titrator using 1 N NaOH in a 0.5 ml syringe. If precipitation of the base occurs the pH is noted.

Another 5.00 ml portion is placed in a water thermostat at 25° and the titrator is changed so that it will work as a pH-stat. The pH at which approximately 20 % of the amino compound is present as the free base is chosen. The calculated amount of water sufficient to prevent precipitation at that pH is added. The pH-stat is switched on and when the chosen pH is obtained L_1 mmoles of alkali have been added *i.e.* L_1 mmoles of the free base are present in the solution. 1—2 g of an organic solvent accurately weighed are then added from a syringe. Alkali is now added by the titrator and a quantity of the amino compound passes from the aqueous layer to the organic layer. When no more alkali is added, (usually after a few minutes), the run is complete.

Table 5. Distribution of lidocaine between cod liver oil and water at 25.0°. L_o = 0.4580.

S	W_2	pH	L_1	L_2	L_2 Corr.	F	Expt. No.
0.3071	10.19	7.40	0.1080	0.1880	0.1877	41.5	I
0.5931	10.22	7.40	0.1035	0.2235	0.2230	38.8	II
1.250	10.27	7.40	0.1045	0.2800	0.2790	35.1	III
2.309	10.33	7.40	0.1005	0.3305	0.3287	36.0	IV
4.607	10.38	7.40	0.1005	0.3815	0.3778	35.5	V
1.004	5.32	7.40	0.1050	0.3205	0.3197	35.9	VI
1.821	15.28	7.40	0.1050	0.2815	0.2800	36.0	VII
2.999	25.28	7.40	0.1120	0.2865	0.2841	34.1	VIII
2.010	35.36	8.00	0.2500	0.3630	0.3610	36.9	IX
1.990	20.33	7.70	0.1660	0.3330	0.3312	36.7	X
1.396	5.25	7.00	0.0449	0.2455	0.2445	35.9	XI
3.053	5.19	6.50	0.0151	0.1885	0.1865	32.6	XII
10.00	10.00	6.50	—	0.0064			
		7.00	—	0.0072			
		7.40	—	0.0080			
		7.70	—	0.0090			
		8.00	—	0.0101			

Example

The pH stability of the instrument (Titrator type TTT 1 with Titrigraph type SBR2/SBUI from Radiometer) was determined in the following way.

15.0 ml of a solution containing 0.2 moles NaH_2PO_4 and 0.2 moles Na_2HPO_4 in 1 liter were placed in a water thermostat at 25.0°. The instrument was adjusted to operate as a pH-stat at the same pH as that of the above solution. The syringe contained N NaOH. When the solution and instrument were in equilibrium 0.100 ml of N HCl was added. After the instrument had restored the initial pH by adding alkali, a further 0.100 ml of acid was added and this was repeated until 4 portions of acid had been neutralized. From 8 similar experiments 31 values for the volume of added alkali were obtained (one value was rejected as erroneous). These 31 values had a standard deviation of $\pm$ 0.010 ml which corresponds to a standard deviation of $\pm$ 0.003 pH units from one measurement to another.

To investigate the usefulness of the method the distribution coefficient of lidocaine between cod liver oil and water was determined with various volumes of both phases. The results are given in Table 5. In experiment I and II the amount of cod liver oil is probably too low to ensure ideal solutions and in experiment XII the value of L_1 is far below that recommended from Tables 1 and 2.

As the cod liver oil contained a small quantity of acid a blank run was carried out. The results are given in Table 5 (bottom) and the L_2 values were corrected accordingly.

From experiments III–XI a value for F of 35.8 is obtained with a standard deviation of $\pm$ 0.8. This reproducibility is in good agreement with the theoretical considerations above.

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