Journal of Chromatography, 92 (1974) 1–7

CHROM. 7302

APPLICATION OF A STATISTICAL METHOD TO COUNTER-CURRENT DISTRIBUTION OF ACIDS AND BASES AND THEIR SEPARATION BY DISCONTINUOUS CHANGE OF pH

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(First received September 18th, 1973; revised manuscript received December 17th, 1973)

SUMMARY

A statistical method is applied to the partition of an acid or a base between two phases in a counter-current apparatus. Four equations are obtained, depending on whether the mobile phase is organic or aqueous. These expressions correlate the number of transfers and the position of the distribution curve maximum with pH, dissociation constant (K_{diss}) and distribution constant (K_r). It was found that two acids (or bases) with a $K_r \cdot K_{diss}$ product in a $1:10^m$ ratio are eluted with numbers of transfers in the same ratio, provided that the pH is constant. In contrast, at two pH values which differ by m they are eluted with an equal number of transfers. A partition method for separation is thus proposed in which the pH of the aqueous mobile phase changes discontinuously, decreasing for the bases and increasing for the acids. The theoretical distribution curve is calculated and two examples are given of the application of the method.

INTRODUCTION

The use of counter-current distribution (CCD) for the separation of mixtures of acids and bases has been proposed by many authors[1], including an upper mobile phase with a discontinuously varying pH (ref. 2). We have shown[2] that the logarithm of the extraction coefficient, defined as the ratio between the concentrations of solute in the aqueous (c_w) and the organic (c_o) phases, was linearly pH dependent. We have also shown as an example that, by lowering the pH, a mixture of bases could be separated and eluted in order of decreasing $K_r \cdot K_b$, where K_r is the partition coefficient between the aqueous and organic phases and K_b* is the dissociation constant. Thus the following equation can be written:

$$\log \frac{c_w}{c_o} = -\text{pH} + \log \frac{K_r \cdot K_b}{K_w}$$

We describe in this paper the introduction of a statistical method into the distribution equation of a solute (acid or base) between two immiscible phases. An expression was found which relates the position of the distribution curve

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* As in the previous paper K_b is used instead of $K_a = K_w/K_b$.

maximum of the solute after n transfers with pH, K_r and K_{diss}. The theoretical distribution curve was obtained from these parameters.

A different expression was obtained, depending on whether the mobile phase was aqueous or organic. The mobile phase may be the upper or the lower, according to the design of the CCD apparatus.

THEORY

Distribution between aqueous (w) and organic (o) phases of a weak monobasic acid

The equilibrium expression when a weak acid HA dissociates is

$$K_a = \frac{[\mathrm{H}^+][\mathrm{A}^-]_w}{[\mathrm{HA}]_w} \tag{1}$$

The concentration of undissociated solute in both phases is given by the expression

$$K_r = \frac{[\mathrm{HA}]_w}{[\mathrm{HA}]_o} \tag{2}$$

From eqns. 1 and 2 we obtain

$$K_r = \frac{[\mathrm{H}^+][\mathrm{A}^-]_w}{[\mathrm{HA}]_o K_a} \tag{3}$$

The total number of moles of solute (n_t) is equal to the sum of moles in the organic phase and those dissociated and undissociated in the aqueous phase:

$$n_t = n_{\mathrm{HA}_o} + n_{\mathrm{A}^-{}_w} + n_{\mathrm{HA}_w}$$

The total concentration, for identical volumes of the two phases, will be

$$c_t = [\mathrm{HA}]_o + [\mathrm{A}^-]_w \tag{4}$$

as the amount of HA_w is negligible compared to that of $\mathrm{A}^-{}_w$ for the pH values used.

Substitution of $[\mathrm{A}^-]_w$ in eqns. 3 and 4 gives $[\mathrm{HA}]_o$, which may be denoted by c_o and is obtained from eqn. 5

$$c_o = \frac{c_t \cdot [\mathrm{H}^+]}{K_a \cdot K_r + [\mathrm{H}^+]} \tag{5}$$

Analogously, $[\mathrm{A}^-]_w$ is obtained by substituting $[\mathrm{HA}]_o$ into eqns. 3 and 4. $[\mathrm{A}^-]_w$ is the approximate total concentration of acid in the aqueous phase, thus:

$$c_w = \frac{K_a \cdot K_r \cdot c_t}{K_a \cdot K_r + [\mathrm{H}^+]} \tag{6}$$

The concentrations of acid in the organic and in the aqueous phases after one partition are thus obtained from eqns. 5 and 6.

Distribution between aqueous (w) and organic (o) phases of a weak monoacidic base

A weak monoacidic base BOH dissociates according to the equilibrium expression

$$K_b = \frac{[\mathrm{OH}^-]\,[\mathrm{B}^+]_w}{[\mathrm{BOH}]_w} \tag{7}$$

and is distributed between two immiscible phases according to equation

$$K_r = \frac{[\mathrm{BOH}]_w}{[\mathrm{BOH}]_o} \tag{8}$$

From eqns. 7 and 8 we obtain

$$K_r = \frac{[\mathrm{OH}^-]\,[\mathrm{B}^+]_w}{K_b[\mathrm{BOH}]_o} \tag{9}$$

and, analogously to the acids, we may write for the bases

$$c_t = [\mathrm{BOH}]_o + [\mathrm{B}^+]_w \tag{10}$$

as $[\mathrm{BOH}]_w$ is negligible compared to $[\mathrm{B}^+]_w$*.

Substituting $[\mathrm{B}^+]_w$ of eqns. 9 and 10 we obtain $[\mathrm{BOH}]_o$, which may be written as c_o and is given as

$$c_o = \frac{c_t \cdot K_w}{K_b \cdot K_r[\mathrm{H}^+] + K_w} \tag{11}$$

$[\mathrm{B}^+]_w$ is obtained by substitution of $[\mathrm{BOH}]_o$ in eqns. 9 and 10 and can be identified with the total concentration of base in the aqueous phase, c_w. Thus

$$c_w = \frac{K_b \cdot K_r[\mathrm{H}^+]c_t}{K_b \cdot K_r[\mathrm{H}^+] + K_w} \tag{12}$$

Hence, the concentrations of a weak base in the organic and in the aqueous phases after one partition are obtained from eqns. 11 and 12.

We shall now apply the statistical method in order to obtain the parameters needed to calculate the theoretical distribution curve after n partitions. The percentage of solute in each phase after one partition can be considered as the probability of finding a molecule in either phase after one transfer. The amounts of solute in each phase are obtained by dividing eqns. 5 and 6, and 11 and 12 by c_t. The probability of finding, after n transfers, some solute in the rth tube (where $r \leqslant n$) is given by the expression

$$P_r = \frac{n!\,m^r s^{n-r}}{r!(n-r)!} \tag{13}$$

where m and s are the fractions of solute in the mobile phase (m) and in the stationary phase (s) after one partition. Eqn. 13 is an integral function. Thus the maximum

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* Strychnine ($K_b = 10^{-6}$) is evenly distributed between chloroform and a buffer at pH 5. The concentration $[\mathrm{B}^+]_w$ obtained from eqn. 7 is a thousand times $[\mathrm{BOH}]_w$.

can be obtained by setting the P_r ratio equal to unity for two consecutive values of r, *e.g.*

$$1=\frac{P_r}{P_{r+1}}=\frac{n!m^r s^{(n-r)}(r+1)!(n-r-1)!}{r!(n-r)!n!m^{(r+1)}s^{(n-r-1)}}=\frac{s(r+1)}{m(n-r)}$$

Assuming that $r \gg 1$ in this expression, we obtain

$$\frac{sr}{(n-r)m}=1 \tag{14}$$

where r is the maximum of the distribution curve of the solute. Substituting eqns. 5 and 6 for the acids and eqns. 11 and 12 for the bases into eqn. 14, depending on whether the mobile phase is organic or aqueous, four different expressions will be obtained for s and m, two for the acids and two for the bases.

Acidic solute and aqueous mobile phase: $s=o$, $m=w$

When the mobile phase is aqueous (upper or lower), substituting in eqn. 14 c_o/c_t of eqn. 5 for s and c_w/c_t of eqn. 6 for m we obtain for an acid

$$K_a \cdot K_r n - K_a K_r r - r[\mathrm{H}^+]=0$$

which may be rewritten as

$$[\mathrm{H}^+]=\frac{K_a \cdot K_r}{r}(n-r)$$

In logarithmic form this becomes

$$\log\frac{r}{n-r}=\mathrm{pH}+\log K_a \cdot K_r \tag{15}$$

Acidic solute and organic mobile phase: $s=w$, $m=o$

When the mobile phase is organic, substituting c_w/c_t of eqn. 6 for s and c_o/c_t of eqn. 5 for m in eqn. 14 we obtain for an acid

$$K_a K_r \cdot r-[\mathrm{H}^+](n-r)=0$$

In logarithmic form this equation may be written as

$$\log\frac{r}{n-r}=-\mathrm{pH}-\log K_a \cdot K_r \tag{16}$$

Basic solute and aqueous mobile phase: $s=o$, $m=w$

If the mobile phase is aqueous and the solute is a base, substituting c_o/c_t of eqn. 11 for s and c_w/c_t of eqn. 12 for m into eqn. 14 we obtain

$$K_b \cdot K_r[\mathrm{H}^+](n-r)-r \cdot K_w=0$$

and hence

$$\log\frac{r}{n-r}=-\mathrm{pH}+\log\frac{K_b \cdot K_r}{K_w} \tag{17}$$

Basic solute and organic mobile phase: $s = w$, $m = o$

In the case of an organic mobile phase with a base as solute, substituting c_w/c_t of eqn. 12 for s and c_o/c_t of eqn. 11 for m into eqn. 14 we obtain

$$(n-r)K_w - K_b \cdot K_r[\mathrm{H}^+]r = 0$$

and hence

$$\log \frac{r}{n-r} = \mathrm{pH} - \log \frac{K_b \cdot K_r}{K_w} \tag{18}$$

Hence eqns. 15, 16, 17 and 18 allow us to correlate experimental conditions (n number of transfers and pH), properties of the solute (K_b or K_a) and of the solute relative to the solvents (K_r) with the position of the maximum of the distribution curve (r).

A simplified form of eqn. 13 (Laplace's approximation)[3] can be used to obtain the distribution curve when these parameters are known:

$$P_x = \frac{e^{-\frac{x^2}{2nms}}}{\sqrt{(2\pi nms)}} \tag{19}$$

where P_x is the percentage of the solute in the tube at a distance x from the maximum. The fraction of solute in the mobile and stationary phases, c/c_t, will be substituted for m and s. These may be the organic or the aqueous fractions and can be obtained from eqns. 5 and 6 for acids and from eqns. 11 and 12 for bases.

For the separation of a mixture of acids or bases two procedures are possible, the first with an aqueous mobile phase and the second with an organic mobile phase. This latter method does not allow the separation of all the components of a complex mixture, *i.e.* with large differences in values of $K_r \cdot K_{\mathrm{diss}}$, as can be inferred from eqns. 15, 16, 17 and 18 if the extraction occurs at a constant pH. Thus two acids (or bases) with $K_r \cdot K_{\mathrm{diss}}$ products in a $1:10^m$ ratio can be eluted by a number of transfers in the same ratio $1:10^m$, at a constant pH.

In contrast, using two pH values differing by m, the acids (or bases) are eluted with an equal number of transfers.

Therefore the best procedure will be the use of an aqueous mobile phase with a discontinuously varying pH. The bases will need increasingly acidic pH values, whilst the contrary will be true for the acids. In this way the elution of substances will occur in order of decreasing $K_r \cdot K_{\mathrm{diss}}$ products (see eqns. 15 and 17.) The substances must be stable at the upper and lower values of the pH range, where the maximum concentrations occur.

This method was successfully used for the separation of tertiary alkaloids of various Strychnos species[2,4].

Partition and separation of acids are exemplified in the following paragraphs.

Example I — Counter-current partition of iodoacetic acid between ethyl acetate (mobile phase) and water at a constant pH. Iodoacetic acid (0.5 g) was dissolved in water (10 ml) and sodium citrate added until pH 3.7 had been reached. This solution was then placed in tube No. 3 of a QVF steady-state distribution apparatus with 123 tubes (volume of both upper and lower phase 10 ml). Citrate buffer (0.2 *M*, pH 3.70) was used as the stationary phase, whilst ethyl acetate was the organic mobile phase. The distribution curve for the substance was determined by UV spectrometry ($\lambda = 270$ nm, upper phase).

After 119 transfers the maximum in the distribution curve was found at tube 89 (see Fig. 1). The value of $K_r \cdot K_a$, obtained from eqn. 16, was found to be $7.7 \cdot 10^{-5}$.

The theoretical distribution curve, obtained from eqn. 19 by substituting c_0/c_t of eqn. 5 for m and c_w/c_t of eqn. 6 for s, can be seen in Fig. 1.

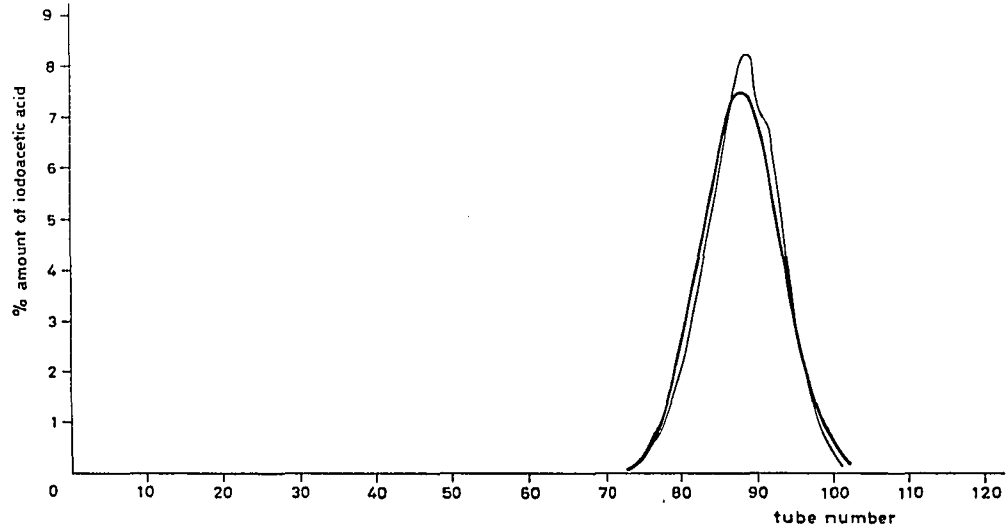

Fig. 1. Experimental (——) and theoretical (━━) distribution curve after 119 transfers of iodoacetic acid between ethyl acetate (mobile phase) and water at pH 3.70.

The partition constant, K_r (0.1), of iodoacetic acid distributed between water and ethyl acetate can be obtained from the product $K_r \cdot K_a$ once K_a ($7.6 \cdot 10^{-4}$) is known.

Example II — Separation of a mixture of monochloroacetic, dichloroacetic, trichloroacetic and monoiodoacetic acids by CCD between a mobile buffered aqueous layer (lower phase) with varying pH and ethyl acetate. A mixture of monochloroacetic (0.5 g), dichloroacetic (0.5 g), trichloroacetic (0.5 g) and monoiodoacetic (0.5 g) acids was dissolved in water (20 ml) and sodium citrate added until pH 2.07 had been reached. The mixture was placed in tubes No. 120 and No. 121 of the apparatus. These acids were distributed between the mobile aqueous layer (lower phase) and ethyl acetate. The extraction was monitored by paper chromatography[5].

The trichloroacetic acid in the lower phase was collected from tube No. 1 in the collector, after 240 transfers at pH 2.07. In contrast, dichloroacetic acid was eluted after 120 transfers at pH 2.50. The monochloroacetic acid remained in the apparatus, but was separated from iodoacetic acid, which stayed at the starting point.

In this case, however, the approximation of eqn. 4 is not valid and thus the product $K_r \cdot K_a$ could not be calculated for the mono-, di- and trichloroacetic acids.

The compounds could be obtained as the free acids by changing the pH of the solutions to 1.0 with hydrochloric acid and extracting three times with ethyl acetate. The residue of the organic phase was extracted with chloroform from few citric acid of the buffer soluble in ethyl acetate at pH 1.0.

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