A Manual of Physical Methods in Organic Chemistry

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Preface

The purpose of this manual is threefold. It is to provide tested and detailed directions for use in a laboratory course at the undergraduate or graduate level, to serve as an introduction to a new technique for the individual researcher, and to enable any scientist to acquaint himself rapidly with a physical method in order to evaluate its usefulness in his work.

The present manual is a translation, revision, and expansion of the manual originally written for use in the Department of Organic Chemistry of the University of Amsterdam. It is natural that no attempt has been made to be as comprehensive as the many-volume *Technique of Organic Chemistry* series, or as thorough as a monograph on any single subject. The manual is intended as a well-tested introduction to the many physical methods confronting the practicing chemist. Its continuing intensive use in the laboratories in Amsterdam and Groningen has shown that the book fills a real need.

HANS WYNBERG

Groningen, Holland, 1964

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1-1 Introduction

Separations based on countercurrent distribution depend on the differences in the partition coefficients of various components with respect to two liquid phases.

Although separatory funnels can be used to effect separations in this manner, the method would become cumbersome. Using the apparatus developed by Craig simplifies the technique greatly; furthermore, complete automation is possible.

The method is especially important for achieving separations under very mild conditions (of temperature and pH, for instance) and thus is ideally suited for mixtures of peptides, hormones, vitamins, antibiotics, and light-, heat-, or air-sensitive compounds. The method is frequently employed in cases where column or gas-phase chromatography or fractional distillation fails.

The method is not successful with:

(*a*) Compounds of limited solubility.
(*b*) Strongly polar compounds (e.g., salts).
(*c*) Completely non-polar compounds (saturated hydrocarbons).
(*d*) Compounds whose molecular weight is above *ca.* 6000.

1-2 Principle

The countercurrent distribution apparatus consists of a train of tubes connected in series. Each unit contains an extraction tube and a decantation tube (Fig. 1-1). Every tube contains L ml. of the lower (stationary) phase (heavier liquid) and U ml. of the upper (mobile) phase (lighter liquid).

Equilibration of the solute between the two liquid phases is achieved by tilting back and forth, rocking from position I to III and back (Fig. 1-2). In position III separation of the two phases is possible, and careful turning to position IV allows the top phase to enter the decantation tube, while the bottom phase remains behind. The top phase is transferred to the next tube in position I. The first tube (known as the 0th tube in the theoretical treatment) is filled with U ml. of the top phase and a new cycle can start.

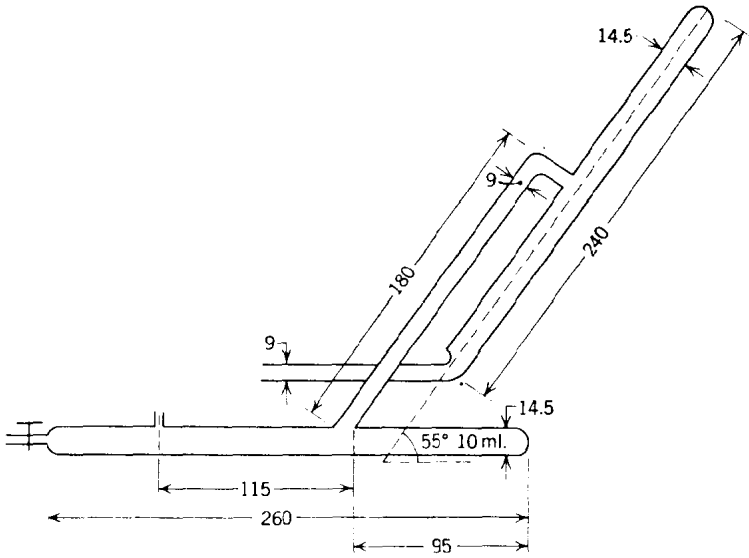

Fig. 1-1. Cell for countercurrent distribution (dimensions in millimeters).

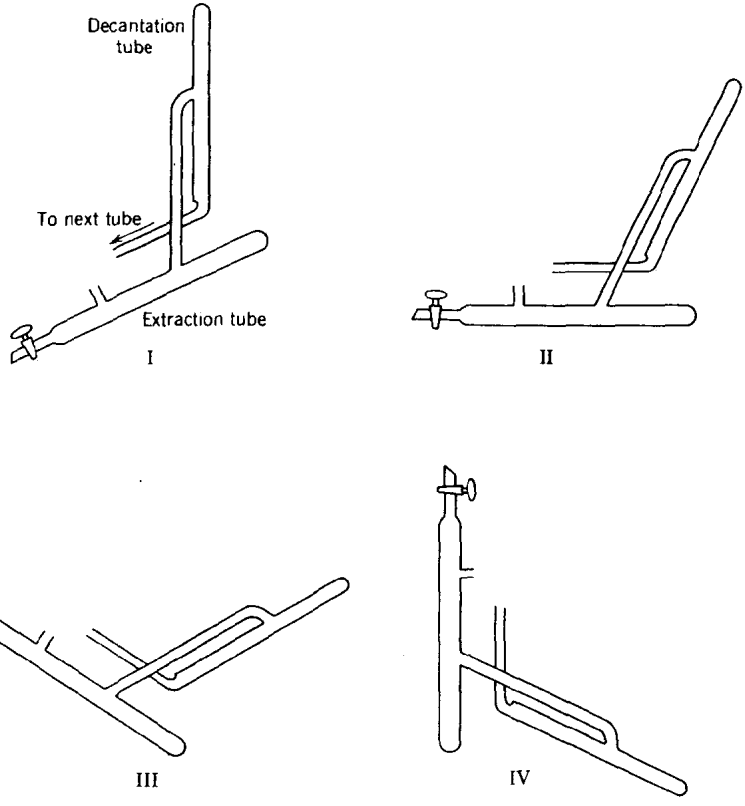

Fig. 1-2. Positions of the cell.

1-3 Theory

1-3a THE SIMPLE CASE

The components of the mixture move with the mobile phase with different speeds depending upon their distribution coefficients. The distribution of a substance over the various tubes can be calculated as follows.

Assume experimental conditions so that, upon equilibration, a substance is distributed equally between the top and bottom phases. After the first cycle each

Table 1-1 Fraction of Solute in Each Tube after n Cycles

Tube	0	1	2	3	4	5	6	7	8	9	10	11	
0	1												$\times 2^{0}$
1	1	1											$\times 2^{-1}$
2	1	2	1										$\times 2^{-2}$
3	1	3	3	1									$\times 2^{-3}$
4	1	4	6	4	1								$\times 2^{-4}$
5	1	5	10	10	5	1							$\times 2^{-5}$
6	1	6	15	20	15	6	1						$\times 2^{-6}$
7	1	7	21	35	35	21	7	1					$\times 2^{-7}$
8	1	8	28	56	70	56	28	8	1				$\times 2^{-8}$
9	1	9	36	84	126	126	84	36	9	1	1		$\times 2^{-9}$
10	1	10	45	120	210	256	210	120	45	10	1		$\times 2^{-10}$
11	1	11	55	165	330	462	462	330	165	55	11	1	$\times 2^{-11}$

of the first two tubes will contain one half of the original substance. After n cycles, the distribution among the first $(n + 1)$ tubes is given by the terms of the binomial expansion: $(\frac{1}{2} + \frac{1}{2})^n$ (see Table 1-1).

1-3b FUNDAMENTAL PROCEDURE

All fractions undergo the same number of transfers during the fundamental procedure. The number of cycles (n) is equal to the number of times that the mobile phase is transferred to the next tube. This number is equal to the number of times that fresh mobile phase is added to the 0th tube. After n cycles $(n + 1)$ tubes will have been used. Tubes are numbered starting with 0 (0, 1, 2, . . . , r).

Assuming that the partition coefficient K is constant, independent of the concentration (linear partition isotherm), then

$$K = \frac{C_{\text{mobile phase}}}{C_{\text{stationary phase}}}$$

Under these conditions the partition coefficient of a substance for a particular solvent is a characteristic physical property.

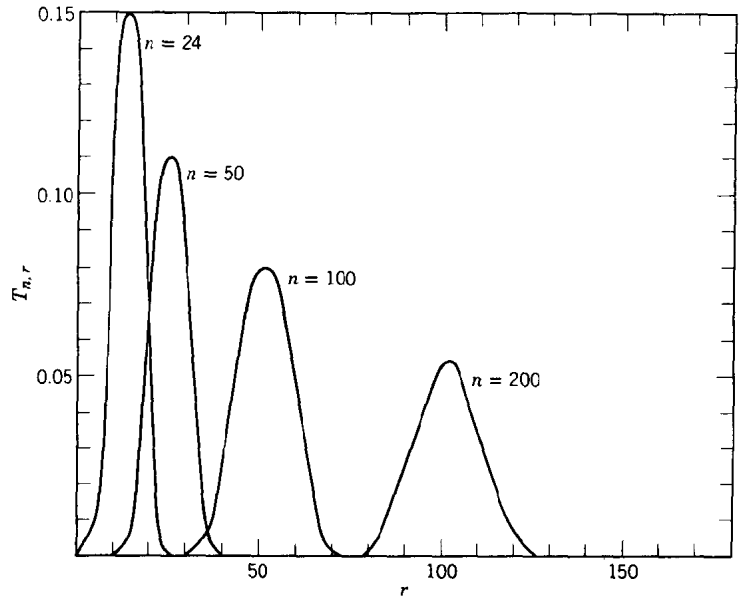

Fig. 1-3. Bandwidth with increasing number of partitions ($K = 1$, $\alpha = 1$).

When the volumes of the mobile and stationary phases are equal, then

$$p = \text{fraction of solute in mobile phase} = \frac{K}{(K+1)}$$

$$q = \text{fraction of solute in stationary phase} = \frac{1}{(K+1)}$$

$$p + q = 1$$

When these volumes are not equal, then

$$p = \frac{\alpha K}{(\alpha K + 1)}$$

$$q = \frac{1}{(\alpha K + 1)}$$

$$p + q = 1$$

where $\alpha = V_{\text{mobile phase}}/V_{\text{stationary phase}}$.

The fraction of solute in each tube can now be calculated (see Table 1-1). It is given by the terms of the binomial expansion $(p + q)^n = 1$. The individual terms of the expansion are equal to

$$T_{n,r} = \frac{n!}{(n-r)!r!} p^r q^{n-r} \tag{1-1}$$

When this function is plotted vs. the number of the tube (r), a theoretical distribution curve is obtained (see Fig. 1-3). The absolute bandwidth increases with an

increase in n (i.e., the solute is distributed over more tubes); however, the bands become narrower relative to one another, since with an increase in n the solute is found in a smaller fraction of the total number of tubes used.

Position of the Maximum. From formula 1-1 it follows that

$$T_{n,r+1} = \frac{n-r}{r+1}\frac{p}{q} T_{n,r} \tag{1-2}$$

The slope of the distribution curve is positive for $T_{n,r}/T_{n,r-1}$ larger than 1, and negative when this fraction is smaller than 1. At the maximum (for n = odd, compare Table 1-1)

$$T_{n,r} = T_{n,r+1}$$

$$\frac{n - r_{\max}}{r_{\max} + 1}\frac{p}{q} = 1 \rightarrow \boxed{r_{\max} = p(n+1) - 1} \tag{1-3}$$

When n is odd, the maximum concentration is found in two tubes, namely in $p(n+1)$ and in $[p(n+1) - 1]$. When n is large (>20), $r_{\max}$ equals pn to a good approximation. (1-3a)

Calculation of the Partition Coefficient from the Distribution Curve. From the position of the maximum we find

$$r_{\max} = \frac{\alpha K}{(\alpha K + 1)}(n+1) - 1$$

$$K = \frac{r_{\max} + 1}{\alpha(n - r_{\max})} \tag{1-4}$$

K may be calculated from the concentration ratios of neighboring cells (formula 1-2):

$$\boxed{K = \frac{r+1}{\alpha(n-r)}\frac{T_{n,r+1}}{T_{n,r}} = \frac{r}{\alpha(n-r+1)}\frac{T_{n,r}}{T_{n,r-1}}} \tag{1-5}$$

1-3c LARGE NUMBER OF TRANSFERS

Formula 1-1 becomes cumbersome for a large (>20) number of transfers. The following method can then be used:

$$d \ln T_{n,r} = \frac{dT_{n,r}}{T_{n,r}} = \left[\frac{(n-r)p}{rq} - 1\right] dr$$

The origin of the coordinate system is taken at the maximum of the partition curve (Fig. 1-4), and the number of the tube counting from the maximum is called x. Then:

$$r = np + x$$

$$n - r = nq - x$$

$$d \ln T_{n,r} = \left[\frac{(nq - x)p}{(np + x)q} - 1\right] dx = \frac{-(p+q)x}{npq + qx} dx$$

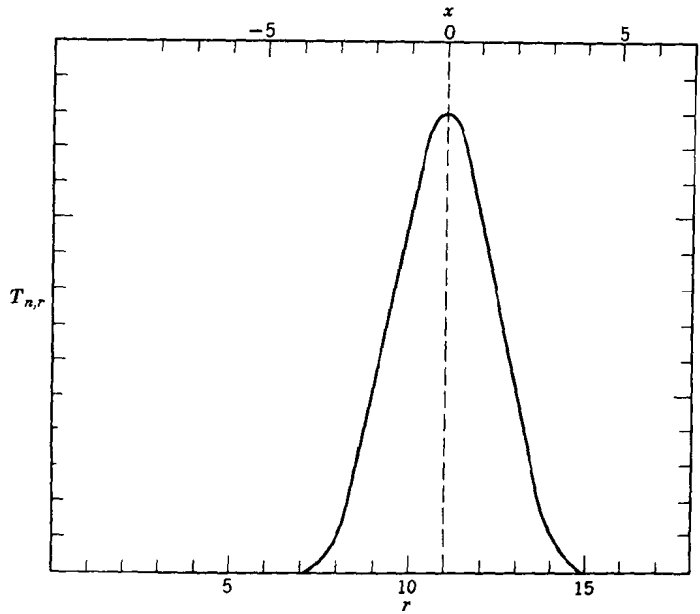

Fig. 1-4. Transition from r to x.

Near the maximum qx is small compared to npq, and we may write

$$d \ln T_{n,r} = \frac{-x}{npq}\, dx \tag{1-6}$$

This equation is not valid further away from the maximum, but then $T_{n,r}$ becomes negligible (for $n > 20$).

Integration of formula 1-6 yields

$$T_{n,r} = C \exp\left(\frac{-x^2}{2npq}\right)$$

C is found by calculating the value of $T_{n,r}$ at the maximum with the aid of formula 1-1 and substituting:

$$\boxed{T_{n,r} = (2\pi npq)^{-1/2} \exp\left(\frac{-x^2}{2npq}\right)} \tag{1-7}$$

1-3d SEPARATION [7]

The ratio of the partition coefficients (separation factor) is of importance in determining the separability of two compounds.

$$\beta \equiv \frac{K_2}{K_1} = \frac{p_2 q_2}{p_1 q_1} \quad (K_2 > K_1)$$

The maxima are found at np_1 and np_2 after n transfers. The more β differs from 1 the easier is the separation. When $\beta = 1$ a separation is clearly impossible.

In the tube containing a maximum amount of the first component we find:

$$(T_{n,r})_{\max} = (2\pi np_1q_1)^{-\frac{1}{2}}$$

For those tubes in which the amount of substance equals $e^{-1}T_{\max}$ the equation

$$e^{-1} = \exp\left(\frac{-x^2}{2np_1q_1}\right)$$

holds. The number of these tubes therefore equals

$$x_e = \pm(2np_1q_1)^{\frac{1}{2}}$$

Thus the width of the "band" between the two e^{-1} limits is

$$L_e = 2(2np_1q_1)^{\frac{1}{2}} = 2.82(np_1q_1)^{\frac{1}{2}} \tag{1-8}$$

Similarly the bandwidths between the 1 % and 10 % limits are

$$\left.\begin{aligned} L_{1\%} &= 6(np_1q_1)^{\frac{1}{2}} \\ L_{10\%} &= 4.3(np_1q_1)^{\frac{1}{2}} \end{aligned}\right\} \tag{1-9}$$

When C and D are the 1 % limit, Fig. 1-5 shows that

$$AE - AB = BE = BD + CE - CD$$
$$CD = BD + CE - AE + AB$$

The overlap therefore equals

$$\begin{aligned} CD &= 3(np_1q_1)^{\frac{1}{2}} + 3(np_2q_2)^{\frac{1}{2}} - np_2 + np_1 \\ &= n^{\frac{1}{2}} \cdot 3(p_1^{\frac{1}{2}}q_1^{\frac{1}{2}} + p_2^{\frac{1}{2}}q_2^{\frac{1}{2}}) - n(p_2 - p_1) \end{aligned}$$

The number of transfers necessary to obtain a 1 % separation is given by ($CD = 0$)

$$(n_{1\%})^{\frac{1}{2}} = \frac{3(p_1^{\frac{1}{2}}q_1^{\frac{1}{2}} + p_2^{\frac{1}{2}}q_2^{\frac{1}{2}})}{p_2 - p_1} \tag{1-10}$$

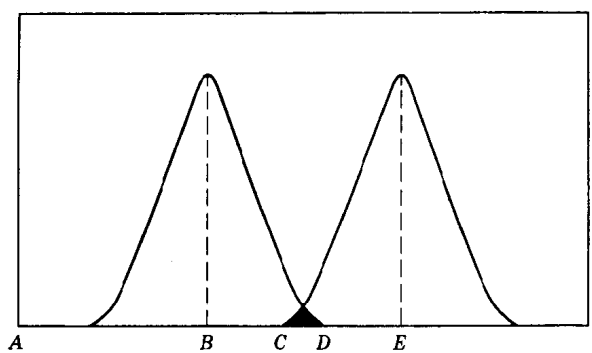

Fig. 1-5. Overlap of two components.

$L_{10\%}$ and $n_{10\%}$ can be calculated similarly:

$$L_{10\%} = \frac{4.3}{6} L_{1\%} = 0.7 L_{1\%}$$
$$n_{10\%} = \frac{2.15^2}{3} n_{1\%} = \tfrac{1}{2} n_{1\%} \tag{1-11}$$

These relationships are valid only when the mixture contains equal amounts of both components (see § 2-3d).

1-3e SINGLE WITHDRAWAL METHOD

The operation can be continued upon completion of the fundamental procedure by collecting the mobile phase of the last tube in a fraction collector after every transfer (see § 3-2). This procedure is closely related to the elution method used in chromatography (see § 2-3).

When n transfers have been completed, the amount of substance *in the mobile phase* of the last tube (the one about to be eluted) is equal to

$$T_{n,r} = \frac{n!}{r!(n-r)!} p^r q^{n-r} p \tag{1-12}$$

Here n varies for every fraction separated (since it is equal to the number of transfers completed at that time), but r is a constant for every fraction and is equal to the total number (R) of tubes in the apparatus.

$$T_{nR} = \frac{n!}{R!(n-R)!} p^{R+1} q^{n-R}$$

T_{nR} can be plotted as a function of n. The change to a continuous function, by moving the origin to the maximum, can now be made just as in § 1-3c. When $n_{\max}$ is equal to the total number of transfers which have been carried out at the moment that the maximum is being eluted,

$$R = p n_{\max}, \quad n_{\max} = \frac{R}{p}$$

Then

$$T_{nR} = \left(\frac{2\pi n_{\max} q}{p}\right)^{-1/2} \exp\left(\frac{-x^2 p}{2 n_{\max} q}\right) \tag{1-13}$$

The bandwidth within the e^{-1} limits may be found as follows:

$$\frac{T_{nR}}{T_{\max}} = e^{-1} = \exp\left(\frac{-x^2 p}{2 n_{\max} q}\right)$$
$$x_e = \left(\frac{2 n_{\max} q}{p}\right)^{1/2} = \frac{2Rq}{p}$$
$$L_e = 2.82\left(\frac{Rq}{p}\right)^{1/2} \tag{1-14}$$

In a similar manner,

$$\left.\begin{aligned} L_{1\%} &= 6\left(\frac{Rq}{p}\right)^{1/2} \\ L_{10\%} &= 4.3\left(\frac{Rq}{p}\right)^{1/2} \end{aligned}\right\} \tag{1-15}$$

The number of tubes needed to achieve a 1% separation is (compare Fig. 1-5)

$$3\frac{(Rq_1)^{1/2}}{p_1} + 3\frac{(Rq_2)^{1/2}}{p_2} - \frac{R}{p_2} + \frac{R}{p_1} = 0$$

$$R^{1/2} \cdot 3(p_2 q_1^{1/2} + p_1 q_2^{1/2}) = R(p_1 - p_2)$$

$$R_{1\%} = 9\left(\frac{p_2 q_1^{1/2} + p_1 q_2^{1/2}}{p_1 - p_2}\right)^2 \tag{1-16}$$

To achieve a 10% separation the coefficient is 4.5 instead of 9. All these relationships are valid only for a mixture containing equal amounts of the two components (see also § 2-3d).

1-3f SEPARATION OF ACIDS FROM BASES

In order to separate weak acids, a solvent mixture is used consisting of a buffer solution and an organic solvent. The ionization of the acid in the aqueous phase [2] is given by

$$A{-}H \overset{K_a}{\rightleftharpoons} A^{\ominus} + H^{\oplus}, \qquad K_a = \frac{[H^{\oplus}][A^{\ominus}]_2}{[AH]_2}$$

Only the undissociated acid will dissolve in the organic phase [1]. So here

$$K = \frac{[AH]_1}{[AH]_2}$$

The "observed partition coefficient" is therefore

$$K' = \frac{[AH]_1}{[AH]_2 + [A^{\ominus}]_2} \quad (K' < K)$$

but

$$[A^{\ominus}]_2 = K_a \frac{[AH]_2}{[H^{\oplus}]_2}$$

Therefore

$$K' = \frac{K}{(1 + K_a/[H^{\oplus}]_2)}$$

If we use a buffer when $[H^{\oplus}] \ll K_a$, then

$$K' = \frac{K[H^{\oplus}]_2}{K_a}$$

or

$$\boxed{\log K' = \log K - \mathrm{pH} + \mathrm{p}K_a} \tag{1-17}$$