A NEW PROCEDURE FOR FRACTIONATION OF MIXTURES BY SOLVENT DISTRIBUTION*

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The value of systematic distribution between immiscible solvents for the resolution of mixtures has long been recognized, but it was not until the work of Craig (1) that its full potentialities were appreciated. With the development of an apparatus for carrying out a large number of simultaneous distributions between immiscible solvents, Craig and Post (2) introduced a new and very effective analytical tool to organic chemists and biochemists. The application of their so-called counter-current techniques to chemical problems has led to a closer realization of the goal of absolute chemical purity and has accounted for the separation and identification of the components of many mixtures. The use of the Craig apparatus, however, has been somewhat limited because the cost has been prohibitive to many laboratories.

In the course of investigations on natural plant growth regulators in this laboratory, a simple automatic solvent distribution apparatus operating on a principle different from that of the Craig apparatus has been devised for use in large scale fractionation procedures. Because the apparatus is inexpensive and requires little attention during the distribution, it should prove to be very useful in the isolation and purification of biological materials. The design is novel in that equilibration of the solute between immiscible solvents is achieved without any mechanical agitation.

Theory and Design of Apparatus

The method consists of a series of liquid-liquid extractions in which one phase flows continuously, in a finely dispersed state, through successive stationary aliquots of the second phase, as shown in Fig. 1.

It should be noted that this type of solvent distribution differs markedly from the counter-current distribution procedure developed by Craig. In the Craig procedure, a finite volume of one phase is equilibrated with a finite volume of the second phase containing the solute. Each layer is then reequilibrated with fresh portions of the solvent pair and the whole process

* Report of a study made under the Research and Marketing Act of 1946. A preliminary report was presented at the meeting of the American Society of Biological Chemists, Atlantic City, New Jersey, April 17-21, 1950 (Federation Proc., 9, 189 (1950)).
is repeated many times. This has been called counter-current distribution by Craig, but the phases do not actually move in opposite directions. Instead, one phase moves past the other so that each fraction of the stationary phase is equilibrated successively with every fraction of the moving phase. True counter-current distribution carried out in discontinuous stages has been used by Martin and Synge (3) in separating acetyl amino acids and also by O'Keeffe, Dolliver, and Stiller (4) for separation of the streptomycins. All of these procedures, including the one described here, are fundamentally different from the continuous counter-current extraction methods used commercially and possess the advantage of more precise mathematical interpretations than the continuous procedures allow.

The distribution described in the present paper cannot be considered a "counter-current distribution" in the same sense as the Craig procedure. Because of the different extraction conditions and the different mathematical interpretations, there is a definite advantage to be gained by using a different name. Consequently, it has been suggested that the present method be termed a "cascade distribution process."

Theoretical Considerations—The mathematical description of the behavior of the apparatus is based primarily on three important assumptions: (a) that there is complete equilibration (or a constant fraction thereof) of the solute between the two phases as the droplets pass through each tube;

1 This suggestion was made by Mr. J. S. Ard of this Laboratory. Justification for the name is found in Webster's International Dictionary: "Cascade: . . . 3. An arrangement of the parts of an apparatus so that fluid passes, or is conceived to pass, from one to another, down the series."

2 The authors are indebted to Mr. J. A. Kies of the Naval Research Laboratory for the mathematical derivation and for valuable advice and assistance in the interpretation of the data.
(b) that the mobile phase is removed continuously, leaving only an insignificant amount in contact with the stationary phase in each tube; and (c) that there is at all times rapid equalization of the solute concentration throughout each aliquot of stationary phase. The data to be discussed show that these assumptions are well justified with the apparatus used.

If one considers the simple case in which 1 volume of the stationary phase (containing solute) is extracted continuously with finely dispersed mobile phase, the quantity of solute picked up \((-dQ)\) by a droplet of volume, \(dv\), passing through volume \(V\), is described by the expression

\[ -dQ = QKdv/V \]  

(1)

where \(Q\) is the quantity of solute uniformly distributed in the stationary phase at any instant and \(K\) is the distribution coefficient.

By integrating Equation 1, we obtain

\[ Q = Q_0 e^{-Kv/V} \]  

(2)

where \(Q_0\) is the quantity of solute originally present and \(v\) is the total volume of mobile phase.

If the mobile phase then passes through a series of \(n\) tubes, each containing a volume of stationary phase equal to the one just described, an expression for the quantity of solute in each tube can be derived as follows: Equation 1 gives the amount of solute entering the second tube \((-dQ_1)\) at the instant when \(Q_1\) is the amount of solute in the first tube. \(Q_1\) is defined in terms of \(Q_0\) as shown in Equation 2. The quantity of solute in the second tube is initially 0 but later is \(Q_2\). The net transfer of solute from the mobile phase to Tube 2 is \(dQ_2\) and thus

\[ dQ_2 = Q_0 e^{-Kv/V}(Kdv/V) - Q_2 Kdv/V \]  

(3)

By integrating Equation 3 between the limits 0 and \(v\), we obtain

\[ Q_2 = Q_0 (Kv/V)e^{-Kv/V} \]  

(4)

Similarly for the third tube,

\[ dQ_3 = Q_0(Kv/V)e^{-Kv/V}(Kdv/V) - Q_3 Kdv/V \]

(5)

and

\[ Q_3 = Q_0 (Kv/V)^2e^{-Kv/V}/2 \]

(6)

It follows that the general expression for the \(n\)th tube is

\[ Q_n = Q_0 (Kv/V)^n e^{-Kv/V} \]

(7)

\[ (n - 1)! \]

In order to simplify the expression, the tube initially containing all the solute is numbered zero and \(Q_n\) is redefined as the fraction of solute per
tube; i.e., $Q_0 = 1$. Equation 7 then becomes

$$Q_n = \frac{(Kv/V)^ne^{-Kv/V}}{n!}$$

(8)

Equation 8 is the well known Poisson exponential distribution equation which is widely used in statistics and engineering. Although the form of the equation is identical, the derivation is entirely different from Poisson's original derivation. The equation, as originally stated, gives the probability of an event happening $x$ times in $n$ trials if the probability of the event happening in a single trial is $p$, provided the product, $np = a$, is kept constant. Equation 8 is not an approximation but is the exact expression of the ideal case.\(^3\)

![Graph](http://www.jbc.org/)

**Fig. 2.** Comparison of theoretical distribution curves for solute with distribution coefficient 0.7. Twenty-four tube binomial expansion distribution, $X$; fourteen tube Poisson distribution, $O$; seven tube Poisson distribution plus seven fractions of mobile phase, $\Delta$ ($v/V = 9$). Data for the binomial distribution taken from Williamson and Craig (7) and for the Poisson distribution from Molina (8).

In spite of the different mathematical treatment, a distribution pattern similar to the binomial expansion pattern is obtained with the “cascade” apparatus by proper choice of solvent ratio and number of tubes. For instance, as shown in Fig. 2, the theoretical curves for the two procedures are essentially the same for a solute ($K = 0.7$) distributed in a twenty-four tube Craig apparatus or a fourteen tube “cascade” apparatus with a vol-

\(^3\) After this paper was submitted for publication, a recent article by Johnson (5) on a similar distribution procedure was brought to the authors’ attention. In the paper cited, the mathematical derivation was based on assumptions comparable to the ones discussed above and the equation derived was of the same general form (the Poisson exponential distribution formula). However, the apparatus proposed by Johnson and Talbot (6) for such a distribution is much more complicated than the present one.
ume ratio \( v/V \) of 9. Actually, one need use only seven tubes and collect seven equal aliquots of the mobile phase as it is removed from the last tube; i.e., the effective length of a series can be extended considerably by collecting fractions of the mobile phase as it leaves the last tube in the series. These fractions have been referred to as “imaginary tubes” by Stene, and he has discussed the process in detail in an article on the theory of systematic extraction (9). Craig (1) has also utilized this means of extending the length of a series in the binomial distribution. The mathematical interpretation of his “single withdrawal” technique is discussed in detail in “Technique of organic chemistry” (1). Because of the wide spread application of the Poisson distribution to problems in industrial inspection, values for the “imaginary tubes” as well as for the stationary tubes in the cascade procedure may be found in published tables. Molina (8) has published such a compilation, and all of the data necessary for drawing theoretical curves are available in this reference. From the tables it is possible to determine how many tubes are required and what volume of upper layer is necessary to achieve any degree of separation of two or more compounds of known distribution coefficients.

Even without the tables one can easily calculate from Equation 8 and the properties of its standard deviation the conditions required for a separation of two compounds, A and B, into fractions containing approximately 84 per cent of Compound A and 16 per cent of Compound B and vice versa. The distance of the mean from the origin, which coincides with the position of the maximum in this distribution, is \( Kv/V \) and the standard deviation, \( \sigma \), is \( \sqrt{Kv/V} \). The volume ratio should be adjusted so that the distribution curves of Compounds A and B will fulfill the following requirements.

\[
K_{av}/V + \sqrt{K_{av}/V} = K_{bv}/V - \sqrt{K_{bv}/V}
\]  

(9)

Once the values of \( K_a \) and \( K_b \) are defined, only one value of \( v/V \) will satisfy Equation 9. If \( K_a = 1 \) and \( K_b = 2 \), Equation 9 becomes

\[
v/V + \sqrt{v/V} = 2v/V - \sqrt{2v/V}
\]  

(10)

and \( v/V \) is found to be equal to 5.76, the volume ratio required for the desired separation of Solutes A and B. For economy of tubes and solvent, the series need only be long enough to retain 84 per cent of Compound A and 16 per cent of Compound B, as shown in Fig. 3. The remainder of the two solutes will pass through the tubes into the upper layer. \( K_{av}/V + \sqrt{K_{av}/V} \) is equal to the number of tubes required for the separation, and, if one substitutes the values given above for \( K_a \) and \( v/V \), the number of tubes is found to be eight. Actually, greater separation can be accomplished by fractionating the mobile phase as it is removed from the last tube. The degree of separation possible when the effluent is divided into eight equal portions is shown in Table I. Any two solutes may be sepa-
Fig. 3. Separation of solutes by distribution. Illustration of the method for predicting separations from the standard deviations of the distribution curves.

**Table I**

Separation of Compounds A and B by Distribution in Eight Tube "Cascade" Apparatus*

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Compound A (K = 1)</th>
<th>Compound B (K = 2)</th>
<th>Purity of combined fractions†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>per cent</td>
<td>per cent</td>
<td>per cent</td>
</tr>
<tr>
<td>Tube 0</td>
<td>0.2</td>
<td>(0)</td>
<td></td>
</tr>
<tr>
<td>&quot; 1</td>
<td>1.5</td>
<td>(0)</td>
<td></td>
</tr>
<tr>
<td>&quot; 2</td>
<td>4.5</td>
<td>(0)</td>
<td></td>
</tr>
<tr>
<td>&quot; 3</td>
<td>8.9 60.7</td>
<td>0.2 4.5</td>
<td>93 7</td>
</tr>
<tr>
<td>&quot; 4</td>
<td>13.4</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>&quot; 5</td>
<td>16.1</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>&quot; 6</td>
<td>16.1</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>&quot; 7</td>
<td>13.8 24.1</td>
<td>4.4 11.0</td>
<td>69 31</td>
</tr>
<tr>
<td>&quot; 8</td>
<td>10.3</td>
<td>6.6</td>
<td></td>
</tr>
<tr>
<td>Effluent 8</td>
<td>7.1 11.3</td>
<td>12.5 17.6</td>
<td>27 73</td>
</tr>
<tr>
<td>&quot; 7</td>
<td>4.2</td>
<td>20.6</td>
<td></td>
</tr>
<tr>
<td>&quot; 6</td>
<td>2.6</td>
<td>18.5</td>
<td></td>
</tr>
<tr>
<td>&quot; 5</td>
<td>1.0</td>
<td>11.3 54.4</td>
<td></td>
</tr>
<tr>
<td>&quot; 4</td>
<td>0.4</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td>&quot; 3</td>
<td>(0)</td>
<td>(0)</td>
<td></td>
</tr>
<tr>
<td>&quot; 2</td>
<td>(0)</td>
<td>(0)</td>
<td></td>
</tr>
<tr>
<td>&quot; 1</td>
<td>(0)</td>
<td>(0)</td>
<td></td>
</tr>
</tbody>
</table>

* Figures taken from Molina (8). This separation is somewhat better than would be achieved in a twenty-four tube Craig apparatus; with the latter, 43.5 per cent of Compound A would be obtained in a purity of 94 per cent and 42.3 per cent of Compound B in a purity of 92 per cent.

† Calculated on the basis of equal amounts of Compounds A and B in the original sample.
rated in this manner, an increasing number of tubes being required as the distribution coefficients approach one another more closely.

Apparatus—The apparatus is shown schematically in Fig. 1. The tubes are filled with the heavier member of an equilibrated solvent pair and the lighter layer is permitted to flow continuously into the entry tube of the first unit (Tube 0) which contains the solute dissolved in an aliquot of heavy layer. The mobile phase enters the tube from below and is dispersed by means of a fritted disk or a glass wool plug. The droplets ascend through the stationary phase and overflow into the next tube. Any number of units may be used, and the volume of the upper layer may be varied at will, depending on the particular distribution conditions desired. As stated above, the effective length of the series may be increased by fractionating the upper layer as it is removed from the last tube. An automatic fraction collector has been found to be very useful in connection with the apparatus.

The tubes in use at present are 18 and 25 cm. long (measured from the bottom of the tube to the overflow). The number of tubes possible is limited only by the height of the supporting column. For the 18 cm. tubes, the height of the entry tube above the side arm is 8 cm. An 11 foot support will hold twenty-five tubes plus suitable containers at the top and bottom of the series for the mobile phase before and after it has passed through the series. The entry tubes for the 25 cm. tubes are correspondingly longer and only fifteen of these can be accommodated on the support. The volume of the tubes can be made greater by merely increasing the diameter, with a corresponding increase in the area of dispersion.
Either fritted disks (Style A) or glass wool plugs\(^4\) (Style B), as shown in Fig. 1, are satisfactory dispersing media. Fritted disks may also be incorporated in Style B, if it is desired. The glass wool is less expensive and is easily replaced, thus obviating the necessity for rigorous cleaning.

For certain distributions, it may be desirable to utilize the lighter liquid for the stationary phase and pass the heavier liquid continuously through the lighter phase. A tube of the design shown in Fig. 4 may be used for this purpose. The dispersion behavior of various solvent pairs in this tube has been observed, although an actual distribution has not been carried out. The same mathematical considerations would apply, except that \(1/K\) would have to be substituted for \(K\) in Equation 8.

![Figure 5](http://www.jbc.org/)

**Fig. 5.** Distribution of acetic acid by the cascade procedure. \(K = 0.37\). Solvent pair made up of equal parts of ethyl acetate, cyclohexane, ethyl alcohol, and water. Solid line, experimental data; broken line, theoretical data.

**EXPERIMENTAL**

*Distribution of Acetic Acid, Solute Remaining in Tubes.* The solvent pair used in this experiment consisted of an equilibrated mixture of equal parts cyclohexane, ethyl acetate, ethyl alcohol, and water. Glass wool dispersion units were used. The first tube (Tube 0) was filled with a solution of 1 ml. of glacial acetic acid in 95 ml. of the heavy phase. Into each of the remaining tubes 95 ml. of the heavier solvent were introduced. The lighter solvent was delivered slowly at a uniform rate (about 300 ml. per hour) into the first dispersing unit until 900 ml. had been collected from the run off of the last tube. Aliquots of each tube were titrated with approximately 0.05 N NaOH, and the fraction of solute per tube was calculated from these titrations and the titration of the original sample. These data are represented by the solid line in Fig. 5. The theoretical curve

\(^4\) A small tuft of glass wool is packed loosely in the bulb of the tube. If the dispersion is not good in a trial run with pure solvents, the plug may be tamped down with a glass rod. Usually this is all the adjustment that is required.
(broken line) was taken from Molina (8), based on a value of 3.5 for $K_v/V$, which is equal to "a" in his Table I. This value for $K_v/V$ was determined from the observed solute concentrations near the peak of the curve and the relationship

$$\frac{Q_n}{Q_{n-1}} = \frac{K_v/V}{n}$$  (11)

Therefore,

$$K_v/V = n \frac{Q_n}{Q_{n-1}}$$  (12)

By calculating $K_v/V$ from each of the experimental points in succession, one can determine easily whether or not the distribution has been successful; too great a spread in values is proof of the fact that equilibrium conditions have not been attained. An approximate value for $K_v/V$ is obtained directly from the position of the peak. Identical results were obtained when this distribution was carried out in the larger tubes (150 ml. volume) with fritted disks as the dispersing agents.

Separation of Benzoic and β-Naphthoic Acids, and Use of "Imaginary" Tubes in Distribution—The effectiveness of the apparatus in the separation of solutes is illustrated in Table II. Benzoic and β-naphthoic acids were chosen for this study because their distribution coefficients differed by a factor of 2, thus affording the possibility of a reasonable separation in a relatively short series of tubes. Although only nine tubes were used (eight plus the zero tube) the effective length of the series was increased to twenty-three by the use of a large volume of mobile phase.

The distribution behavior of the two acids was determined experimentally by weight, titration, and ultraviolet absorption. Theoretical and experimental data for each solute are summarized in Figs. 6 and 7.

Table II

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Benzoic acid ($K = 0.25$)</th>
<th>β-Naphthoic acid ($K = 0.42$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubes 1-4</td>
<td>97</td>
<td>(0)</td>
</tr>
<tr>
<td>&quot; 5-7</td>
<td>225</td>
<td>20</td>
</tr>
<tr>
<td>Tube 8</td>
<td>76</td>
<td>23</td>
</tr>
<tr>
<td>&quot; Effluent, 1st 500 ml.</td>
<td>(0)</td>
<td>3</td>
</tr>
<tr>
<td>&quot; next 1500 ml.</td>
<td>(0)</td>
<td>793</td>
</tr>
<tr>
<td>&quot; last 800 ml.</td>
<td>163</td>
<td>189</td>
</tr>
</tbody>
</table>

* Solvents, benzene, 5 parts; n-hexane, 5 parts; methanol, 8 parts; water, 2 parts.
percentage of β-naphthoic acid in each sample was determined spectro-
photometrically and the benzoic acid values were obtained by weight dif-
fERENCE. The titration data merely served to confirm the other data. The
solutions for the standard spectrophotometric curves were made up of
weighed amounts of Fraction 4 of the upper layer, the purest β-naphthoic
acid available, and the contents of Tube 4, which was the best fraction of
benzoic acid.

The theoretical data for the effluent fractions were obtained from Table
II of Molina (8) as follows: When the last fraction of mobile phase had
been collected, \( \frac{v}{V} \) was 2750/95 and \( \frac{K_v}{V} \) for benzoic acid was found to
be 7.2 (calculated from the peak values as described previously). There-
fore \( K \) (benzoic acid) was 0.25. After the first fraction was collected \( \frac{K_v}{V} \)
was \( \frac{(0.25 \times 500)}{95} \), or 1.3. In Molina’s Table II, the accumulation of all
the terms of the distribution beyond the eighth term (i.e., the ninth term)
for this value of \( a \) was found to be negligible. After 1 liter of upper layer
had been collected, \( \frac{K_v}{V} \) was 2.6 and the ninth term (\( a = 2.6 \)) was 0.15
per cent. This process was repeated for each fraction of upper layer. Es-
sentially the same procedure was used to obtain the theoretical data for
β-naphthoic acid except that $K_v/V$ for this compound could not be determined from the peak values. When $v/V$ was 2750/95, 95.6 per cent of the β-naphthoic acid had accumulated in the effluent fractions. In Table II (8) the ninth term of $a = 14.7$ was 95.6 and, therefore, $K = 0.5$ (since $a = K_v/V$).

This value of $K(\beta$-naphthoic acid) is higher than the value of 0.391 found by Craig (1) for β-naphthoic acid in the same solvent pair. It is well known that the distribution coefficients of solutes are extremely sensitive to small variations in solvent composition, and the use of solvents from different sources could easily account for this discrepancy.

There are several possible explanations for the experimental deviations from the theoretical curves in Figs. 6 and 7. The greatest discrepancy between theoretical and experimental values occurred in the fractions containing very small amounts of benzoic acid. This was not surprising, since the analytical method was not sufficiently sensitive for benzoic acid in the presence of large amounts of β-naphthoic acid. In addition, the solutes were not absolutely pure. Tubes 0 and 1 contained, besides benzoic acid, a substance insoluble in ethyl alcohol which had an abnormally low titration-weight ratio. Another source of error was the variation in tube volume, which amounted to as much as 5 per cent for some of the tubes. This caused the retention of variable amounts of upper layer in the tubes, which was not accounted for in the calculations. For more precise work the tube volumes should be as uniform as possible. For isolation work, in which the active component occurs in extremely low concentrations, this is obviously not an important consideration.

The apparatus has been used successfully in the fractionation of plant extracts for various biologically active materials. Fractions, 60 γ of which are required for stimulation of plant growth, have been purified by this technique (10) until they showed activity in amounts as low as 0.1 to 1.0 γ.

**DISCUSSION**

In order to achieve theoretical or nearly theoretical results for any distribution, many factors must be considered. It has been impossible to study all of these in detail during the short time the apparatus has been in use. Many problems have been solved empirically and the over-all results have been satisfactory for the distributions studied. Obviously, every fractionation offers individual problems which must be solved as they arise. The results to date have been sufficiently satisfactory to indicate that most, if not all, of these problems will be amenable to solution.

Considerable thought has been given to the possibility of predicting optimum conditions for the distribution of a given solute. The rate of molecular diffusion for a solute in a given solvent pair is an inherent property
FRACTIONATION OF MIXTURES

that cannot be changed experimentally. However, the length of time the two liquids are in contact can be varied, because this depends on the drop velocity and the length of the tube. The drop velocity, which is defined by Lamb's modification of Stokes' law (11), is proportional to the difference in densities of the two phases and also to the size of the droplet

\[ V = \frac{2}{3a^2g} \frac{(\rho - \rho')}{\eta} \frac{(\eta + \eta')}{(2\eta + 3\eta')} \quad (13) \]

where \( a \) is the radius of the droplet, \( g \) is the gravitational acceleration, \( \rho \) and \( \rho' \) are the densities of the stationary phase and the mobile phase, and \( \eta \) and \( \eta' \) are the viscosities of the stationary phase and the mobile phase. Thus the finer dispersions provide a longer time of contact as well as a larger surface for solute distribution. Of even greater importance to the extent of solute distribution is the factor of frictional drag on the droplet responsible for creating convection currents in the interior of the drop. The circulation within the droplet is of considerable magnitude and it can be shown that the thoroughness of internal mixing is proportional to the length of the path of rise and inversely proportional to the diameter of the droplet (for any given velocity). A theoretical discussion of this phenomenon is found in Lamb's text on hydrodynamics previously mentioned. Sherwood, Evans, and Longcor (12) investigated the problem of liquid-liquid extraction from single drops experimentally and found that the solute was transferred much more rapidly than could be predicted from molecular diffusion alone.6

When the matter of solute distribution is considered from all angles, one concludes that the finest dispersion possible is the most desirable. Unfortunately there are certain experimental limitations on the fineness of the dispersion because of the high resistance to liquid flow offered by fritted disks of low porosity. The limit of dispersion required could be determined, though somewhat laboriously, with fritted disks of graduated porosities. In the experiments described, this was unnecessary, since nearly theoretical results were obtained with the dispersing units first tried. The disks used in Style A tubes were very coarse disks of variable porosity. It is estimated that the pore size was approximately 100 to 200 \( \mu \).

The fineness of dispersion depends, to a certain extent, on the physical characteristics of the solvent pair as well as on the dispersing device. Distributions with certain solvent pairs, notably water-chloroform, water-benzene, and water-butanol, may prove to be unsatisfactory because of the failure of the lighter phase to disperse properly, regardless of the dispersing

6 This fact must have been ignored or its full potentialities not realized in many studies of liquid-liquid extraction.
device used. Fortunately, there are many available combinations of solvents that do disperse easily and therefore this is no great drawback in the use of the apparatus. If it is necessary to use a solvent combination which does not disperse well, the dispersion may be improved by the addition of small amounts of detergent to the aqueous phase.

Another important factor in solute equilibration is the tube length, which can be increased within reasonable limits to improve equilibration conditions. It is possible that the apparatus could be made somewhat more compact by introducing a spiral baffle in the tubes to increase the path of travel of the droplets, but the turbulence created by the droplets rising freely through the column of liquid might be depressed so greatly that no advantage would be gained by this manipulation.

Proper tube proportions will also depend on the relative densities of the phases. If the difference in densities is great, the entry tube will have to be lengthened to take care of the increased liquid head required to force the lighter phase through the system. For most distributions, an entry tube 50 per cent longer than the height of the heavier liquid in the tube is satisfactory.

The volume of stationary phase in the tubes should be uniform throughout the series and should be adjusted so that a minimum amount of mobile phase is retained in each tube.

The rate of flow may be fairly rapid but should not be rapid enough to cause excessive washing over of the stationary phase from one tube to the next. Flow rates varying from 250 to 500 ml. per hour have been found satisfactory. It should be noted that the term "rate of flow" used here refers to the rate of entry of the lighter phase, rather than to the velocity of rise of the individual droplets. The latter is dependent upon the relative densities of the two phases and the size of the droplets, and is independent of the over-all rate of flow of the lighter phase through the apparatus. The more rapidly the lighter phase enters the tubes, the more numerous are the droplets formed per unit time at the fritted disks.

Solvent pairs made up of mixtures of three or more components usually disperse more satisfactorily than mixtures of water and one organic solvent. These mixtures possess a further advantage in that the distribution coefficients of solutes may be adjusted by merely altering the relative proportions of the constituents; e.g., in equal parts of ethyl acetate, cyclohexane, ethyl alcohol, and water, $K$ (benzoic acid) is equal to 2.4. If the proportions of solvents are changed to 1 part of ethyl acetate, 3 parts of cyclohexane, 2 parts of ethyl alcohol, and 2 parts of water, the distribution coefficient is decreased to about 1.

Although the apparatus was designed primarily to handle large quan-
tities of material with a minimum of labor, it is believed to be adaptable for very precise analytical work on a micro or semimicro scale if the necessary time and effort are expended to determine the various sources of error.

SUMMARY

A new procedure for the fractionation of mixtures by distribution between immiscible solvents has been described. The method involves a series of liquid-liquid extractions in which one phase flows continuously through successive stationary aliquots of the second phase.

Factors affecting solute distribution between the mobile (dispersed) phase and the stationary phase have been discussed. Distribution of a solute between immiscible liquids under these conditions is much more rapid than would be expected from molecular diffusion alone. This is not surprising in view of theoretical considerations of circulation within the drop brought about by frictional drag on the surface of the moving droplet. At the same time convection currents in the external liquid also aid in establishing equilibrium conditions.

The mathematical expression for the distribution of a solute by the procedure described is based on the assumption that complete solute equilibration is achieved between the droplets of mobile phase and the stationary phase in each tube. This expression is the Poisson exponential equation. The data show that the assumption of solute equilibration is well justified.

The apparatus has been used successfully in the purification of plant extracts and is proposed as a useful laboratory tool for all types of biochemical fractionations in which solvent distribution appears feasible.

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