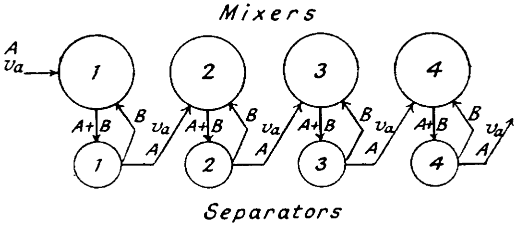
353. *The Separation of Mixtures by Continuous Extraction Processes. Part II. The Continuous Step-wise Extraction of a Fixed Quantity of a Mixture of Solutes.*

By John D. A. Johnson.

The theoretical behaviour of a solute introduced all at once into the first of a series of vessels containing two immiscible solvents so arranged that one solvent passes along the system continuously is considered. It is shown that the solute moves in the form of a wave of gradually diminishing amplitude, the speed being determined, other things being constant, by the distribution coefficient of the solute between the two liquid phases. The extent to which two or more solutes might be expected to be separated is deduced, and this method of separation of the components of mixtures is compared with the continuous counter-current process recorded in Part I.

In Part I (*J.*, 1950, 1068) the mechanism of the separation of solutes subjected to distribution between two liquids flowing in counter-current has been considered. The present communication deals with the behaviour of solutes distributed between two solvents in a series of vessels through which, however, only one of the liquids passes continuously. The principle of the process is illustrated in Fig. 1. The vessels are, for simplicity, supposed to be of equal volume, although this is not essential; each vessel contains the same volume of " static " solvent phase, although this too is not a necessary feature. Moving solvent enters the first vessel and, after mixing with the static solvent, passes to a separator, whence the static phase passes back to the same vessel and the moving solvent proceeds to the next one in the series. It is assumed that each of the solvent phases is mutually saturated with the other before use, so there are no volume changes on mixing.

Fig. 1.



A = *moving solvent.* B = " *static* " *solvent.*

If a solute is placed in the first vessel after each of the vessels has acquired its quota of moving solvent, it will become distributed through the system in a manner which can be readily calculated. The problem is akin to that of a partition chromatogram column, which Martin and Synge (*Biochem. J.*, 1941, **35**, 1358) have treated as made up of a series of " theoretical plates " on which a degree of equilibration is achieved. They further showed that, with the ordinary rates at which the solvent moves through the column, diffusion plays only a negligible rôle. The faster the solvent moves the less the complicating effect of diffusion, but the less likely also that distribution equilibrium will be attained, since diffusion and the passage of solute from one phase to another both depend on the kinetic behaviour of the solute. In the system now being considered diffusion plays no part, since the solute can pass from one vessel to the next only in its solvent vehicle. Consequently, if desired, the rate at which the solute is passed from vessel to vessel can be made so low that there can be no doubt whatever about the essential equilibration of the contents of each vessel.

The chief feature of the process now being described is that it can deal with small quantities of material in very dilute solution, or with larger quantities in much more concentrated solution. Moreover, there is no upper limit to the size or number of vessels. It is shown below that with extended systems the behaviour is strictly analogous to that of perfectly operated partition-chromatogram columns, and that solutes can be separated as precisely as in the latter.

The symbols used in this communication have the following significance :

V = volume of each mixing vessel,
v = volume of " static " solvent present in each vessel,
$p_1, p_2, \ldots p_n$ = concentration of solute in moving solvent in vessel 1, 2, 3 . . . . n,
$q_1, q_2, \ldots q_n$ = concentration of solute in static solvent in vessel 1, 2, 3 . . . . n,
k = distribution coefficient of the solute = p/q,
x = amount of solute introduced into the system, and
v_a = feed rate of the moving solvent.

The solutes are considered to behave ideally, *i.e.*, k is assumed to be independent of concentration.

At any time, t, the amount of solute remaining in the *first vessel* is given by

$$(V - v)p_1 + vq_1 = (V - v)p_1 + vp_1/k = p_1(V - v + v/k) = x - \int_0^t v_a p_1 \mathrm{d}t$$

Differentiating, we have

$$(V - v + v/k)\mathrm{d}p_1/\mathrm{d}t = - v_a p_1$$

and since initially

$$p_1(V - v + v/k) = x$$

the general solution is

$$p_1 = kx \, . \, \mathrm{e}^{-kv_a t/[kV + v(1 - k)]}/[kV + v(1 - k)] \quad . \quad . \quad . \quad . \quad . \quad (1a)$$

Or, writing

$$k/[kV + v(1 - k)] = b$$

we have

$$p_1 = bx \, . \, \mathrm{e}^{-v_a tb} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1b)$$

In the *second vessel* at time t the following relation holds :

$$-\int_0^t v_a p_2 \mathrm{d}t + \int_0^t v_a p_1 \mathrm{d}t = (V - v)p_2 + vp_2/k$$

whence

$$-v_a p_2 + v_a p_1 = (V - v + v/k)\mathrm{d}p_2/\mathrm{d}t$$

Introducing the value of p_1 given in (1) it can be shown that :

$$p_2 = v_a b^2 xt \, . \, \mathrm{e}^{-v_a tb} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

Similarly for *the third and the fourth vessel* the concentrations in the moving solvents are :

$$p_3 = 0{\cdot}5 \; v_a{}^2 b^3 xt^2 \, . \, \mathrm{e}^{-v_a tb} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

and

$$p_4 = 0{\cdot}167 \; v_a{}^3 b^4 xt^3 \, . \, \mathrm{e}^{-v_a tb} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

Whence, in general

$$p_n = (v_a{}^{n-1} b \;\; xt^{n-1}/\underline{|n - 1}) \, . \, \mathrm{e}^{-v_a bt} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (5)$$

Choosing arbitrary values for the parameters in order to find how the concentration changes with time in the several vessels of a series, we get (for $v_a = 1$, $V = 10$, $v = 5$, $k = 1$) the data depicted in Fig. 2. This shows that the concentration maximum built up seems to travel like a wave of gradually diminishing amplitude throughout the series of vessels. It is to be noted, however, that, at any rate for the particular values of the parameters chosen, the maximum concentration in the successive vessels does not diminish rapidly, so that even with the tenth vessel, the maximum concentration is about 70% of that reached in the fifth. It should be observed that, in choosing $v = 5$ and $V = 10$, it is supposed that each vessel is half-full of static solvent, which, as will be shown presently, is not the best condition for the operation of a system.

The time at which the concentration in a given vessel is a maximum can be calculated by differentiating equation (5) and equating to zero. This gives

$$t_{(\max.)} = (n - 1)/v_a b \quad \ldots\ldots\ldots\ldots \quad (6)$$

from which it appears that the concentration maximum passes along at a uniform rate for a given system. As might have been expected, the greater the feed rate of moving solvent, the earlier the moment at which the maximum appears in a given vessel.

The maximum concentration in a given vessel is calculated by combining equations (5) and (6), whence

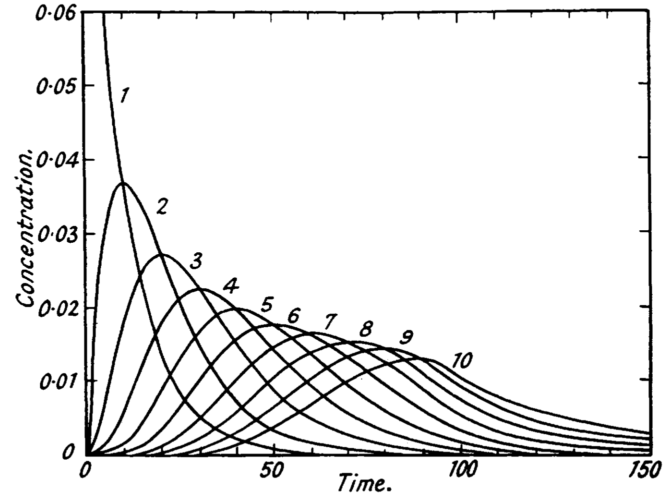
$$p_{n(\max.)} = xb(n - 1)^{n-1} \,.\, \mathrm{e}^{1-n}/|\underline{n - 1} \quad \ldots\ldots\ldots \quad (7)$$

and the ratio of maximum concentrations in successive vessels is found by dividing this expression by one in which $(n + 1)$ replaces n. This gives

$$p_{n(\max)}/p_{(n+1)(\max.)} = [(n - 1)/n]^{n-1}\,\mathrm{e}.$$

By introducing various values of n into this expression a measure of the rate at which the " wave " dies down may be deduced; it is found by such substitutions that the decrement is surprisingly low, which indicates that the solute has a great tendency " to keep together " as it passes along an extensive system, although it must be quite clear that, theoretically, solute must be present indefinitely in all vessels from the moment it is introduced into the system. The concentration in vessels remote from that carrying the maximum is, however, negligibly small and the exact disposition of solute is deduced later.

FIG. 2.



Concentration–time relation for a series of vessels for the arbitrary values : $v_A = 1$; $V = 10$; $v = 5$; $k = 1$.

It is clearly necessary to qualify the statement often made, in connection with partition chromatography in tubes and with paper chromatography, that an ideal solute moves in a band which (when k is constant) shows no tendency to spread. These bands must spread and in general become more diffuse the further they travel along a system. It is true that in a very extended system this effect will be almost imperceptible.

At the moment when the concentration in a given vessel reaches its maximum, such concentration is equal to that in the preceding vessel. This can be shown by observing that, when the concentration in the nth vessel reaches its maximum [equation (6)], the concentration in the $(n - 1)$th must, from (5), be

$$\begin{aligned} p_{n-1} &= (xv_a{}^{n-2}b^{n-1}t^{n-2}/|\underline{n - 2}) \,.\, \mathrm{e}^{-v_a bt} \\ &= xb(n - 1)^{n-1} \,.\, \mathrm{e}^{1-n}/|\underline{n - 1} \end{aligned}$$

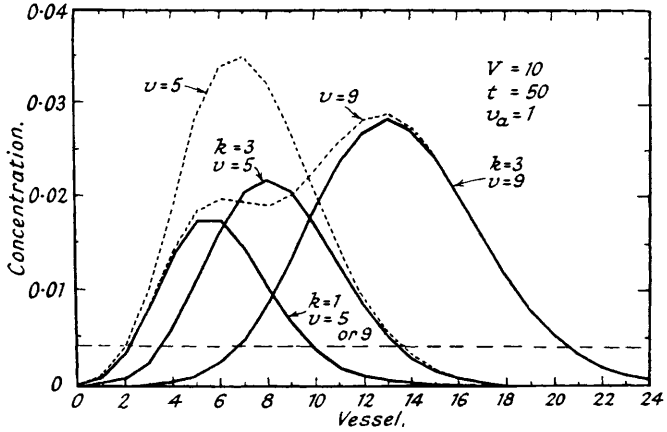
when the appropriate value of t is introduced. This expression for p_{n-1} is identical with (7), and explains why the curves given in Fig. 2 intersect as shown. It appears, therefore, that when the concentration in a vessel is higher than it is in any other vessel, it is itself already declining.

Separation of Two Solutes.—From equation (6) it is clear that the time at which the maximum concentration in a given vessel is reached depends on the distribution coefficient of the solute, the volume of the vessels, the volume of static solvent present, the flow rate of the mobile phase, and the number of the vessel in the series. For a given set-up, therefore, the time is dependent on k, indicating the possibility of separating two or more solutes. However, the *ratio* of $v : V$ is important for the efficiency of separation. To illustrate this, suppose that the volume of the vessel is 10, that v_a is 1, and that the separation of two solutes having $k = 1$ and 3, respectively, is being attempted. In one case, let the ratio of $v : V$ be 0·5, and in a second case be 0·9. The concentrations in each of the vessels of a long series at time $t = 50$, for example, can be calculated from equation (5), and Fig. 3 illustrates the result.

It is seen that when $v : V = 0{\cdot}9$ the peak concentrations of the two solutes are separated to a greater degree than when $v : V = 0{\cdot}5$. If the two solutes were of the same nature, say were both acids, the apparent total acidity of the contents of the various vessels could be represented by the sums of the separate concentrations of the two solutes, the broken lines in the Fig. 3 representing the results of such summations. With the lower ratio $v : V$ the presence of two solutes could be inferred from the slight kink in the total acidity curve, but it is evident that

FIG. 3.

Dependence of the degree of separation of solutes on the volume ratio of solvents.



little separation of the solutes would result by $t = 50$. When $v : V$ is 0·9, however, the " kink " is much more pronounced and appreciable separation of the solutes has occurred.

If v is supposed to become equal to V, equation (5) becomes

$$p_n = (x v_a^{\,n-1} k^n t^{n-1} \,.\, e^{-k v_a t/V}) / (V^n \underline{|n - 1})$$

and the maximum concentration in a given vessel, obtained by differentiating this expression, is reached when

$$t_{(\text{max.})} = V(n - 1)/k v_a$$

The interpretation of this is, that when the volume of " static " solvent in each vessel approaches the volume of the vessel itself, the time at which the maximum concentration of a given solute is reached in a specified vessel is inversely proportional to the distribution coefficient of the solute. In other words, the greater the value of k, the earlier the maximum appears in a given vessel, as might have been surmised. More important, *the maximum of concentration passes along the system at a uniform rate, which is proportional to the distribution coefficient, like a gradually dying wave and, with two or more solutes, the relative distances travelled in the system by these maxima bear a simple relationship to one another, being again proportional to the distribution coefficients of the solutes.* (It should be remembered that in making these deductions the maximum concentration has been assumed to be that in the vessel which has just reached its maximum, and this has been shown to be the same as that existing in the preceding vessel at the same moment. With an extended series of vessels the difference between the nth and $(n - 1)$th vessels is so slight that the conclusions just reached become almost exact.)

In these calculations, it has been assumed that through a series of vessels, almost full of static solvent, a thin stream of moving solvent has been passed, and this approximates closely to partition-chromatogram columns in which the hold-up of the developing phase is small compared with the actual volume of the static phase and its support. It has been indicated above that when the ratio $v : V$ is low the separation of solutes is not so effective, whence it may be concluded that most efficient operation of partition-chromatogram columns requires close packing of the columns. Similarly, since paper chromatography closely resembles chromatography with such columns, swamping the paper with the developing liquid should be carefully avoided. (The strict analogue of paper chromatography is a series of vessels not quite filled with static solvent, through which the moving solvent passes, carrying the solutes along with it. The vessels are successively filled by the moving solvent. This arrangement is insensibly different from that just considered, when the ratio $v : V$ approaches unity.)

It can readily be shown that a system of many vessels is most efficient when v approaches V. Thus, from equation (6), the vessel n_1 in which the maximum of concentration of solute 1 having a distribution coefficient k_1 is to be found, is given by

$$n_1 = \frac{k_1 v_a t}{k_1 V + v(1 - k_1)} + 1$$

and a similar relation holds for a second solute. The best separation of the two solutes will be when

$$\frac{n_1}{n_2} = \left[\frac{k_1 v_a t}{k_1 V + v(1 - k_1)} + 1\right]\left[\frac{1}{\dfrac{k_2 v_a t}{k_2 V + v(1 - k_2)} + 1}\right]$$

is a maximum or a minimum. Obviously, the separation will be better with a larger number of vessels, so the unity terms may be neglected :

$$\frac{n_1}{n_2} = \frac{k_1}{k_2}\left[\frac{k_2 + f(1 - k_2)}{k_1 + f(1 - k_1)}\right], \text{ where } f = v/V$$

Differentiating n_1/n_2 with respect to f shows no maximum or minimum for values of f between 0 and unity; and since when $f = 0$, $n_1/n_2 = 1$, and when $f = 1$, $n_1/n_2 = k_1/k_2$, the maximum separation of two solutes follows the use of volumes of " static " solvent almost equal to those of the containing vessels.

To some extent this could have been expected on general grounds, since such a method of operation of the system is equivalent to the extraction of a solute from one solvent by a large number of extremely small quantities of a second solvent.

It is important to note that pairs of solutes having $k < 1$ will move relatively slowly compared with those having $k > 1$, yet nevertheless two solutes having $k < 1$ can be separated just as efficiently as two solutes having $k > 1$ provided that the ratio of their distribution coefficients is sufficiently large. However, it would in such a case be desirable practically to reverse the rôle of the solvents, the distribution coefficients applicable being then the inverse of the conventional values, so that a more speedy passage of the solutes along the system occurs with just as efficient a separation.

Shape of the Curve connecting Number-of-vessel with the Concentration of its Contents.—So far, in considering the separation of solutes we have been concerned mainly with the extent to which the peak concentrations of the solutes are separated from one another. It is clear from Fig. 3 that appreciable concentrations of solute exist in the vessels on either side of that in which the maximum concentration occurs, and that two solutes may exist together in appreciable, and more or less equal, concentrations in the vessels between those carrying their maxima. It is, therefore, of interest and importance to know something of the shape of the curve connecting concentration with the number-of-vessel, since the imperfection of separation of two solutes depends on the extent to which the curves for the solutes are superimposed. The common area under both curves is a measure of this imperfection and it should be made as small as possible.

Furthermore, we have supposed that the degree of separation of two solutes is directly connected with the degree of separation of the peaks of concentration and this will only be justified if it can be shown that the shape of the " concentration waves " is unconnected with the value of k.

The ratio of the maximum concentration p_r to the concentrations in the vessels in front of and behind the vessel in which the maximum has been reached can be derived for a given time as follows. For the vessel situated one in advance of the vessel having the maximum concentration, the concentration is :

$$
\left.
\begin{aligned}
& & p_{n+1} &= v_a{}^n b^{n+1} x t^n \,.\, e^{-v_a bt}/|\underline{n} & & \\
&\text{giving the ratio} & p_{n+1}/p_n &= v_a bt/n & &= (n-1)/n \text{ [from equation (6)]} \\
&\text{Similarly,} & p_{n+2}/p_n &= v_a{}^2 b^2 t^2/[(n+1)n] & &= (n-1)^2/[n(n+1)] \\
&\text{and} & p_{n+3}/p_n &= v_a{}^3 b^3 t^3 [n(n+1)(n+2)] & &= (n-1)^3/[n(n+1)(n+2)] \\
& & &\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots & & \\
& & p_{n-1}/p_n &= (n-1)/v_a bt & &= 1 \\
& & p_{n-2}/p_n &= [(n-1)(n-2)]/v_a{}^2 b^2 t^2 & &= (n-2)/(n-1) \\
& & p_{n-3}/p_n &= [(n-1)(n-2)(n-3)]/v_a{}^3 b^3 t^3 = & & [(n-2)(n-3)]/(n-1)^2 \\
&\text{or, generally,} & p_{n+r}/p_n &= (n-1)^r/[n(n+1)(n+2)\ldots\ldots(n+r-1)] & & \text{for the } r\text{th vessel in front of that in which the maximum concentration occurs} \\
&\text{and} & p_{n-r-1}/p_n &= [(n-2)(n-3)\ldots\ldots(n-r+1)]/(n-1)^r & & \text{for the } (r+1)\text{th vessel behind that in which the maximum concentration ocurs.}
\end{aligned}
\right\}\quad (8)
$$

The concentrations are, therefore, not quite symmetrically distributed about the *two* vessels in which the concentrations are equal. In general, the concentration in the $(n + r)$th vessel is slightly higher than it is in the $[n - (r + 1)]$th vessel, but with extended systems the curves become more and more symmetrical as time proceeds. The general position is shown in Fig. 3.

It may be supposed that in partition chromatography, including paper chromatography, a similar state of affairs exists. If a given reagent can detect a solute only when its concentration is above a critical value indicated by the broken horizontal line in Fig. 3, the advanced edge of the solute spot detected will appear less sharp than the retreating edge because for the latter the approach to the region of non-detectability is more abrupt. This might account for " diffuse heads " in paper chromatography, while variations in k might explain " tailing " (as several workers have suggested). A variation in k would also tend to produce a sharpened front. With very extended systems such as paper chromatograms, however, the difference between the two sides of the peak is very slight and another explanation may have to be sought. It is also worth note that, since in paper chromatography one is dealing with minute amounts of material—altogether often only a few times the minimum detectable quantities—it may be unwise to rely on measurements of areas of spots for quantitative work; for at points not very remote from the centre of the spot there may be undetectable quantities of the solute, inconsiderable in themselves but forming, nevertheless, a fair proportion of the total amount of solute present.

The most important feature to be observed from equation (8) is that the relative concentrations of a solute present in a specified vessel and that carrying the peak concentration depend only on the number of the latter vessel in the system, whence it follows that the distributions of all solutes about the vessel containing the peak concentration are the same. This means that the shapes of the curves connecting concentration with number-of-vessel depend only on the value of n. In the table are given the concentrations, in all vessels relative to the maximum, for selected values of n (in which vessel the maximum occurs), and these data apply whether the system is being operated under the optimum conditions or not. *Since it has already been shown that maximum separation of peaks occurs when* $v = V$, *it follows that there is less overlap of the concentration waves when this condition applies and that, indeed, it is a condition for most efficient operation of the system.*

Vessel.	$n = 20$.	$n = 40$.	$n = 60$.	$n = 80$.	$n = 100$.
$n - 34$	—	—	—	—	0·001
$n - 33$	—	—	—	—	0·002
$n - 32$	—	—	—	—	0·003
$n - 31$	—	—	—	0·001	0·005
$n - 30$	—	—	—	0·002	0·007
$n - 29$	—	—	—	0·003	0·010
$n - 28$	—	—	—	0·004	0·014
$n - 27$	—	—	0·001	0·006	0·020
$n - 26$	—	—	0·002	0·009	0·026
$n - 25$	—	—	0·003	0·014	0·036
$n - 24$	—	—	0·005	0·020	0·047
$n - 23$	—	—	0·007	0·028	0·061
$n - 22$	—	—	0·011	0·039	0·079
$n - 21$	—	0·001	0·017	0·053	0·100
$n - 20$	—	0·003	0·026	0·071	0·126
$n - 19$	—	0·005	0·039	0·093	0·156
$n - 18$	—	0·009	0·056	0·121	0·191
$n - 17$	—	0·016	0·077	0·155	0·230
$n - 16$	—	0·027	0·106	0·194	0·275
$n - 15$	0·001	0·045	0·142	0·240	0·325
$n - 14$	0·002	0·070	0·186	0·292	0·379
$n - 13$	0·005	0·105	0·238	0·350	0·436
$n - 12$	0·011	0·151	0·299	0·412	0 498
$n - 11$	0 026	0 211	0·368	0·480	0·559
$n - 10$	0·055	0·283	0·444	0·549	0·623
$n - 9$	0·104	0·368	0·524	0·620	0·686
$n - 8$	0·179	0·463	0·606	0·692	0·747
$n - 7$	0·284	0·564	0·688	0·759	0·803
$n - 6$	0·414	0·667	0·767	0·822	0·856
$n - 5$	0·562	0·765	0·838	0·878	0·901
$n - 4$	0·712	0·853	0·900	0·926	0·940
$n - 3$	0·847	0·924	0·948	0·962	0·970
$n - 2$	0·947	0·974	0·983	0·987	0·990
$n - 1$	1·000	1·000	1·000	1·000	1·000
n	1·000	1·000	1·000	1·000	1·000
$n + 1$	0·950	0·975	0·983	0·987	0·990
$n + 2$	0·860	0·927	0·951	0·962	0·970
$n + 3$	0·743	0·861	0·905	0·927	0·940
$n + 4$	0·613	0·780	0·846	0·881	0·903
$n + 5$	0·486	0·691	0·780	0·828	0·858
$n + 6$	0·379	0·598	0·708	0·770	0·809
$n + 7$	0·270	0·508	0·632	0·707	0·755
$n + 8$	0·190	0·422	0·557	0·642	0·698
$n + 9$	0·129	0·342	0·482	0·575	0·640
$n + 10$	0·084	0·273	0·412	0·510	0·580
$n + 11$	0·053	0·212	0·348	0·447	0·522
$n + 12$	0·033	0·163	0·288	0·389	0·466
$n + 13$	0·019	0·122	0·236	0·333	0·412
$n + 14$	0·011	0·090	0·191	0·283	0·360
$n + 15$	0·006	0·065	0·152	0·238	0·312
$n + 16$	0·003	0·046	0·120	0·198	0·268
$n + 17$	0·001	0·032	0·093	0·162	0·228
$n + 18$	—	0·022	0·077	0·132	0·193
$n + 19$	—	0·015	0·058	0·106	0·162
$n + 20$	—	0·010	0·043	0·085	0·134
$n + 21$	—	0·006	0·032	0·067	0·111
$n + 22$	—	0·004	0·023	0·052	0·091
$n + 23$	—	0·002	0·017	0·040	0·073
$n + 24$	—	0·001	0·012	0·031	0·059
$n + 25$	—	—	0·008	0·024	0·047
$n + 26$	—	—	0·006	0·018	0·037
$n + 27$	—	—	0·004	0·013	0·029
$n + 28$	—	—	0·002	0·010	0·023
$n + 29$	—	—	0·001	0·007	0·018
$n + 30$	—	—	—	0·005	0·014
$n + 31$	—	—	—	0·004	0·010
$n + 32$	—	—	—	0·003	0·008
$n + 33$	—	—	—	0·002	0·006
$n + 34$	—	—	—	0·001	0·004
$n + 35$	—	—	—	—	0·003
$n + 36$	—	—	—	—	0·002
$n + 37$	—	—	—	—	0·001

Degree of Separation of Solutes.—A system of the type now being considered could be operated in two ways, analogous to two of the procedures used in chromatography in columns, whether of the adsorption, ion-exchange, or partition types. On the one hand, a mixture of solutes could be introduced into an extended system of vessels and the solutes isolated from the emergent liquid phase. Naturally, if water were the moving phase the solute appearing first in quantity would be that with the highest value of k whatever the amount of this solute compared with that of others. It is true that if this solute were present in very small amount, it might be contaminated with large amounts of solutes of lower distribution coefficient present in predominant quantities, but some concentration of the former solute would nevertheless be effected if the effluent liquid were cut at the appropriate point in the process.

The second method of operating the system would be to introduce the mobile phase until the various solutes were distributed throughout the system and then, after having examined the contents of each vessel, to work up the contents of appropriate vessels on the basis of this examination. There are advantages and disadvantages in both methods, but the method of choice is the former since it is necessary to keep a watch only on the effluent liquid phase; and, most important of all, such a system is considerably more efficient than the other, because full advantage is taken of all the vessels in the system, whereas in the second method some of the solutes will undergo separation by relatively few vessels.

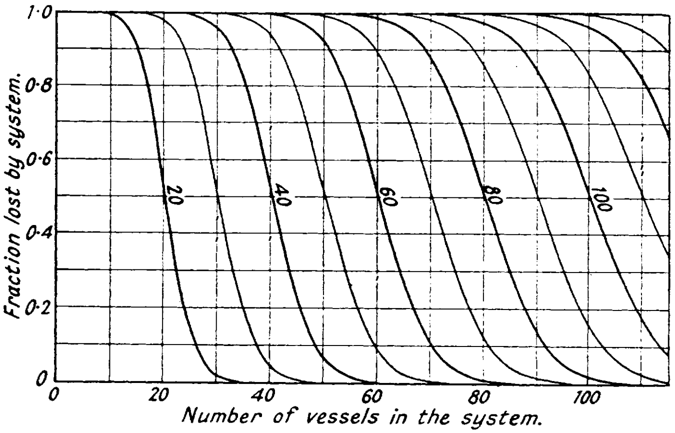
Some idea of the separations to be expected using the second method may be gained from the data in the Table. If the system comprises about 140 vessels and the process has been conducted for such a time that the peak concentration of a solute having the highest value of k_1 is in vessels 99 and 100, then the peak concentration of a second solute having $k_2 = 0{\cdot}8k_1$ will be in vessels 79 and 80. If, furthermore, we assume (and this will be the general assumption throughout this paper in assessing efficiency of separation) that the solutes are present in equal amounts in the system, then vessels 80—100 contain mixtures of various amounts of these two solutes. Thus concentration, in the mobile phase, of the solute of lower value of k in vessel 89 is 0·575 of the peak value, and the concentration of the other solute is 0·559 of its peak value in the same solvent. There is thus an extensive overlap of the two solutes, even with a system comprising 140 vessels. By working up the contents of vessels 90—140 for one solute and the contents of the remaining vessels for the other, the two substances could be obtained approximately 86% pure. Even this is quite good when the closeness of the distribution coefficients is considered. (Operating according to the first method this purity is achieved with 95 vessels.)

The actual point at which to divide the contents of the system is not easily decided when the two solutes are present in unequal amounts and, for this and other reasons already given, this method of operation is not recommended. Attention will, therefore, be directed to the alternative procedure.

Theoretically, even from the outset, *all* solutes emerge from the system, but at different rates. Only after some time will the concentration of the solute of highest distribution coefficient become detectable. Thereafter it will rise to a maximum and then fall to zero after infinite time. The problems to be solved include the point at which to make the " cut," and the minimum number of vessels necessary to permit reasonable separation of solutes.

The rate of emergence of a solute is $v_a p_N$ where p_N is its concentration in the moving solvent

FIG. 4.



(*Values of $v_a bt$ are given on the curves.*)

in the last vessel N of the series. The total amount Q which has left the vessel by time t is given by

$$Q = \int_0^t v_a p_N \mathrm{d}t$$

which, by equation (5) becomes

$$Q = [v_a{}^N b^N x / \underline{|(N-1)}] \int_0^t t^{N-1}\, \mathrm{e}^{-v_a bt} \, . \, \mathrm{d}t$$

The integral can be obtained by reduction and, denoting the fraction of the solute introduced into the system which has left by time t by F, we get

$$\mathrm{F} = \left[1 - \frac{\mathrm{e}^{v_a bt} \text{ expanded to } N \text{ terms}}{\mathrm{e}^{v_a bt} \text{ to an infinite number of terms}} \right] \quad . \quad . \quad . \quad . \quad . \quad (9)$$

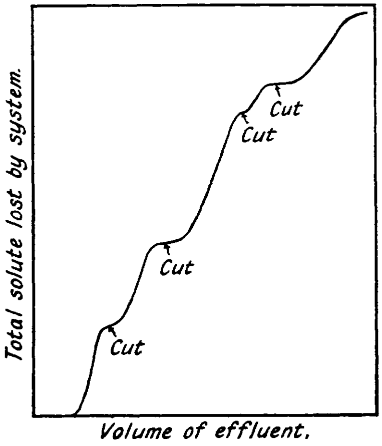
Evaluation of this expression is tedious and in Fig. 4 the only curves which have been calculated are those for $v_a bt = 20$, 40, 60, 80, and 100. Fortunately it is simple to interpolate values for F and the remaining curves in Fig. 4 have been derived in this way.

For a given system, the various amounts F_1, F_2 . . . . . of solutes having distribution coefficients k_1, k_2 . . . . . which have left the system by time t could be calculated from equation (9), but it is simpler to use Fig. 4 especially if the ratio of these amounts is sought. We shall suppose, in the first place, that the system is operated in the most efficient manner, whence the exponents in equation (9) reduce to $kv_a t/V$, in which the only variable is the distribution coefficient if we are considering a given time. Consequently, the various relations in Fig. 4 can be regarded as referring to solutes having values of k determined by the values of $v_a bt =$

kv_at/V recorded on the curves. Thus if we wish to consider the fractions of two solutes having $k = 1{\cdot}0$ and $2{\cdot}0$ which have emerged from a system of 40 vessels we can refer to curves for $kv_at/V = 20$ and 40, or 25 and 50, and so on, realising that the precise pair which must be chosen to arrive at the solution of our problem will be completely determined by the values of v_a, V, and t. To illustrate the use of Fig. 4, suppose that two solutes having $k = 2$ and 3 are introduced in equal amounts into a system of 50 vessels (each having a volume of 10 units) and that the feed rate of the moving solvent is 1 unit. The fractions of the two solutes which would have left the system by $t = 200$ would be (since $kv_at/V = 40$ and 60) 0·070 and 0·915 respectively. Thus, if these were the only solutes present, the material which would have emerged by this time would be $0{\cdot}915 \times 100/(0{\cdot}915 + 0{\cdot}070) = 92{\cdot}9\%$ pure. If the cut were made at this moment and the passage of mobile phase continued, the second solute could be isolated $\dfrac{(1{\cdot}000 - 0{\cdot}070) \times 100}{(1{\cdot}000 - 0{\cdot}070) + (1{\cdot}000 - 0{\cdot}915)} = 91{\cdot}6\%$ pure. If on the other hand the cut were made much earlier or later, the two components would be isolated in widely different purities. Thus if t were 300, kv_at/V would be 60 and 90, giving, as the fractions eliminated, 0·915 and 1·00; the two materials then being obtainable 52·3% and almost 100% pure respectively. The amount of the substance having the latter purity would be, however, only 8·5% of that introduced into the system. If we define as the best degree of separation, that which leads to the isolation of both solutes in the same degree of purity, then the cut should be made at such a time that the amount of the one solute which has left the system is equal to the amount of the other which remains in the system. This can be determined by examination of the appropriate curves in Fig. 4, and it will be seen that the curves corresponding to $t = 200$ (giving $kv_at/V = 40$ and 60) are not far from the ideal for the example given. In practice, of course, the precise moment for the cut can be ascertained in two ways. On the one hand the actual total amount of all solutes which has left the system can be plotted against time and the cut made when the curve takes the trend which indicates that a second solute is emerging (Fig. 5), or a chemical or physical property of the effluent can be plotted against volume (or time, if the feed rate of the mobile phase is steady) and the cut made appropriately. The greatest uncertainty will be for solutes having similar k values, or when the amount of one solute is considerably greater than that of another.

FIG. 5.

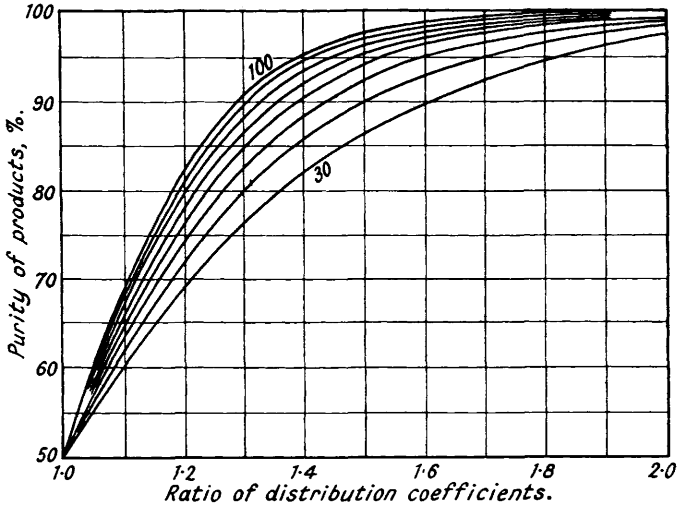
General pattern of discharge diagram.



We shall next consider the number of vessels required to achieve good separation. Martin and Synge (*loc. cit.*) have given some idea of the theoretical-plate efficiency of the columns studied by them, and Stein and Moore (*J. Biol. Chem.*, 1948, **176**, 337) have stated that their columns have an efficiency equivalent to 2000 theoretical plates. The sharp separation of many amino-acids from one another in paper chromatography is eloquent testimony to the theoretical-plate efficiency of this simple technique. To gain some idea of the value of the system now being considered, let us suppose that it comprises 100 vessels. Fig. 4 shows that curves for $v_abt = kv_at/V = 90$ and 110 will be suitable pairs for consideration since both solutes would emerge equally pure. The actual purity would be 84·3%. Such a purity would refer to solutes whose distribution coefficients were in the ratio of 110 : 90 = 1·22. Similarly, curves for 80 and 122 form a suitable pair, corresponding to a distribution coefficient ratio of 1·525 and a purity of 98·4%. By proceeding in this way, the relation between distribution-coefficient ratio and purity can be calculated for a system of 100 vessels, and similar relations for smaller systems can readily be deduced. Fig. 6 illustrates the results of such computations and may be used to design apparatus for separating mixtures of solutes.

As might have been expected, the longer the system, the better the separation, and with 100 vessels the performance should be very good indeed. Thus solutes having a distribution coefficient ratio of 1·3 should be obtained over 90% pure and, if the ratio is 1·6, the purity would be over 99%. With systems as short as 30 vessels products of 90% purity would result if the k ratio were 1·6. This is near the lower limit of usefulness and it can be assumed that for the effective separation of solute mixtures, at least 50 vessels should be used and 50—100 would probably satisfy most demands. It is to be appreciated of course that if the desire is to isolate pure solutes, the efficiency of this operation being a secondary consideration, the cut can be made at an earlier point than usual (which will give the first solute in a purer state), a second fraction of solutes in approximately equal amounts can then be taken and, after the second cut, the second solute can be isolated in a high degree of purity from the third fraction of emergent solvent phase. The second fraction can then be re-cycled through the system. Alternatively, the cut can be made in the usual way and the less pure fractions re-cycled separately. Thus for a 30-vessel system and solutes having a k ratio of 1·6, the products will be 90% pure after the first run. If these are now re-cycled and the cut made at the same time as before the products would be $90 \times 100/91 = 99\%$ pure. Operating in this way is, of course, tantamount to having a longer system. Re-passage of solute through n vessels can give a purer product than is obtainable with $2n$ vessels.

FIG. 6.



The numbers of vessels in the series are 30, 40, 50, 60, 70, 80, 90, *and* 100 *for the curves ranging from the lowest to the topmost, respectively.*

In the discussion just presented it has been assumed that the system has been operated in the most efficient manner, that is, that the moving solvent has comprised only a vanishingly small proportion of the contents of each vessel. In practice, of course, it would usually be difficult to operate the system unless the moving phase occupied at least a small fraction of the volume of each vessel, because the separators must be capable of dealing effectively and continuously with the mixed solvent phases. If the separators were continuous centrifuges, they could almost certainly deal with a solvent volume ratio of 9 : 1 which, however, is probably the lowest value to which it should fall. Using finite volumes of moving solvent causes concentration waves for different solutes to crowd together somewhat and the extent to which this lowers efficiency can be evaluated as follows.

Denoting the appropriate values of $kv_at/[kV + v(1 - k)]$ (*i.e.*, v_abt) derived from Fig. 4 for, say, 100 vessels by P_1 and P_2, so that

$$k_1v_at/[k_1V + v(1 - k_1)] = P_1 \text{ and } k_2v_at/[k_2V + v(1 - k_2)] = P_2$$

it can readily be shown that

$$\frac{k_1}{k_2} = \frac{P_1}{P_2} + \frac{k_1(P_1 - P_2)[V - v]}{P_2v} \quad . \quad . \quad . \quad . \quad . \quad . \quad (10)$$

In discussing the most efficient systems, v was supposed equal to V, whence this expression reduced to $k_1/k_2 = P_1/P_2$ and this was the basis of the deductions given earlier. It is now seen that in practice the ratio k_1/k_2 corresponding to a certain purity which may be ascertained from Fig. 4 is actually dependent on k_1. For given values of P_1 and P_2 ($P_1 > P_2$), the ratio k_1/k_2 is greater than P_1/P_2 if $v \neq V$. The greater the value of k_1, the greater the ratio k_1/k_2. Thus the purity corresponds to P_1 and P_2 when the ratio of distribution coefficients is higher than indicated in Fig. 6, and the effect is most noticeable with high values of k. Thus, taking $P_1 = 113{\cdot}1$ and $P_2 = 87{\cdot}5$ from Fig. 4 for a 100-vessel system, and $v = 0{\cdot}9V$, we get

$$k_1/k_2 = 1{\cdot}292 + 0{\cdot}0325k_1$$

If k_1 is 2, the ratio is 1·357; if k_1 is 10, it is 1·617.

The effect is also more noticeable if P_1 and P_2 correspond to higher purities. Thus it is most desirable to operate with each vessel filled as much as possible with the static phase. This will throw a strain on gravity-type separators such as those described in Part I, since for each volume of mobile phase passing through them there must be at least ten volumes of mixed solvents. Centrifugal separators would be able to deal adequately with this problem and an operation need not last more than a few hours.

The time and the volume of mobile solvent phase taken for a separation can be ascertained from Fig. 4. If the time is defined as that required for the elimination of 99% of the last solute to be discharged from the system, the value of P which corresponds to the number of vessels used can be read off. Thus if the system comprise 100 vessels, P is approximately 123, whence v_at can be calculated from the expression $P = v_abt$ if the lowest distribution coefficient of the mixture of solutes is known approximately. If $v = 0{\cdot}9$, $V = 9$, and $k_{\text{min.}} = 0{\cdot}5$, v_at becomes 2337. The rate of flow of the mobile phase being known, t can be calculated. The expression v_at is the total volume of the mobile phase used and it amounts, in the example just quoted, to 2·337 times the total volume of the 100 vessels, which is not excessive.

Comparison of the Continuous Counter-current and Continuous Step-wise Techniques of Separation of Solutes.—In the continuous counter-current method of separating two solutes by distribution between solvents, the solutes move in opposite directions but the transfer is not direct. Each part of solute stands a considerable chance of being temporarily transported in a direction opposite to that of its general movement.

The process is not unlike the behaviour of a solute diffusing from a point of higher concentration which itself possesses movement of translation. This to-and-fro movement improves the solute separation so that the system is superior to a system operating on the continuous step-wise plan. By comparing Fig. 6 of this communication with Fig. 9 of Part I, it will be seen that a system of 35 vessels arranged in counter-current fashion is as good as one of 100 vessels in which the moving solvent flows step-wise. The relative advantages and disadvantages of the two systems can be summarised as follows. The continuous counter-current arrangement requires a less extended system, and two solutes emerge simultaneously from opposite ends; it does not involve the difficulties of separating the two solvent phases inherent in operating the step-wise system under conditions approximating to the optimum. On the contrary, to take full advantage of the counter-current method, the flow rates of the two solvents must be very closely controlled.

Experiments describing the application of the continuous step-wise process will be described later.

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