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Chromatography and Countercurrent Distribution: Features of the Optimum Phase–Volume Ratio

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Abstract

It is commonly believed that the optimum solvent ratio in countercurrent distribution and chromatography may be expressed as $V = V_U/V_L = (K_{D_1} K_{D_2})^{-1/2}$. Here V_U and V_L are the volumes of mobile and stationary phases. This expression, proposed by Bush and Densen and widely accepted, leads to optimum separations only under special conditions. Under most commonly-encountered situations in chromatographic and countercurrent systems, better separations may be achieved by reducing V to the lowest practicable level. Measures of separation effectiveness include resolution, extent of separation, total percent impurity, and quantity factor, the latter two of which are herein developed. Computer simulation is used for testing existing separation parameters and developing new ones on a rational and scientific basis.

Finding suitable stationary and mobile phases in countercurrent distribution (CCD) and liquid chromatography (LC) is a critical problem. When systems are found in which the physical and chemical properties, as well as economic factors in preparative fractionations, are appropriate, separations may be carried out. The effectiveness of the fractionation will depend upon several additional factors. Of these the choice of cutpoint in the distribution profile and solvent-volume ratios are considered in this work. All data are the result of mathematical computation and computer simulation.

CHOICE OF CUTPOINT

In either preparative or analytical work, separation of two components from one another requires at least three boundaries. These are at the leading and trailing edges of the distribution profiles and between the two solute profiles. The latter of these is the cutpoint under discussion.

When choosing this point in a distribution profile, two factors must be considered. The resulting fractions should be high in purity and should contain relatively large amounts of the desired components This is especially true for a preparative experiment. To express these concepts of purity and quantity mathematically, the following notation for a two-solute two region system is used.

m_{ij} = the number of moles of Component i in Region j;
$i = 1,2; \quad j = 1,2.$
m_{it} = total number of moles of Component i.
m_{tj} = total number of moles in Region j.
m_{tt} = total number of moles of both solutes.

Here the t represents summation over the missing subscript.

The concept of "total percent impurity" (TPI) will be used as a measure of quality. In the following discussion it is assumed that Component 1 is favored in Region 1 and Component 2 is favored in Region 2. The impurity of Region 1 is defined as m_{21}/m_{t1}, that is, $m_{21}/(m_{11} + m_{21})$. Then

$$\text{TPI} = 100\left(\frac{m_{21}}{m_{t1}} + \frac{m_{12}}{m_{t2}}\right) \tag{1}$$

The total amount of solutes partitioned into their appropriate regions is represented by the "quantity factor" (QF):

$$\text{QF} = m_{11} + m_{22} \tag{2}$$

An unstated goal of many separations is the maximization of QF and the minimization of TPI. Although more than one boundary between the profiles may be utilized, the present discussion is restricted to single cutpoints in order to fulfill the first of these goals. No cutpoint which meets both of these goals is known (*1*). Glueckauf (*2*) proposed that a cut be made such that the impurities in each region were equal, i.e., $m_{21}/m_{t1} = m_{12}/m_{t2}$. The utility of this approach was discussed for separations where the components were almost completely resolved. As Said (*3*) pointed out, an analytic expression cannot be derived which predicts this cutpoint for actual chromatographic zones of the normal distribution type when $m_{1t} \neq m_{2t}$. Under this condition the "optimum" cutpoint is difficult to locate.

When equal amounts of the two solutes are to be separated, the "impurities equal" cutpoint occurs at the geometric mean of the retention volumes and tends to give low values of TPI.

Rietema (*4*) has proposed an "efficiency number," E, for evaluating separations, and Rony (*5*) has described a similar term which he called "extent of separation," ξ. Some forms of these terms for a two-component two-region separation, using the aforementioned notation, are

$$E = \left| \frac{m_{11}}{m_{1t}} - \frac{m_{21}}{m_{2t}} \right| = \xi = \left| \frac{m_{11}}{m_{1t}} + \frac{m_{22}}{m_{2t}} - 1 \right| \tag{3}$$

In elution chromatography, for which CCD is a model, it was proposed that the optimum cutpoint is that which maximizes ξ. For Gaussian distribution profiles this point corresponds to the intersection of the normalized profiles (*6*).

The third cutpoint discussed is that at the intersection of the two molar distribution profiles. This cutpoint maximizes QF because it assigns to Region 1 all fractions containing a majority of Solute 1 and to Region 2 all fractions containing a majority of Solute 2. No assumptions are made about the shape, height, or width of the distribution profiles. This makes the intersection an extremely useful cutpoint when multiple inputs are used or when the partition coefficients are known to vary with concentration. In certain cases a mathematical expression can be written for the location of the intersection cutpoint. When a single input of solute is introduced and the conditions are ideal, i.e., constant K_D, V_U, V_L, and no imperfect transfer effects, the volume of effluent at the intersection can be written

$$V_X = \frac{V_{R_1}\sigma_2^2 - V_{R_2}\sigma_1^2 + \sigma_1\sigma_2\sqrt{(\sigma_2^2 - \sigma_1^2)2\ln\left(\frac{m_{1t}\sigma_2}{m_{2t}\sigma_1}\right) + (V_{R_2} - V_{R_1})^2}}{\sigma_2^2 - \sigma_1^2} \tag{4}$$

The transfer number n_X at the intersection is

$$n_X = V_X/V_U \tag{5}$$

where

$$\sigma = \left(\frac{V_L V_R}{K_D}\right)^{\frac{1}{2}} \tag{6}$$

$$V_R = R(V_U + V_L/K_D) \tag{7}$$

and R = the number of tubes in the apparatus. Throughout the manuscript the convention $K_{D_1} > K_{D_2}$ is used.

The above equations are valid for effluent profiles. In the so-called "fundamental method of operation" where a limited number of transfers is performed such that the solute profiles remain in the apparatus, the solute profiles intersect when

$$\binom{n}{r} p_1^r q_1^{n-r} = \binom{n}{r} p_2^r q_2^{n-r} \tag{8}$$

where

$$p = \frac{VK_D}{1 + VK_D}$$

$$q = \frac{1}{1 + VK_D}$$

$$V = V_U/V_L$$

and n = number of transfers.

Solving Eq. (8) for r gives the serial number of the tube where the molar profiles intersect

$$r = n\left(\frac{\ln \frac{p_1}{p_2}}{\ln \frac{q_2}{q_1}} + 1\right)^{-1} \tag{9}$$

The equation for finding the intersection of ideal frontal curves has already been described (*7*). For intermediate cases or when nonideality occurs, computer simulation may be used to predict the intersection cutpoint.

When a total-amount curve such as the solid line in Fig. 1 is encountered, in which it is impossible to get estimates for the retention volumes and the standard deviations, the total curve must be resolved into its individual components. Procedures for resolving curves which are assumed to be Gaussian or skewed Gaussian have been described (*8*). The curves shown in Fig. 1 correspond to the experiment with the molar solute ratio of 10.0/1.9 in Table 1. The three cutpoints discussed above are indicated on the figure and are: A, the intersection of the normalized distribution profiles; B, the intersection of the molar profiles; and C, the cutpoint which gives equal impurities.

Table 1 shows the results of three computer simulations. The initial conditions are the same for the three experiments except for the number of moles of solute 2, m_{2t}. The mass balance for the cutpoint which gives the

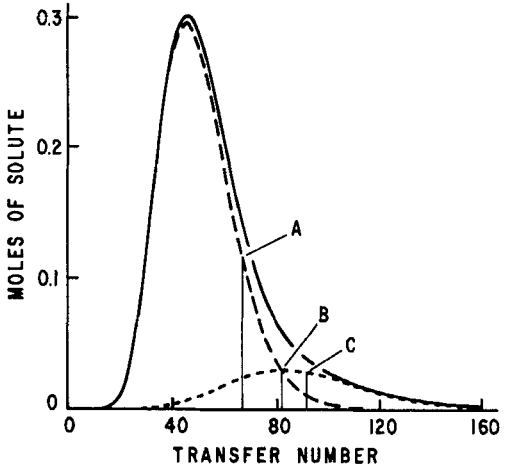

FIG. 1. Location of cutpoints. See text and Table 1.

minimum total percent impurity was calculated and is included for reference. Of the three discussed, Glueckauf's cutpoint gave the lowest TPI for unequal solute ratios, but highest for equal solute ratios. As was predicted, the highest value of QF was always found for the intersection of the molar profiles cutpoint. Values are calculated for ξ in Table 1. In the case where the solute ratio is 1, the two intersection cutpoints are equivalent. When the solute ratio is different from 1, the same cutpoint for the intersection of the normalized distribution profiles applies since ξ is independent of initial solute amounts. For effluent profiles where the conditions of ideality are met, the cutpoint which optimizes ξ is therefore always found by Eq. (4) with the term m_{1t}/m_{2t} omitted.

The validity of ξ as a universal index of separation has been questioned (*1*). The quantities recovered and the purities of each component and TPI experiments shown in Table 1 are clearly different. Yet following the procedure for determination of ξ optimum, one would have to conclude that the same separation had occurred in all three cases. It would appear that ξ is not meaningful for comparing experiments of this type. Likewise, ξ is inappropriate within a given experiment ($m_{1t} \neq m_{2t}$) for indicating a good cutpoint. The clearest example of this is found in Table 1 where the solute ratio is 10.0/1.9. The cutpoint that corresponds to the highest value of ξ also corresponds to the lowest values of QF and the highest value of TPI, indicating that this cutpoint is the poorest of the three.

TABLE 1

Comparison of Cutpoints

$\frac{m_{1t}}{m_{2t}}$	Description of cutpoint	Cutpoint No.	TPI	m_{11}	m_{21}	m_{12}	m_{22}	ξ	QF
$\frac{10.0}{1.90}$	Intersection of normalized profiles	65/66	50.6	8.63	0.34	1.37	1.56	0.68	10.2
	Intersection of molar profiles	81/82	26.8	9.74	0.78	0.26	1.12	0.56	10.9
	Impurities equal	90/91	18.7	9.91	1.04	0.09	0.86	0.44	10.8
	Minimum total percent impurity	110/111	14.5	9.99	1.50	0.01	0.40	0.21	10.4
$\frac{10.0}{10.0}$	Intersection of normalized profiles	65/66	31.7	8.63	1.81	1.37	8.19	0.68	16.8
	Intersection of molar profiles	65/66	31.7	8.63	1.81	1.37	8.19	0.68	16.8
	Impurities equal	63/64	32.2	8.35	1.57	1.65	8.43	0.67	16.8
	Minimum total percent impurity	69/70	31.3	9.07	2.33	0.93	7.67	0.67	16.7
$\frac{10.0}{19.0}$	Intersection of normalized profiles	65/66	36.6	8.63	3.44	1.37	15.56	0.68	24.2
	Intersection of molar profiles	59/60	34.2	7.68	2.18	2.32	16.82	0.65	24.5
	Impurities equal	53/54	33.2	6.36	1.23	3.64	17.77	0.57	24.1
	Minimum total percent impurity	52/53	33.2	6.10	1.10	3.90	17.90	0.55	24.0

Resolution is often used as a measure of separations. Problems in the use of this concept have been discussed (*9*). It appears that there is no single concept that fully measures the amounts and purities of separated components. Similarly a cutpoint cannot be found which minimizes the impurity and maximizes the quantity of the separated components recovered. The "best separation" is that which meets the desired goals.

SOLVENT VOLUME RATIO

One of the easiest parameters for an experimenter to adjust in liquid extraction is the solvent–volume ratio. The expression of Bush and Densen (*10*) for the solvent–volume ratio

$$V = V_U/V_L = (K_{D_1}K_{D_2})^{-\frac{1}{2}} \tag{10}$$

gives the best separation under certain conditions. In CCD this ratio gives the best separation when the fundamental method of operation is used and when the number of transfers is a constant. In this procedure a limited number of transfers is performed such that essentially no solute leaves the instrument. Often the number of transfers performed is equal to the number of tubes. For example, in a 50-tube CCD instrument in which 50 transfers are to be carried out, the optimum solvent–volume ratio is given by Eq. (10). Computer simulations of this case were performed using partition coefficients of $K_{D_1} = 1.5$ and $K_{D_2} = 1.0$. From Eq. (10) the optimum solvent ratio is 0.8. The results are summarized in Table 2 for the intersection cutpoint. Clearly, 0.8 is the best solvent volume ratio. The results of performing 70 transfers in the 50-tube distributor are also given. Again for 70 transfers, 0.8 is the best solvent volume ratio.

TABLE 2

Comparison of Solvent–Volume Ratios for a Constant Number of Transfers in the Fundamental Procedure[a]

V	n	QF	TPI	n	QF	TPI
1.0	50	1.52	47.8	70	1.57	40.4
0.8	50	1.52	47.6	70	1.60	39.6
0.5	50	1.51	48.9	70	1.59	40.9
0.125	50	1.37	62.6	70	1.43	56.1

[a] $K_{D_1} = 1.5$, $K_{D_2} = 1.0$, $R = 50$.

In a more exhaustive study of solvent–volume ratios (*11*), Eq. (10) was verified by simulations of a 200-tube instrument when 200 transfers were carried out.

Grushka's derivation of the Bush and Densen relation (*12*) agrees with this conclusion. However, it will be shown that the Bush and Densen ratio is not an optimum for all CCD operations, but only for the special case that Grushka considered, i.e., the number of transfers is a constant and is less than the number of tubes in the CCD train. In addition, Eq. (7) of Ref. *12* is somewhat misleading because n_{req} cannot always be carried out with the tubes or stages at hand. These relationships will be discussed later in this report.

In the fundamental procedure, the maximum number of transfers performed before some fraction of solute leaves the instrument can be calculated. The distribution of a solute profile in a CCD train is shown in Fig. 2. The quantity t is a factor times the standard deviation of the solute

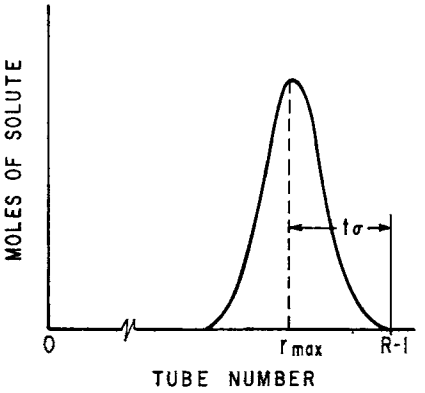

FIG. 2. Distribution profile inside the countercurrent distributor.

profile such that some fraction, $F(t)$, of the solute is still inside the instrument. For instance, when $t = 2.326$, 99% of the solute is inside the CCD train. The peak maximum is given by

$$r_{max} = np$$

and the standard deviation is

$$\sigma = \sqrt{npq}$$

The maximum number of transfers before some fraction of solute leaves the instrument occurs when

$$r_{max} = R - 1 - t\sigma$$

Substituting and solving for $\sqrt{n}$ gives

$$\sqrt{n} = \frac{-t\sqrt{pq} \pm \sqrt{t^2pq + 4p(R-1)}}{2p}$$

Only the positive root is meaningful. In terms of K_D

$$\sqrt{n} = \frac{-t + \sqrt{t^2 - 4(R-1)(1+VK_D)}}{2\sqrt{VK_D}} \tag{11}$$

The three simulations in Table 2 were extended to the maximum value of n for the fundamental procedure. The results are presented in Table 3.

The solvent volume ratio of 0.125 gives the lowest TPI and 0.8 the highest total TPI. The observed value of n from the computer simulations agrees exactly with the value calculated from Eq. (11). The values for resolution within the distributor R_s' give additional evidence that the solvent

TABLE 3

Comparison of Solvent Volume Ratios for a Variable Number (Eq. (11)) of Transfers in the Fundamental Procedure

V	n^a	n^b	TPI	$R_s'^c$	$R_s'^d$
0.8	72	72	39.0	0.41	0.43
0.5	89	89	35.4	0.44	0.47
0.125	229	229	29.8	0.54	0.52

[a] Computer simulation
[b] Equation (11).
[c] Equation (12).
[d] Equation (13).

volume ratio of 0.125 has given the best separation. The observed value of R_s' is found by

$$R_s' = \frac{r_{\max_1} - r_{\max_2}}{2\sigma_1 + 2\sigma_2} \tag{12}$$

The numerator of Eq. (12) has only one significant figure in our 50-tube experiment, which explains the discrepancy between the observed and predicted values for resolution. The predicted values of resolution are given by

$$R_s' = \frac{0.5\sqrt{nV}(K_{D_1} - K_{D_2})}{\sqrt{K_{D_1}(1 + VK_{D_2})} + \sqrt{K_{D_2}(1 + VK_{D_1})}} \tag{13}$$

When computing the observed value of resolution, an estimate of the standard deviation must be made. The normal density function with a standard deviation of 1 and area of 1 has a height of 0.3989. Assuming the observed distributions are nearly Gaussian, σ can be estimated by

$$\sigma = \left(\frac{0.3989}{\text{observed height}}\right) \text{area} \tag{14}$$

Calculations of δ from Eq. (14) agree very well with theoretical values of σ from the binomial distribution $\sigma = \sqrt{npq}$. A comparison of σ's is made in Table 4 using data from the simulations in Table 3.

Equation (14) is especially valuable for estimating σ's when the distribution profile is narrow.

The results of Table 3 show that the Bush and Densen equation, Eq. (10), does not give the best separation when n is a variable. Since resolution, Eq. (13), is proportional to $\sqrt{n}$, smaller values of V allowing more transfers lead to better R_s' and TPI values. Equation (13) is rather misleading because it appears that as $V \to 0$, $R_s' \to 0$. For $V = 0$, of course, no separa-

TABLE 4

Theoretical and Calculated Values of the Standard Deviation of Solute Profiles

	$V = 0.125$, $n = 229$		$V = 0.5$, $n = 89$		$V = 0.8$, $n = 72$	
	$K_D = 1.0$	$K_D = 1.5$	$K_D = 1.0$	$K_D = 1.5$	$K_D = 1.0$	$K_D = 1.5$
σ^a	4.76	5.52	4.49	4.68	4.23	4.25
σ^b	4.76	5.52	4.45	4.67	4.22	4.23

[a] Equation (14).

[b] Binomial distribution $\sqrt{npq}$.

tion is possible but the behavior of R_s' as V becomes a very small number must be examined by incorporating the concept of n as a variable. Substituting for n from Eq. (11) into Eq. (13) gives

$$R_s' = \frac{(\sqrt{t^2 + 4(R-1)(1+VK_{D_1})} - t)(K_{D_1} - K_{D_2})}{4\sqrt{K_{D_1}K_{D_2}}(1+VK_{D_1}) + 4K_{D_1}(1+VK_{D_2})} \tag{15}$$

The K_D in Eq. (11) is K_{D_1}; that is, the faster moving peak. Equation (15) is plotted in Fig. 3 using parameters from the previously discussed system; that is, $K_{D_1} = 1.5$, $K_{D_2} = 1.0$, $R = 50$, and $t = 2.326$. The values of R_s' for $V \leqslant 0$ are not physically realistic but the shape of the curve is of interest because in some systems the maximum will occur at a $+V$ value. The maximum value of Eq. (15) occurs at

$$V = \frac{t}{K_{D_1}(R-1)^{\frac{1}{2}}}\left(\sqrt{\frac{K_{D_1}}{K_{D_2}}} - 1\right)^{\frac{1}{2}} + (K_{D_1}K_{D_2})^{-\frac{1}{2}} - \frac{2}{K_{D_1}} \tag{16}$$

The first term is small compared to the last two, so that for $K_{D_1} \geqslant 4K_{D_2}$, the maximum R_s' will occur at a $+V$ value. When $K_{D_1} < 4K_{D_2}$, however, the smallest possible solvent volume ratio will give the highest value of R_s'.

Up to this point we have been considering the fundamental procedure method where the solute remains in the CCD apparatus. In the single withdrawal method of operation, transfers are performed until the solutes have been eluted from the instrument. For a given number of tubes, R, the single withdrawal procedure gives a better separation than the fundamental procedure because more transfers are performed.

Using arguments similar to those in the derivation of Eq. (11), we can calculate the number of transfers necessary to elute all but a given percent of solute from the instrument. In this case we want $r_{\max} = R - 1 + t\sigma$. The expression relating n and V is

$$\sqrt{n} = \frac{t + \sqrt{t^2 + 4(R-1)(1+VK_D)}}{2\sqrt{VK_D}} \tag{17}$$

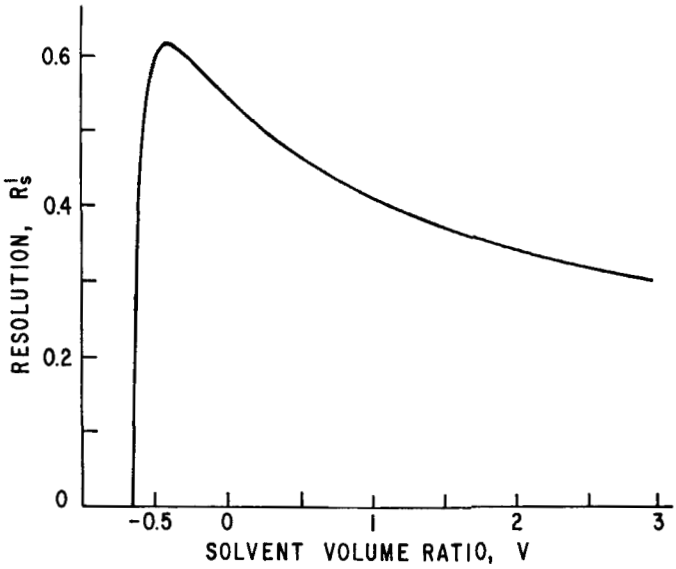

FIG. 3. Effect of solvent ratio on resolution inside the distributor.

Equation (17) is plotted in Fig. 4 for $K_D = 1$, $R = 50$, and $t = 2.326$. In calculating Eq. (17) the slower-moving peak with partition coefficient K_{D_2} should be used. Computer simulations of the single withdrawal method were performed using the same parameters as in the fundamental procedure simulations. Equation (17) was tested for both partition coefficients. As shown in Table 5, the simulations agree well with the prediction from Eq. (17).

The values of σ in Table 5 from the computer simulation are found by Eq. (14). The theoretical σ is given by

$$\sigma = \left[\frac{R}{VK_D}\left(1 + \frac{1}{VK_D}\right)\right]^{\frac{1}{2}} \tag{18}$$

The equation for resolution when operating in the single withdrawal method is given by

$$R_s = \frac{R^{\frac{1}{2}}(K_{D_1} - K_{D_2})}{2K_{D_1}(VK_{D_2} + 1)^{\frac{1}{2}} + 2K_{D_2}(VK_{D_1} + 1)^{\frac{1}{2}}} \tag{19}$$

Here the behavior of R_s, the resolution of the peaks in the effluent as a function of V, is clear. As V becomes smaller, R_s increases. Figure 5 shows a plot of R_s vs V for the aforementioned parameters. Clearly the smallest solvent–volume ratio will give the highest resolution. Values of R_s calcu-

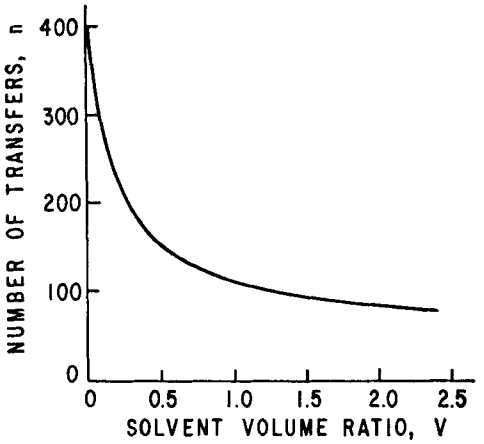

FIG. 4. Number of transfers required to elute 99% of solute.

TABLE 5

Number of Transfers Required for Near Complete Removal of Solute from CCD Train for Various Solvent Volume Ratios

	$K_D = 1.0$				$K_D = 1.5$			
V	n^a	n^b	σ^c	σ^b	n^a	n^b	σ^c	σ^b
0.125	602	601	60.00	59.54	420	420	41.09	41.12
0.5	193	193	17.32	17.19	147	148	12.47	12.39
0.8	141	142	11.85	11.77	112	113	8.74	8.67

[a] Equation (17).
[b] Computer simulation.
[c] Equation (18).

lated and observed and TPI values are given in Table 6.

Bush and Densen described a method of operation which is between the single withdrawal and the fundamental procedure. In this intermediate case, transfers are performed until the intersection of the molar profiles is at the last tube in the apparatus. The number of transfers required is found by rearranging Eq. (9). In Eq. (20), R is fixed and n is the variable:

$$n = R\left(\frac{\ln \frac{p_1}{p_2}}{\ln \frac{q_2}{q_1}} + 1\right) \tag{20}$$

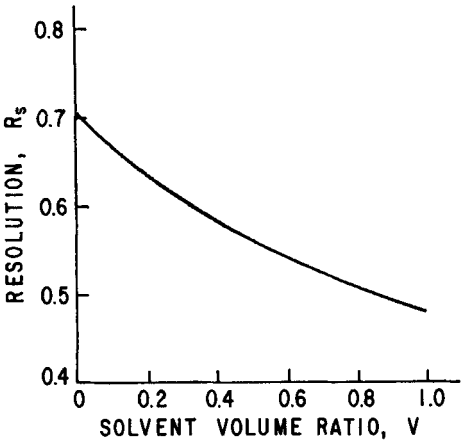

FIG. 5. Effect of solvent–volume ratio on resolution in the CCD output profile.

TABLE 6

Comparison of Solvent–Volume Ratios for Single Withdrawal Procedure

V	TPI	R_s[a]	R_s[b]
0.125	18.3	0.651	0.659
0.5	26.0	0.541	0.559
0.8	30.8	0.514	0.506

[a] Computer simulation.
[b] Equation (19).

Carrying out the number of transfers given by Eq. (20) results in an effluent fraction of m_{11} and m_{21} and a fraction remaining in the apparatus of m_{22} and m_{12}. An alternative statement of Eq. (10) is $p_1 = q_2$, so that Eq. (20) simplifies to $n = 2R$ for the solvent–volume ratio given by Eq. (10). Simulations of the intermediate case were done for $R = 50$, $K_{D_1} = 1.5$, and $K_{D_2} = 1.0$. The results are summarized in Table 7 and indicate that the optimum solvent–volume ratio for the intermediate case is not that given by Eq. (10).

Clearly Eq. (10) is not an optimum for CCD in either the fundamental procedure method, the single withdrawal method, or for the intermediate case. The same conclusion that low values of V give the best separation also holds for partition chromatography for which countercurrent distri-

TABLE 7

Comparison of Solvent–Volume Ratios for the Intermediate Case

			Inside		Outside			
V	n^a	n^b	m_{22}	m_{12}	m_{11}	m_{21}	TPI	QF
1.0	91	91	0.83	0.17	0.83	0.17	33.8	1.66
0.8	100	100	0.84	0.16	0.84	0.16	31.2	1.69
0.5	132	132	0.86	0.12	0.88	0.14	26.0	1.74
0.125	375	375	0.90	0.09	0.91	0.10	18.3	1.82

[a] Equation (20).
[b] Computer simulation.

bution is a model. In partition chromatography this corresponds to keeping the void volume small.

In practice an experimenter must consider factors such as time and cost of separations. A resolution of 1.0 will be sufficient, especially in analytical work. From experimental values of partition coefficients the value of V that will give a resolution of 1.0 can be predicted. Setting the left-hand side of Eq. (19) to 1.0 and solving for V gives a very cumbersome expression to evaluate. If, however, the approximation

$$(K_{D_1}V + 1)^{\frac{1}{2}} \approx (K_{D_2}V + 1)^{\frac{1}{2}} \approx [(K_{D_1}K_{D_2})^{\frac{1}{2}}V + 1]^{\frac{1}{2}}$$

is made, the expression for V which gives a resolution of 1.0 is quite simple:

$$V = \frac{\frac{R}{4}\left(\frac{K_{D_1} - K_{D_2}}{K_{D_1} + K_{D_2}}\right)^2 - 1}{(K_{D_1}K_{D_2})^{\frac{1}{2}}} \tag{21}$$

For a value of R_s other than 1.0,

$$V = \frac{R\left(\frac{K_{D_1} - K_{D_2}}{K_{D_1} + K_{D_2}}\right)^2 - 4R_s^2}{4(K_{D_1}K_{D_2})^{\frac{1}{2}}R_s^2} \tag{22}$$

Equations (21) and (22) can give negative results, indicating that a separation giving unit resolution is impossible for the given K_{D_1}, K_{D_2}, and R. In this case the experimenter may wish to increase R to get the desired resolution. A lower bound for R can be found from Eq. (19) by setting $R_s = 1.0$ and $V = 0$:

$$R = \left(\frac{K_{D_2} + K_{D_1}}{K_{D_1} - K_{D_2}}\right)^2 \tag{23}$$

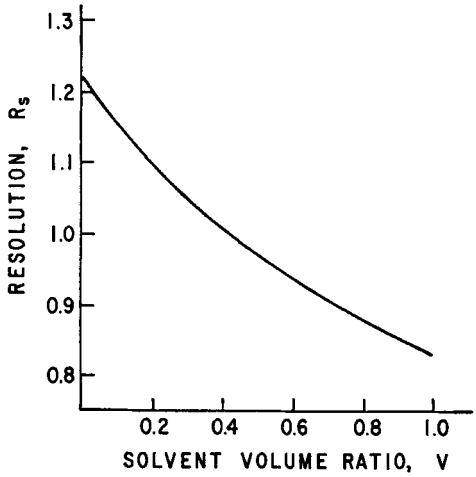

FIG. 6. Effect of solvent–volume ratio on resolution, intermediate case.

Consider the experimental parameters presented in Table 6. Evaluation of Eq. (21) gives a negative volume, so R must be increased to obtain unit resolution. This agrees with Fig. 5 which also shows that unit resolution is impossible for $R = 50$. From Eq. (23) the lower bound on R is 100 tubes. A realistic choice of R for this separation would be 150 tubes. Figure 6 shows that with the proper choice of V, a resolution of 1 is well within reason for $K_{D_1} = 1.5$, $K_{D_2} = 1.0$, and $R = 150$. After selecting V and R, the number of transfers required is given by Eq. (17).

Small solvent–volume ratios require many transfers and consequently a long time. This is a definite disadvantage. A point in favor of small solvent–volume ratios is that the total amount of mobile solvent is reduced. The total upper phase volume required to complete a single withdrawal experiment is

$$V_{U_{\text{req}}} = nV_U = nVV_L$$

Substituting for n from Eq. (15), we get

$$\frac{V_{U_{\text{req}}}}{V_L} = \frac{t^2 + 2(R-1)(1+VK_D) + t\sqrt{t^2 + 4(R-1)(1+VK_D)}}{2K_D} \tag{24}$$

This equation is plotted in Fig. 7 for the parameters from the above discussion with $K_D = 1.0$, $R = 50$, and $t = 2.326$.

While theoretical considerations may indicate low values of V, several other factors limit the lowest volume ratio that can be used in CCD.

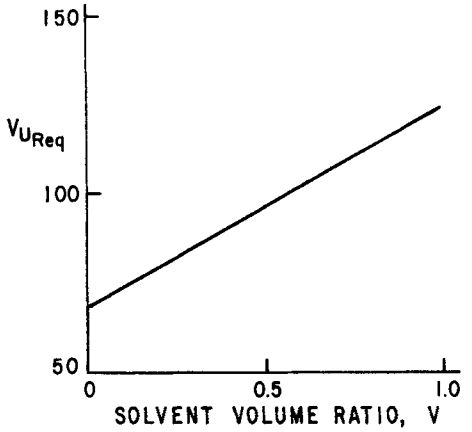

FIG. 7. Volume of mobile phase required to complete a single withdrawal operation.

Phase diagrams of systems studied in our laboratory (*13*) show, for example, that when the solvent ratio is low, the isopycnic region is encountered at low solute levels. Also, the experiment can be altered by the fraction of upper phase retained in each tube (*14*). Using a larger V_U would lessen this effect. So while theoretical predictions and computer simulations indicate that V_U should be very small, practical aspects must be considered.

CONCLUSION

A cutpoint cannot be found which maximizes both the TPI and QF of the solutes. The intersection cutpoint was selected to compare experiments because it is relatively easy to find, maximizes the quantity factor while giving low values of the total percent impurity, and is meaningful for all sizes and shapes of distribution profiles. In general, the lowest possible solvent–volume ratio, not the Bush and Densen ratio, will give the best separation.

Computer programs for simulation of countercurrent distribution are available from this laboratory upon request (*15*).

SYMBOLS

E efficiency

K_D partition coefficient, concentration of solute in upper phase/concentration of solute in lower phase

m_{ij} amount of Component i in Region j
n number of transfers
n_X number of transfers to the intersection of the molar distribution profiles
p probability of finding a solute molecule in the upper (mobile) phase
q probability of finding a solute molecule in the lower (stationary) phase
QF quantity factor
R number of tubes in the apparatus
r serial number of a tube within the apparatus
r_{max} tube containing the maximum amount of solute
R_s resolution of effluent solute profiles
R_s' resolution of solute profiles within the apparatus
t ordinate of cumulative normal distribution,

$$F(t) = \int_{-\infty}^{t} \frac{1}{\sqrt{2\pi}} e^{-X^2/2}\, dX$$

TPI total percent impurity
V solvent volume ratio $= V_U/V_L$
V_U volume of upper (mobile) phase
V_L volume of lower (stationary) phase
V_R retention volume, the accumulated volume of effluent to the solute peak
V_X accumulated volume of effluent to the intersection of the molar distribution profiles

Greek Letters

σ standard deviation
ξ extent of separation

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