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Computer Calculation of Counter-Double-Current-Distribution Curves*

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Summary

A practical method is reported for the calculation by digital computer of counter-double-current-distribution curves of ideal solutes. The method is applicable for the calculation of batchwise operations of the distribution train when the solute is loaded in any number of tubes at any position in the train. Application of the method permits the calculation of the distribution of solutes in the train and in the effluent fractions. The results of calculations of the effects of several modes of operation of the counter-double-current-distribution train are presented. The distribution of a mixture of isoleucine and valine center-loaded into 38 tubes of a 58-tube train is presented as an example.

INTRODUCTION

Counter-double-current distribution (CDCD) is a discontinuous liquid–liquid extraction technique similar to countercurrent distribution (CCD). In CDCD both phases move but in opposite directions at each transfer, while in CCD only one phase moves.

The development of a practical device for CDCD (*1*) provided a means by which large quantities of materials could be fractionated by liquid–liquid extraction. Fractionation of 10-g quantities of α and β chains of hemoglobin (*1,2*) and of 2-g quantities of 4S transfer RNA (*2*) have been achieved by this technique.

The usefulness of CDCD would be increased if a method were

* This investigation was supported in part by a grant (AM 02493) from the National Institutes of Health.

available for the calculation of the solute distribution in the train and in the emerging effluents when more than one tube is loaded with solute or when the sample is loaded off-center. We have used a digital computer simulation of the CDCD process to predict the distribution patterns of ideal solutes* of different partition coefficients. The effect of some of the common variables of CDCD on the distribution patterns has been studied, including the effect of a multiple tube load and an off-center load. The discussion is limited to batchwise operation of the CDCD machine.

EXPERIMENTAL

Materials

All solvents were distilled before use and all were reagent grade. The L-valine was obtained from Mann Research Laboratories and was chromatographically pure. The L-isoleucine was obtained from the Sigma Chemical Company and it was free of alloisoleucine and chromatographically pure.

Counter-Double-Current Distribution

The system used was *n*-butanol/5% hydrochloric acid (50:50 v/v) (3). The amino acids were dissolved in lower phase to give a concentration of 6×10^{-6} *M* for each amino acid. The partition coefficients, measured by the ninhydrin reaction (*4*), were 0.810 for isoleucine and 0.366 for valine. The sample was loaded in the center 38 tubes of a 58-tube CDCD train. The upper phase volume was 20 ml and the lower phase volume was 10 ml. CDCD was carried out for 100 transfers. The effluent tubes and the upper and lower phases of the tubes in the train were then analyzed. The effluent fractions were concentrated on a rotatory evaporator and each fraction was analyzed on an amino acid analyzer (*5*).

THEORY AND CALCULATIONS

Only a brief outline of the theory of CDCD will be presented here, as the basic theory has been presented by Stene (*6*). Con-

* An ideal solute is defined as a solute whose distribution coefficient is independent of solute concentration.

sider the case where the upper phase enters on the left and moves to the right and the lower phase enters on the right and moves to the left. The tubes are numbered from left to right. The amount of solute in any tube r after n transfers is the sum of the material which is contained in the upper phase of the $r-1$ tube at $n-1$ transfers and in the lower phase of the $r+1$ tube at $n-1$ transfers. The amount of material in the upper phase of the $r-1$ tube at $n-1$ transfers equals $[\alpha K/(\alpha K+1)](T_{n-1,r-1})$, where α is the ratio of upper phase volume to lower phase volume, K is the partition coefficient, and $T_{n-1,r-1}$ is the solute in tube $r-1$ at $n-1$ transfers. Similarly, the amount of material in the lower phase of tube $r+1$ at $n-1$ transfers equals $[1/(\alpha K+1)](T_{n-1,r+1})$. Thus the quantity of solute in tube r at n transfers is that shown in Eq. (1).

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

Stene (6) showed that when all the solute remained in the train the process could be described by

$$T_{n,r} = \frac{n!\,[\alpha K/(\alpha K+1)]^{[(n+r)/2]}[1/(\alpha K+1)]^{[(n-r)/2]}}{[(n+r)/2]!\,[(n-r)/2]!} \tag{2}$$

and that $T_{n,r} = 0$, if $[(n+r)/2]$ is not an integer.

If the solutes leave the distribution train in either of the effluent phases, three equations are required to describe the distribution of the solute in the machine at n transfers. The amount of solute in the first tube on the right of the train (fresh lower phase introduced) is given by Eq. (3), that in the center tubes by Eq. (1), and that in the first tube on the left of the train (fresh upper phase introduced) by Eq. (4).

$$T_{n,r} = \frac{\alpha K}{\alpha K+1}(T_{n-1,r-1}) \tag{3}$$

$$T_{n,r} = \frac{1}{\alpha K+1}(T_{n-1,r+1}) \tag{4}$$

The amount of solute in the upper phase (UPn) emerging from the train at the nth transfer is given by Eq. (5). The amount of solute in the lower phase (LPn) emerging at the nth transfer is given by Eq. (6).

$$\mathrm{UP}n = \frac{\alpha K}{\alpha K + 1}\,(T_{n-1}, N) \qquad N = \text{total tubes in the machine} \quad (5)$$

$$\mathrm{LP}n = \frac{1}{\alpha K + 1}\,(T_{n-1}, 1) \quad (6)$$

Although equations have been derived for the calculation of the patterns of the emerging solutes, the manual solution of these equations is time consuming and tedious. The equations of Com-

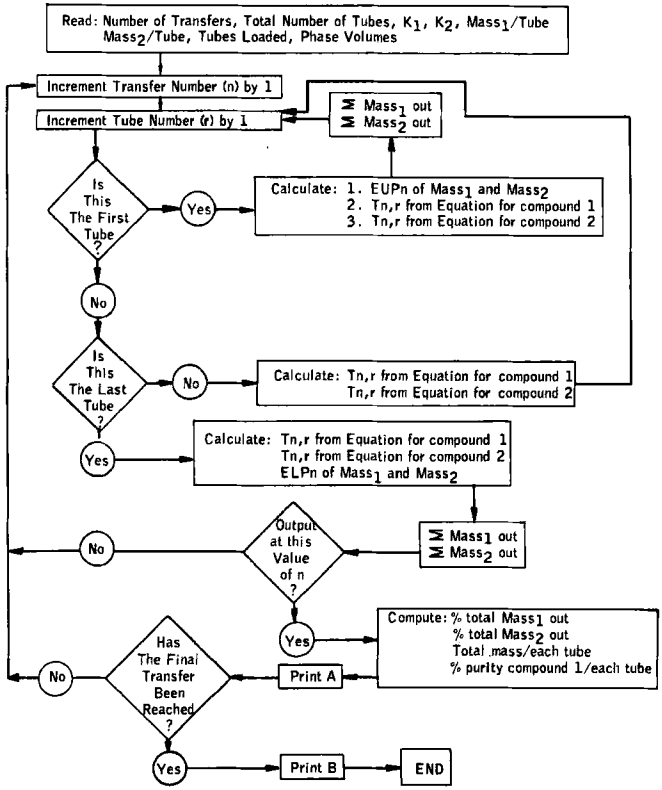

FIG. 1. Flow diagram of the Fortran computer program used to perform calculations of CDCD curves. A: Print the transfer number, print the percents of solutes 1 and 2 removed from the train, and for each tube in the train print the total mass, the mass of solute 1, the mass of solute 2, and the percent purity of solute 1. B: Print for each fraction of each effluent phase at the prescribed transfer intervals the mass of each solute, the total mass, and the percent purity of each solute.

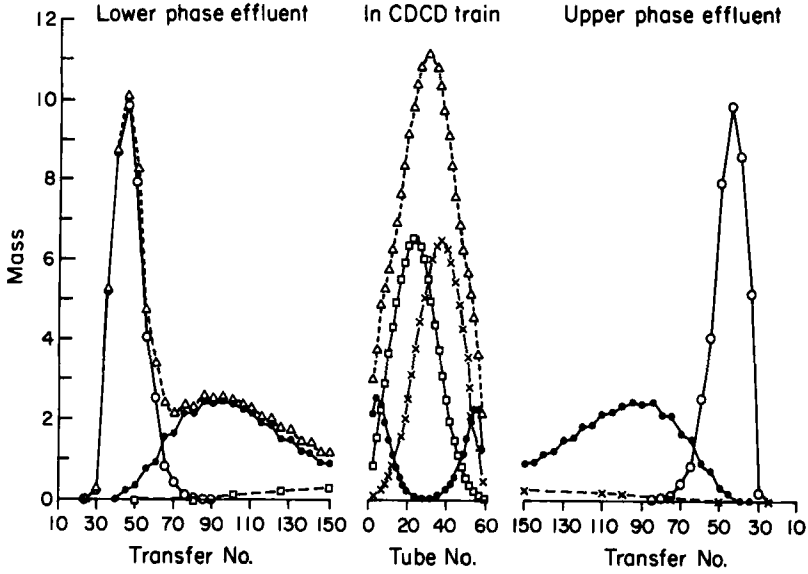

FIG. 2. Calculated CDCD curves after 150 transfers of a 2-tube center-loaded 58-tube train. One hundred mass units of each solute were loaded in each tube. $\beta = 20$, ○——○ ($K_1 = 0.233$, $K_2 = 4.47$); $\beta = 3.0$, ●——● ($K_1 = 0.557$, $K_2 = 1.72$); $\beta = 1.2$ ($K_1 = 0.912$, □——□; $K_2 = 1.095$, ×——×); total mass, △---△.

pere and Ryland (7) do not permit calculation of the solute concentration within the distribution train, and the approach of Hibbits (8) does not lend itself to easy calculation of multiple tube loading or off-center loading.

The calculation of distribution patterns is made by computer calculation of the amount of solute in each tube at each transfer by repetitive use of Eqs. (1), (3), (4), (5), and (6). The calculations were made on a Control Data 160G computer using the program outlined in the block diagram shown in Fig. 1.

RESULTS

The distribution pattern of the solute concentration in the CDCD train and in the upper and lower phase effluent is a function of the parameters of Eq. (2) and of the position where the solute is loaded into the machine. Some calculated CDCD patterns are shown in Figs. 2 and 3. Several calculated plots of percent removal versus

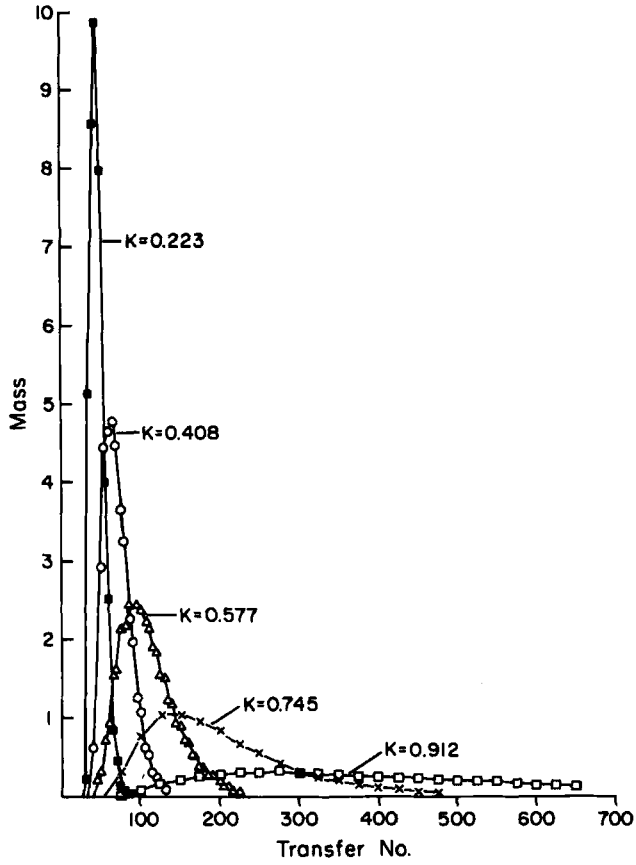

FIG. 3. Calculated CDCD lower phase effluent curves of a 2-tube center-loaded 58-tube train. $K = 0.223$, ■——■; $K = 0.408$, ○——○; $K = 0.577$, △——△; $K = 0.745$, ×——×; $K = 0.912$, □——□.

transfer number are shown in Fig. 4. The curves shown in Fig. 4 are quite similar to those reported by Hibbits (8).

These plots demonstrate the effect of the partition coefficient on the migration of the solutes in the distribution train. The peak of the solutes with a partition coefficient of 1 does not move. The peaks of the solutes with partition coefficients greater than 1 move with the upper phase, and peaks of the solutes with partition coefficients less than 1 move with the lower phase. As the deviation of K from 1 becomes greater, the material moves faster and is con-

centrated in fewer fractions. A marked skewing is observed for solutes with a partition coefficient near 1. The distribution pattern of a material with a partition coefficient $1/K$ is a mirror image of the pattern of a material with a partition coefficient of K (center-loaded, $\alpha = 1$).

The ratio* β of the partition coefficients of the solutes in a binary mixture is a measure of the ease of separation of the two solutes by liquid–liquid extraction techniques (9). If $\beta = 1$, there can be no fractionation of the solutes in the system; and if $\beta > 1$, some purification is possible.

CDCD runs were simulated for β values of 1 to 100. Two solutes of equal mass were loaded in the center tubes of a 58-tube CDCD machine. Partition coefficients were selected such that $\alpha K_1 \cdot \alpha K_2 = 1.000$. The simulation of the distribution was continued until the calculated purity of the effluent solute reached a constant value.

The effect of the β value on the purification of the low K solutes is shown in Table 1 for center loads of 2, 21, and 41 tubes in a

* This β ratio is by definition equal to or greater than 1.

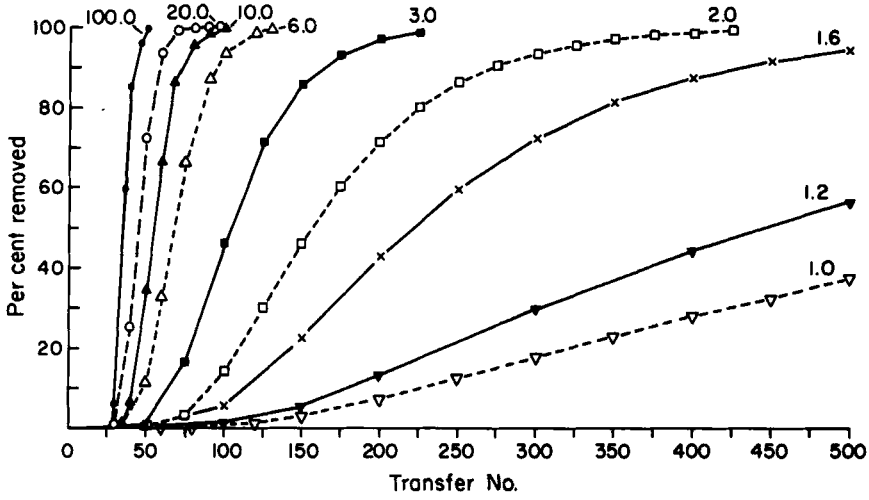

FIG. 4. Calculated plots of percent removal versus transfer number for a 2-tube center-loaded 58-tube train. The partition coefficients are selected so that $\alpha K_1 \cdot \alpha K_2 = 1.000$. $\beta = 100$, ●——●; $\beta = 20$, ○——○; $\beta = 10$, ▲——▲; $\beta = 6$, △——△; $\beta = 3$, ■——■; $\beta = 2$, □——□; $\beta = 1.6$, ×——×; $\beta = 1.2$, ▶——▶; $\beta = 1.0$, ▽——▽.

TABLE 1

Calculated Percent Purity of Low K Solute in Lower Phase Effluent[a]

No. of tubes center-loaded \ β value	1.1	1.2	1.4	1.6	1.8	2.0	3.0	>3.0
2	80	93.4	99.3	99.9	99.98	99.99	100.0	100.0
21	—	92	99	99.8	99.9	99.98	100.0	100.0
41	—	—	—	—	—	99.6	100.0	100.0

[a] An equal mass mixture of the given β value was center-loaded into the appropriate number of tubes in a CDCD train and then distributed until 99% of each solute had left the train. The percent purity is calculated as that of the total solute emerging in the lower phase. Identical values were found for the percent purity of the high K solute in the upper phase effluent.

58-tube machine. The results of a detailed study of the effect of the number of tubes initially loaded on percent purity are shown in Fig. 5. Since the system is symmetrical, the purity of the high K material is the same as that of the low K material.

Thus when solutes are distributed under the conditions given

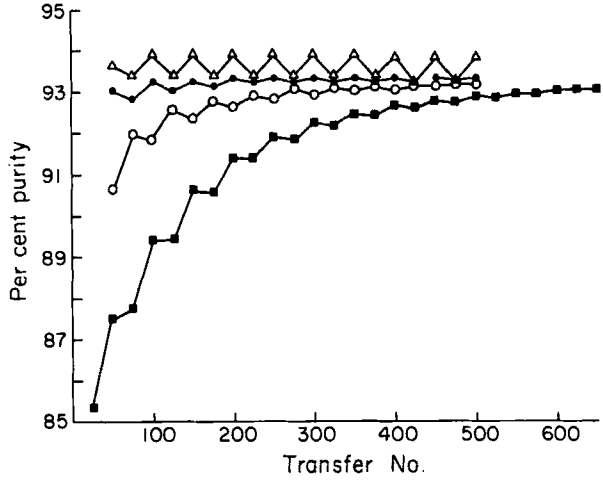

FIG. 5. Calculated CDCD effluent purity curves of a center-loaded binary mixture of $\beta = 1.2$ ($K_1 = 0.912$, $K_2 = 1.095$). The percent purity is that calculated for the single fraction collected at that transfer. Two-tube load, △—△; 5-tube load, ●—●; 11-tube load, ○—○; 21-tube load, ■—■.

in Table 1, a significant portion of the tubes in the train may be loaded with solute with little effect on the final purity of the material. Especially striking is the 99+% purity of the purified materials when a binary mixture with an overall K of 1 and a $\beta \geq 2$ is center-loaded into 70% of the tubes of the train.

The concentration of the purified material is much higher when multiple tube loading has been used. For example, the effluent solute from a 21-tube load is 10 times as concentrated as that from a 2-tube load. The higher concentrations of purified material will generally simplify its isolation.

The efficiency of the separation for solutes with given values of β is dependent upon the effective distribution coefficient of the solute mixture. This can be illustrated by examination of Fig. 3. Note the patterns of the solutes of $K = 0.223$ and $K = 0.408$. The β value for these two solutes is about 1.8 and the K of the mixture equals 0.335. If all the solute of $K = 0.223$ were collected, it would have a 59% purity; and if all the solute of $K = 0.408$ were collected, it would have a 50% purity. Selective cuts to improve the purity would require the sacrifice of solute. When the total overall solute $K = 1$ and $\alpha = 1.8$, the purity of each solute is 99% (Table 1).

The degree of fractionation attainable by CDCD is a direct function of the number of transfers the mixture undergoes. Loading off-center and/or increasing the number of tubes in the CDCD train are useful methods of increasing the number of transfers in which the mixture participates prior to emerging in the effluent. Figure 6 shows that offsetting the initial load of material from the center of the train increases the percent purity of one of the purified materials with a concurrent decrease in yield. Examination of Fig. 7 shows that increasing the number of tubes in the train results in increased fractionation and a simultaneous decrease in concentration of the purified material.

It might appear that the combination of CCD and CDCD would be a powerful method of fractionation. The mixture would be subjected to the fundamental CCD process, and then the distributed material would be directly subjected to CDCD. Since the distribution train available in our laboratory has this potential, several simulations of this type of operation were made.

A comparison of the CDCD simulation and initial CCD followed by CDCD is shown in Fig. 8. These plots and the results of a number of other simulations of the combination of CCD and

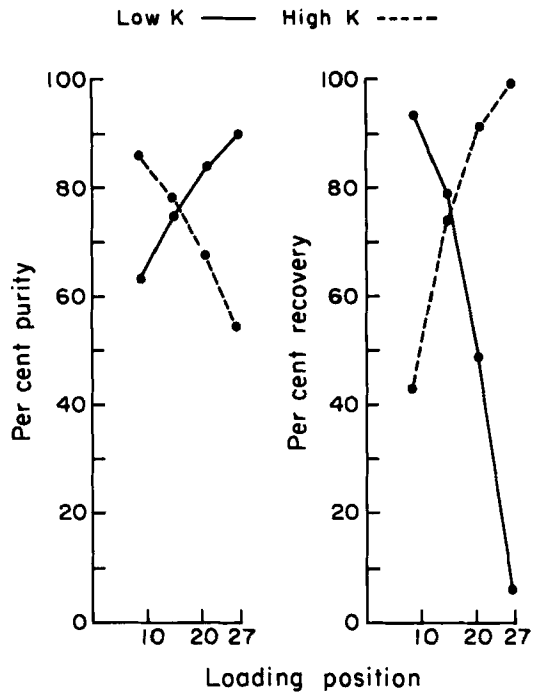

FIG. 6. Calculated percent purities and percent recoveries as a function of loading position. The results shown are from simulated distributions of a 50:50 mixture of two solutes with $\beta = 1.2$ ($K_1 = 0.912$, $K_2 = 1.095$) loaded at the designated position in a 27-tube CDCD train. The values given are those values observed when 99% of the solutes have left the train. The percent purity values and the percent recovery values are those found in the lower phase effluent for the solute of $K = 0.912$ and in the upper phase for the solute of $K = 1.095$. Low K material, ●——●; high K material, ●---●

CDCD do not give any indication that the combination of CCD and CDCD is significantly superior to CDCD.

The results of the counter-double-current distribution of the mixture of isoleucine and valine are shown in Fig. 9. The discrepancy between the calculated and observed curves of the effluents is probably due to imperfect volume transfer and small errors in the measurements of the partition coefficient. A series of calculations have demonstrated that the concentration in the effluent phases can be quite sensitive to these variations. The small deviations in the calculated and observed purities of the amino acids isolated

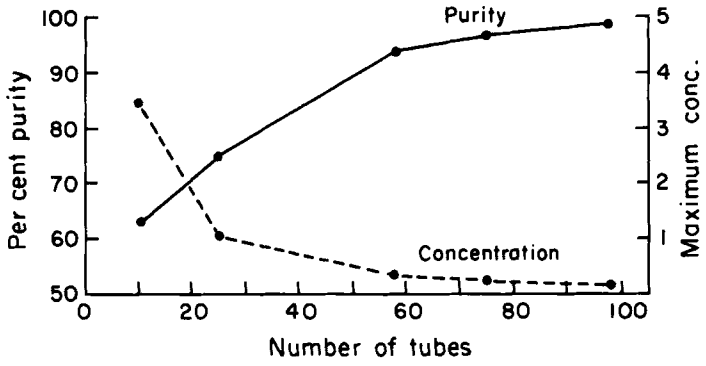

FIG. 7. Effect of train length on the percent purity (●——●) and maximum concentration (●- - -●) of a 2-tube center-loaded solute mixture of $\beta = 1.2$.

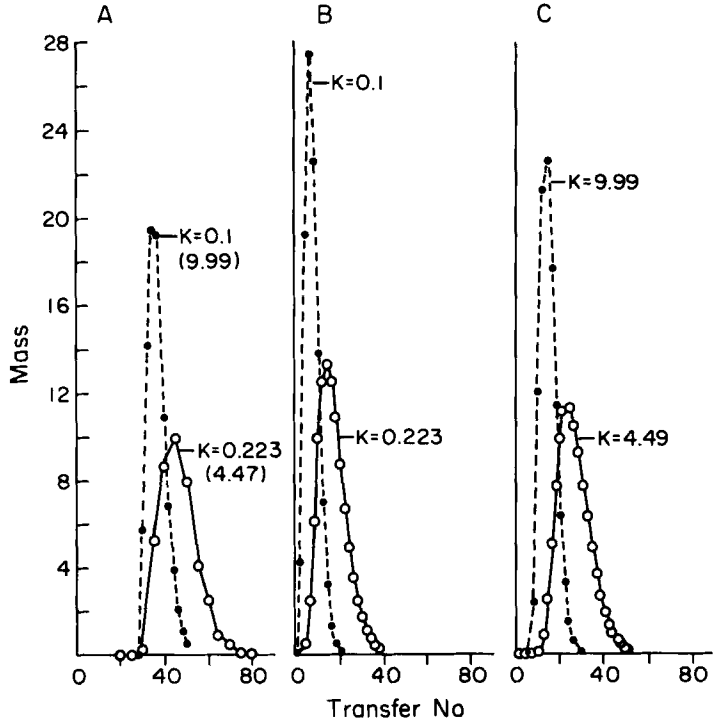

FIG. 8. Comparison of effluent curves from CDCD and from a combination of CCD and CDCD. One hundred mass units of each of four components with partition coefficients of 0.10, 0.223, 4.49, and 9.99 were loaded in each tube. (A) CDCD, 2 tubes center-loaded; (B) lower phase effluent, 2 tubes loaded at front of machine, 50 transfers by CCD followed by CDCD; (C) upper phase, other conditions identical to B.

Per cent purity

	Calc.	Found
Valine	99.87	99.46
Isoleucine	99.48	99.58

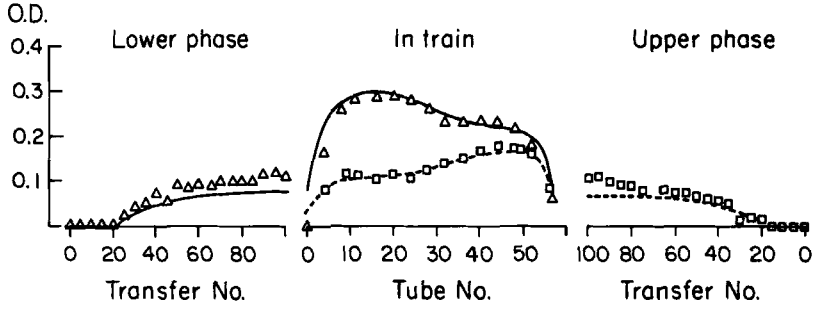

FIG. 9. CDCD of a 38-tube load of an equal molar mixture of isoleucine and valine after 100 transfers. Calculated: lower phase, solid line; upper phase, dashed line. Experimental: upper phase, □; lower phase, △. See experimental section for details.

from the effluent fractions are also probably due to the above-mentioned factors.

DISCUSSION

The results presented here as well as the calculations of Hibbits (*8*) and experiments of Craig and co-workers (*1,9*) indicate that CDCD can be a powerful means of fractionation. If both components of a binary solute mixture are desired in the highest purity, the effective overall K should equal 1, the β value should be as large as possible, and the material should be center-loaded. The simulation techniques used here permit the calculation of CDCD curves when multiple tube loading and/or off-center loading is used. Multiple tube loading can greatly increase the capacity of the train with little loss in the purity of the products. The capacity of the system is demonstrated by an emerging solute of greater than 99% purity ($\beta = 2.0$) from loading 41 out of 58 tubes (see Table 1). If we assume a load of 50 mg/cc in lower phase and a train with a 25-ml lower phase capacity per tube, then 51 g of a binary mix-

ture can be loaded in 41 tubes. A less obvious advantage of multiple tube loading (i.e., single-time bulk loading) over a continuous feed operation is that the concentration of ideal solutes does not increase in a multiple-tube-loading situation, whereas the solute concentrations do increase in a continuous-feed operation (*6,9,10*). Although this increase of solute concentration can be useful in concentrating minor components of mixtures, the partition coefficients of the solute are likely to become concentration dependent at the high concentrations of solute (*9*).

The lack of a good method for determining a running partition coefficient for a solute in CDCD operations presents a problem in the exact calculation of the CDCD curve. Partition coefficients vary slightly from distribution to distribution as a result of the accumulated small variations in the runs. Thus the assessment of purity by comparing the experimental and calculated curves is not as rigorous as that in CCD, particularly since the resolution of two solutes of similar partition coefficients both less than or both greater than 1 is not as great in CDCD as in CCD.

Purity checks can be made by measuring the distribution coefficients across the peaks as done in CCD (*9*). Purity checks may be made on the effluent material if each fraction of the effluent has some of the alternate phase added to it before the determination of the partition coefficient.

Sometimes it is not practicable to compute the CDCD curves. When only two or three tubes are center-loaded and the partition coefficients are known, Craig's method (*1*) of doubling the normal curve of error and normalizing to the tube of maximum concentration gives a curve which appears to be identical to the computer-calculated curve if less than 20% of the solute has left the distribution train.

The computer simulation technique described in this article provides a means for the calculation of CDCD curves only for ideal systems. An ideal CDCD system is defined as one in which (1) the partition coefficients are independent of concentration and/or of the presence of other solutes in the mixture, (2) the volume and composition of the solvent of each phase at each transfer is invarient, (3) equilibrium has been reached in each tube at each transfer, and (4) no chemical modification of the solutes or solvent has occurred during the distribution.

Several workers have proposed techniques for the calculation of

CCD curves which take into account variables 1 and 2 (*11–13*). These techniques should be equally applicable to CDCD.

The calculation of CDCD curves of solutes with concentration-dependent partition coefficients should be possible by including the approach of Williams and Craig (*12*) with the program described in this article. The effect of variation in phase volume and phase composition on the CDCD curves could be calculated by combining the approach of Eddy and Showell (*11*) with the approach of Rothbart and co-workers (*13*) and inserting these into the CDCD program. A program combining all these approaches should provide a means for the calculation of CDCD curves of nonideal solutes in nonideal solvents with nonideal transfer patterns.

Acknowledgments

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