

**COUNTERCURRENT DISTRIBUTION**

BY H. L. ROTHBART AND R. A. BARFORD,  
*U.S. Department of Agriculture, Eastern Regional  
Research Center, Philadelphia, Pennsylvania*

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## I. INTRODUCTION

### A. HISTORICAL

In the early decades of this century, chemical engineers developed liquid extraction systems for separation and purification of materials through processes they called countercurrent extraction. Lewis (54) in 1916 discussed countercurrent principles for the extraction of solids or gases by liquids. Evans (30) considered analogies between extraction and distillation, and Hunter and Nash (44) applied some of the plate concepts of distillation to liquid-liquid extraction. The use of extraction was warranted in those cases where (1) solutes to be separated did not differ in volatility sufficiently for separation by distillation, (2) the solutes were heat sensitive, and (3) economic savings, usually related to energy, could be achieved. Sherwood and Pigford (83) relate the use of extraction for separation of aromatic from paraffinic hydrocarbons in the petroleum industry which corresponds to case 1 above. The biochemist Craig (24) has pioneered in the separation of ther-

mally labile antibiotics by countercurrent distribution. An example of case 3 was provided by Othmer and Trueger (62) for solvent extraction of ethanol and acetone from dilute aqueous solution. Distillation from the extracts apparently required less energy than the distillation from aqueous solution.

Many of the extraction systems described in the engineering literature are continuous and may be summarized as in Fig. 12.1. Extracts 1 and 2 are enriched in particular components which may then be separated from the solvents, usually by distillation, although sometimes by precipitation, and the solvents are then recycled through the extraction apparatus. Cornish et al. (21) in 1934 described a multiplate "column" of 210 units suitable for laboratory use. It had a central feed tube and alternate mixing and settling chambers; thus, only half of the 210 units could be said to be efficient for the extraction of the solutes. The contents of both extracts and the "column" were analyzed at the end of the process.

Syngé (88) in 1939 proposed the use of sequential liquid-liquid extraction for the separation and analysis of amino acid derivatives resulting from hydrolysis and other chemical treatment of proteins. Martin and Syngé (55) in 1941 described a 40-unit "train" useful for the separation of some acetyl derivatives of amino acids. Solute was fed into the apparatus near the center tube and solutes removed from the extracts at either end of the "train." The discontinuous apparatus is depicted in Fig. 12.2. They pointed out that in the future they would operate by adding the mixture at one end in order that "the effective length of the column would be twice that under the conditions of operation described. . . ." In 1944, Craig (22) reported on the use of this latter approach and the construction by Post of a 20-tube stainless steel device to carry out the process which he called "countercurrent distribution." A schematic view of this approach is given in Fig. 12.3. Most of the modern countercurrent distributors are constructed of glass (24), but one of Craig and Post's early metal distributors is displayed at the Smithsonian Museum of Science and Technology in Washington, D.C. Many other devices for multiple extraction have been developed, including the semicontinuous apparatus of Signer and Arm (82), the steady-state distributor de-

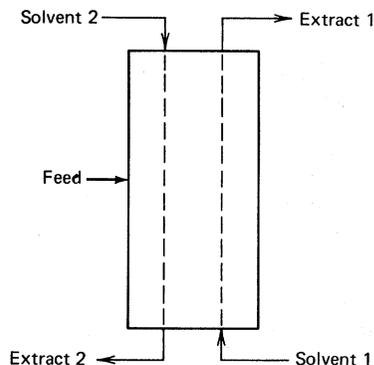


Fig. 12.1. Schematic diagram of a continuous extractor. Denser liquid, solvent 2, is pumped into the top and collected at the bottom. Solutes to be separated (feed) are pumped into center region. Provisions are made for intimate contact within the extractor and separation of each extract from the solvent at the ends of the extractor.

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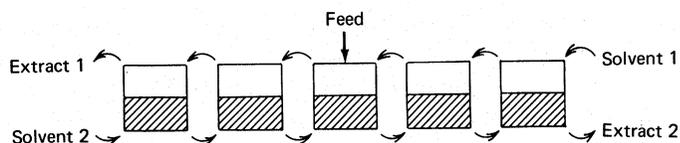


Fig. 12.2 Schematic diagram of a discontinuous extractor. Denser liquid, solvent 2, is pumped into the end tube, equilibrated by shaking, and after settling is passed on to the next tube. The less dense solvent 1 passes in the opposite direction after each equilibration.

scribed by Alderweireldt (3), the “coil planet centrifuge” for countercurrent chromatography of Ito et al. (48) and its subsequent modifications (48a), and the thin-layer countercurrent distribution apparatus described by Albertsson (2).

At about the same time the laboratory equipment was developed, fundamental mathematical approaches for countercurrent distribution were conceived and published. During the course of investigation of chromatography for the separation of amino acids, Martin and Synge (56) viewed the chromatographic column as a series of extraction tubes. Their mathematical approach recognized that the solute distribution profile could be described by the binomial distribution. In the limit, solute distribution profiles could be approximated by the Gaussian distribution which they suggested for chromatographic output curves. Craig (22) referred to their approach but chose to relate curves from countercurrent distribution to the mathematical treatment of linear diffusion against a concentration gradient. He used an essentially Gaussian approximation to the discontinuous curve. At about the same time, Stene (86), who was considering systematic extraction for the analysis of polluting inorganic salts drawn into water mains, published a monumental work (in English) which made use of probability theory for the

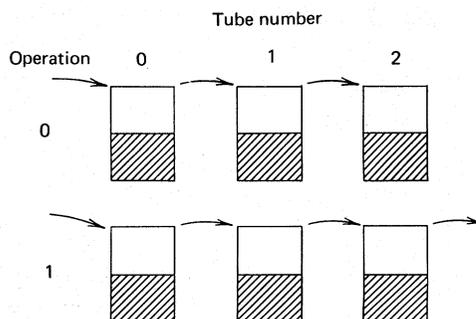


Fig. 12.3. Schematic diagram of countercurrent distribution (CCD). Solute dissolved in mobile (upper) phase is introduced into tube 0 and equilibrated with the stationary (lower) phase. After shaking and settling, the next operation is started by transferring the mobile phases to the next higher-numbered tubes. Fresh mobile phase is introduced into tube 0.

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description of many of the forms of sequential extraction that have been cited in this section.

### B. SCOPE OF THE PRESENT CHAPTER

In the chapter on liquid extraction, solvent systems and other factors affecting the distribution of primarily inorganic ions and complexing agents were explored. It was demonstrated that differences in the distribution of solutes could be used to develop separations. If the nature of the distributions of two solutes between immiscible phases is vastly different, one or a few extractions may be used to provide a nearly complete separation. Mixtures of solutes with distribution properties similar to one another require more complex strategies if acceptable separations are to be achieved. Stene differentiated between continuous and discontinuous processes such as those depicted in Figs. 12.1 and 12.2. He further called the type of process shown in Fig. 12.2 countercurrent extraction, since the two phases are mobile and pass each other traveling in opposite directions. Craig called this process counter-double-current distribution (CDCD) and differentiated it from the process more typically encountered in analytical chemistry and shown in Fig. 12.3. This latter process was called countercurrent distribution (CCD) by Craig. The mechanical genius of Post (24,66), who built apparatus, and the extensive biochemical knowledge, the applications, and the publications of Craig (26) have led to broad dissemination of this latter terminology. For this reason, we use countercurrent distribution (CCD) to describe the process depicted in Fig. 12.3. In this process, solute is introduced into tube number 0 on operation number zero. The tube is shaken to speed equilibration, and the phases are allowed to settle and separate. The lower phase ideally remains in the original tubes throughout the course of an experiment. The upper phase is transferred to the next (higher serial number) tube after phase separation. The equilibration and phase-separation steps are repeated and the mobile phase transferred again. Solute may be introduced into the original tube on only the first or many successive operations. When the number of transfers is equal to the number of tubes and, at the end of the process, the solute is distributed in all containers in both phases, the approach is called the "fundamental process." However, there is nothing particularly fundamental about it.

When the number of transfers exceeds the number of tubes, solute is removed from equilibrium contact at the end of the extraction train (also called a distributor), and thus solute is eventually eluted. This approach is called "single withdrawal." Both Stene (86) and Martin (56) viewed chromatography as a semicontinuous process of this sort with a discontinuous stationary phase and a continuous mobile phase. Mayer and Tompkins (58) developed a plate-equilibrium theory of ion-exchange chromatography based upon the completely discontinuous model. A related approach, extraction chromatography, has been widely applied in recent years to the

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separation of heavy metals and preparation of radioisotopes. Studies such as those on the effect of column loading (93) and the effect of the support properties (61) are aiding the optimization of separations by this approach. An excellent discussion of this method has been published (14) but will not be considered further here. Although there are limitations to viewing chromatography through such approaches (35), the models have been useful. A major thrust of this chapter will be to consider countercurrent distribution, CCD, as defined by Craig, as it relates to analytical chemistry and glean some insights into analogous separation processes. The process of counter-double-current distribution, CDCD, as defined by Craig, will also be approached in some detail, although closed-form analytical expressions describing this distribution have not been developed. There are a number of continuous or semicontinuous separation devices analogous to CCD and CDCD, such as the elution centrifuge and the coil planet centrifuge (48). Although these will be introduced in this chapter, a more detailed exposition has been reserved for another chapter.

The major emphasis of this chapter is on those discontinuous sequential extraction techniques for which a significant literature has developed. A number of other extraction schemes have been proposed, but these have not yet made major impact upon the analytical literature and so are not covered in this Treatise.

## C. SEQUENTIAL EXTRACTION IN ANALYSIS

The relatively large size of the typical tube or stage in extraction makes this tool particularly useful for preparative work. In alternate years until 1976, progress in extraction was reported in the April (reviews) issues of *Analytical Chemistry*. Much of the review was devoted to sample isolation and purification. In most of these approaches, a relatively selective method of detection for some solute is intended; however, a limited number of interfering solutes must be removed prior to use of the detector. Other approaches are used when chemical separations are significant in the "analysis determining step." In this condition, exemplified by chromatography, a relatively indiscriminant detector is used, one which ideally does not distinguish between the solutes to be determined, although it must differentiate the solutes from the solvents present during the detection step. Detectors such as those based on refractive index are typical of this type. The analysis then becomes dependent upon the separation of the solute to be determined from all other solutes. Even an analytical balance can be used as the detector, after the solvent has been evaporated, for the determination of solutes which are almost identical. This strategy has proved exceptionally useful for closely related materials such as homologs, positional and stereo isomers, and even enantiomers. Detailed reviews of applications (2,25,26,68a) and theory (8,86) have been published.

The impact of sequential extraction in analysis has been limited by the

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greater utility of liquid chromatography. In order to separate two closely related compounds or many less similar compounds, a large number of tubes (equilibrium stages, plates) is required. The cost of equipment, space required, and time needed for a separation by countercurrent distribution far exceeds those of liquid chromatography. Why then use CCD for analysis, in view of this relative inefficiency? There may be solvent systems of particular utility which may not be achievable in liquid-liquid chromatography, although recent developments in chromatography using bonded phases have made this a less significant factor. These developments are covered in another chapter. There are special solvent transport conditions, such as transfer programming, which may be used to isolate solutes in particular tubes. This approach is not reasonably achievable in other separation processes. In some cases, solutes are adversely affected by the supposedly "inert supports" encountered in liquid chromatography. In such cases, CCD may provide a useful alternative; the high plate numbers achievable in countercurrent chromatography, discussed in another chapter, make this an attractive alternative. The separation of a variety of biological cells and organelles transported by liquids, in some cases much like classical CCD, has been accomplished by Albertsson (2) by a process known as interfacial distribution and is discussed later in this chapter. The separations are useful both analytically and preparatively and are not readily achievable by other means.

Thus, although sequential extraction has been limited in utility, it does provide special analytical capability in a wide variety of separations, is useful in preparative work even on the industrial scale, and provides a useful model for study of some of the underlying phenomena in continuous systems.

### II. COUNTERCURRENT DISTRIBUTION (CCD)

#### A. IDEAL DISTRIBUTION WITHIN EXTRACTION TRAIN

##### 1. Binomial Distribution

Consider  $X_0$  millimoles of solute originally dissolved in  $v_M$  milliliters of solvent which is identical to the mobile phase. This is equilibrated, by shaking with  $v_S$  milliliters of stationary phase. At equilibrium,

$$K = \frac{(X_0 - X_M)/v_S}{X_M/v_M} = \frac{X_S/v_S}{X_M/v_M} = \frac{1}{K_D} \quad (1)$$

where  $X_M$  and  $X_S$  are the numbers of millimoles of solute in the mobile and stationary phases, respectively, at equilibrium;  $K$ , the distribution coefficient in concentration units, is the reciprocal of that often used in CCD which is denoted  $K_D$ . The choice of form is arbitrary but has been presented in this manner for consistency with chromatographic usage and to simplify

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comparisons with liquid chromatography in succeeding sections. An alternative form of the equation uses subscripts  $U$  and  $L$  for upper and lower phases, respectively. This will be used in the section on CDCD, since both phases are mobile and have to be specified. Some authors use subscripts indicating organic and water phases. This form is not used since both phases may be organic in character.

The fraction of the original solute in the mobile phase after equilibration is denoted  $p$  and is

$$p = \frac{X_M}{X_0} = \frac{v_M}{Kv_S + v_M} \quad (2)$$

and the fraction in the stationary phase is

$$1 - p = q = \frac{X_S}{X_0} = \frac{Kv_S}{Kv_S + v_M} \quad (3)$$

If the mobile phase is removed from the equilibrium stage and a fresh sample of mobile phase, which initially does not contain solute, is added and equilibrated, the fraction of solute remaining in the stationary phase,  $q$ , is distributed between the phases. At equilibrium, the fraction in the mobile phase is  $qp$ , and the fraction in the stationary phase is  $q^2$ . In general, after an equilibration, the fraction of solute in the mobile phase of a tube is calculated by multiplying the fraction of total solute in that tube by  $p$ . Similarly, the fraction of solute in the stationary phase of that tube may be calculated by multiplying the fraction of total solute in the tube by  $q$ .

In countercurrent distribution, the mobile phase which contained the fraction  $p$  of original material is transferred to the next tube in the instrument and equilibrated. If this tube is identical to the previous tube, the solute is distributed similarly with  $p^2$ , the fraction of initial solute in the mobile phase, and  $pq$ , the fraction in the stationary phase. In typical countercurrent distributors, there are usually between 20 and 200 tubes, which are also called equilibrium stages or plates. These are usually numbered from zero to the  $r$ th tube, and each set of transfer operations, which takes place simultaneously, is numbered from zero to the  $n$ th operation. This numbering from zero has been used to simplify the mathematical construction to correlate with well-developed statistical derivations. Table 12.I summarizes a series of equilibrations and transfers. Eventually, solute that is initially in the zeroth tube is transported to higher-numbered tubes. As long as  $p$  is not 0 or 1, a band of solute results from this process which is broader and lower in concentration (or fraction) than the initial solute band. Separation of solutes from one another depends upon differences in the values of  $p$ . Examples are depicted in Figs. 12.4 and 12.5.

The pattern of distribution in Table 12.I is readily apparent to individuals familiar with the binomial distribution. The fraction of original solute in each tube is represented by the binomial expansion of  $(p + q)^n$ , in which  $n$  is the

**TABLE 12.I**  
**Fraction of Original Solute as a Function of Tube Number  $r$  and Transfer Number  $n$**

$n$	Comment	Fraction in					
		$r = 0$	$r = 1$	$r = 2$	$r = 3$	$r = 4$	$r = 5$
0	Before equilibration						
	Mobile	1					
	Stationary	0					
	At equilibrium						
	Mobile	$p$					
	Stationary	$q$					
1	After transfer						
	Before equilibration						
	Mobile	0	$p$				
	Stationary	$q$	0				
	At equilibrium						
	Mobile	$pq$	$p^2$				
	Stationary	$q^2$	$pq$				
							$q + p = (q + p)^1 = 1$
2	After transfer						
	Before equilibration						
	Mobile	0	$pq$	$p^2$			
	Stationary	$q^2$	$pq$				
	At equilibrium						
	Mobile	$pq^2$	$2p^2q$	$p^3$			
	Stationary	$q^3$	$2pq^2$	$p^2q$			
							$q^2 + 2pq + p^2 = (q + p)^2 = 1$
3	After transfer						
	Before equilibration						
	Mobile	0	$pq^2$	$2p^2q$	$p^3$		
	Stationary	$q^3$	$2pq^2$	$p^2q$			
	At equilibrium						
	Mobile	$pq^3$	$3p^2q^2$	$3p^3q$	$p^4$		
	Stationary	$q^4$	$3pq^3$	$3p^2q^2$	$p^3q$		
							$q^3 + 3pq^2 + 3p^2q + p^3 = (q + p)^3 = 1$
4	After transfer						
	Before equilibration						
	Mobile	0	$pq^3$	$3p^2q^2$	$3p^3q$	$p^4$	
	Stationary	$q^4$	$3pq^3$	$3p^2q^2$	$p^3q$		
	At equilibrium						
	Mobile	$pq^4$	$4p^2q^3$	$6p^3q^2$	$4p^4q$	$p^5$	
	Stationary	$q^5$	$4pq^4$	$6p^2q^3$	$4p^3q^2$	$p^4q$	
							$q^4 + 4pq^3 + 6p^2q^2 + 4p^3q + p^4 = (q + p)^4 = 1$
5	After transfer						
							$q^5 + 5pq^4 + 10p^2q^3 + 10p^3q^2 + 5p^4q + p^5 = (q + p)^5 = 1$

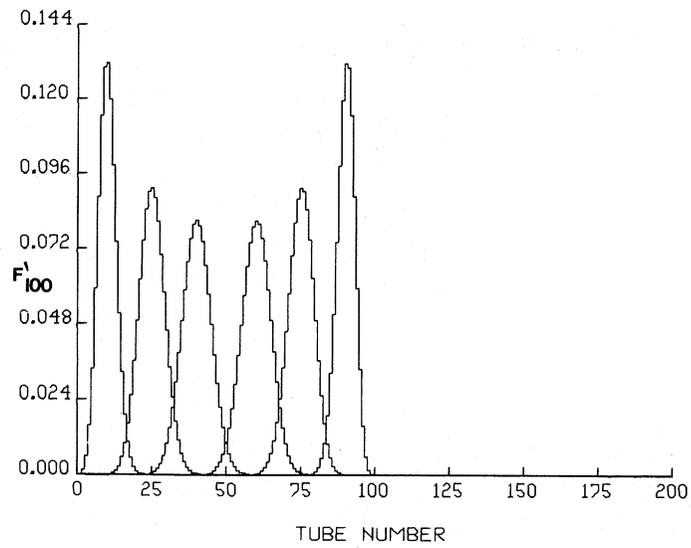


Fig. 12.4. Computer simulation of countercurrent distribution of six solutes with  $K$  of 9.00, 3.00, 1.50, 0.667, 0.333, and 0.111, respectively, in a 200-tube distributor with  $v_M = v_S = 40$  ml ( $n = 100$ ;  $F'_{100}$  plotted vs.  $r$ ).

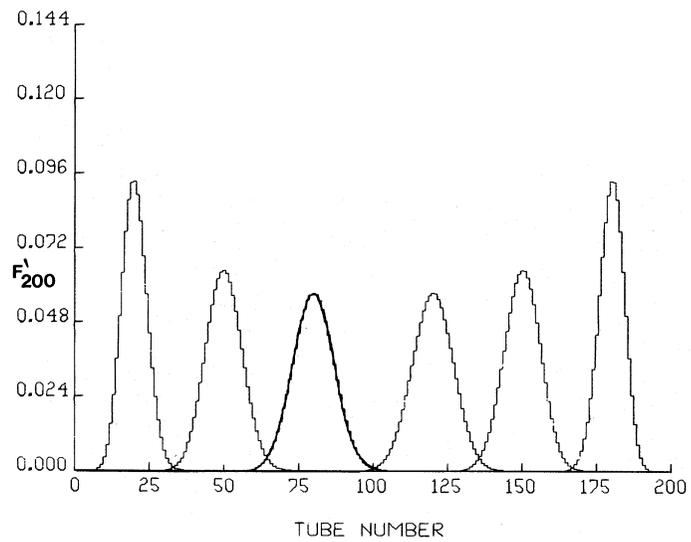


Fig. 12.5. Computer simulation of countercurrent distribution ( $n = 200$ ,  $F'_{200}$  plotted vs.  $r$ ). Conditions otherwise are those described for Fig. 12.4. Solute with  $K = 1.50$  is represented with both a histogram and a continuous approximation to the discrete distribution.

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number of transfers. The fraction of solute in tube  $r$  after  $n$  transfers ( $F'_{n,r}$ ) can be calculated straightforwardly from the binomial distribution:

$$F'_{n,r} = \frac{n!}{r!(n-r)!} p^r q^{n-r} \quad (4)$$

For example, the fraction of solute in tube 3 after transfer number 3 is  $[3!/3!(0)!] p^3 = p^3$ . (It should be remembered that  $0! = 1$  by definition through the gamma function of statistics.)

The factorial part of the expression is equal to the coefficient of the binomial distribution term. For example, in tube 2 after transfer number 3, the binomial coefficient is 3, as can be validated by examination of Table 12.1.

Standard statistical texts (10) demonstrate that the mean and standard deviation of the binomial distribution can be determined from the first and second derivatives, respectively, of the moment-generating function which describes the distribution. The mean is the most probable value and in countercurrent distribution corresponds to the tube in which the fraction of solute is a maximum,  $r_{\max}$  (see Fig. 12.4). From statistics,

$$r_{\max} = np \quad (5)$$

The distance, in tubes, from  $r_{\max}$  to the inflection point is the standard deviation  $\sigma'$  and may be calculated from

$$\sigma' = \sqrt{npq} \quad (6)$$

### 2. GAUSSIAN APPROXIMATION

It is convenient to approximate the discontinuous binomial distribution by the continuous Gaussian distribution. At this point, it can be stated that about 95% of a solute is contained within tubes  $\pm 2\sigma'$  from  $r_{\max}$ :

$$F'_{n,r} \cong \frac{1}{\sqrt{2\pi npq}} \exp \left[ \frac{-(r_{\max} - r)^2}{2npq} \right] \quad (7)$$

which can be compared with a typical form of the Gaussian,

$$f(x) = \frac{1}{\sigma\sqrt{2\pi}} \exp \left[ \frac{-(\mu - x)^2}{2\sigma^2} \right] \quad (8)$$

in the latter of which  $\mu$  is the most probable or mean value (the peak value) which corresponds to  $r_{\max}$ ; and  $\sqrt{npq}$  corresponds to the standard deviation  $\sigma$ . Calculation of solute profiles from the binomial distribution is tedious because of the factorials and the need to calculate each point. This is made easier if a computer is used either for calculation of the binomial expression or, alternatively, if the countercurrent process is simulated by computer. This technique is used extensively throughout this chapter, and Fig. 12.4 is an example of a computer-simulated distribution. Alternatively, the Gaussian approximation may be used to produce rapidly a diagram of the distribu-

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tion. It should be noted that the diagram is continuous, which contrasts with the discrete binomial distribution represented as a histogram. In Fig. 12.5, one of the solute bands is represented as both a histogram and as a continuous approximation to the discontinuous form. It can be seen that the continuous curve is an excellent approximation. The agreement between the two forms improves when  $n \gg r \gg 1$ .

An example of fractionation of two mucopolysaccharides by countercurrent distribution is shown in Fig. 12.6. The distributor was a miniaturized version of the early stainless steel device constructed by Post. It was made principally of plastic and was about 12 cm long. Each tube contained about 3.0 ml of mobile and 0.8 ml of stationary phase. The solvent was an aqueous polymer system which separated into two phases upon settling (68). The system was useful for analysis in studies of metabolism and biological functions of the mucopolysaccharides.

Another example of countercurrent distribution for the separation of biological materials is shown in Fig. 12.7. After 1000 transfers, gramicidin, a

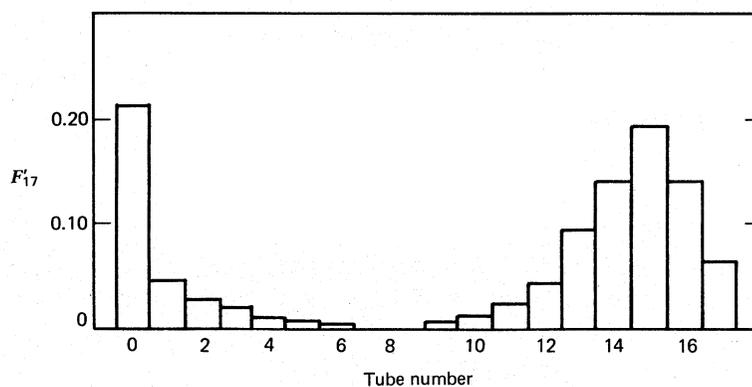


Fig. 12.6. Countercurrent distribution of hyaluronic acid (tubes 0–4) and chondroitin 6-sulfate (tubes 10–17),  $n = 17$ . Solvent composed of 2.67% dextran, 5.15% polyethylene glucol, 0.10  $M$   $\text{NaH}_2\text{PO}_4$ , and citric acid (pH 3.5) (68).

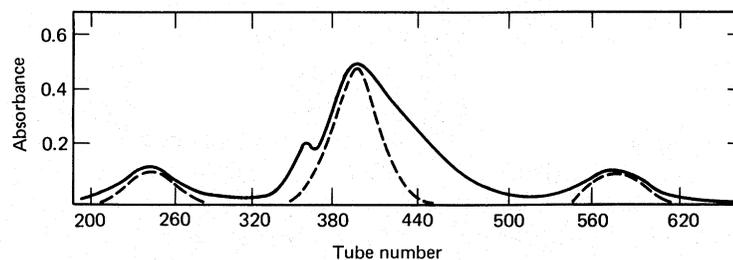


Fig. 12.7. Countercurrent distribution of a mixture of gramicidins after 1000 transfers. Solid curve is experimental; dashed curve is calculated. Solvent system: methanol/water/benzene/chloroform (23/7/15/15). There was 10 ml of each phase in each tube, contained in a 500-tube distributor (72). Solute was loaded into the first 10 tubes at the start.

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polypeptide antibiotic from *Bacillus brevis*, was fractionated into three distinct regions. The three dashed profiles are theoretical values for that system if the distribution coefficients are evaluated from the values of  $r_{\max}$ . On the basis of this disagreement with theory and additional transfers, it was shown that the sample of gramicidin contained at least four and probably five distinct gramicidins (72).

### B. IDEAL ELUTION FROM THE EXTRACTION TRAIN

#### 1. Calculation of Solute Profile in Effluent

##### a. PEAK MAXIMA

Solute is transported through the distributor until it reaches the last tube. Succeeding transfers result in transport of whatever is in the mobile phase of the last tube out of the instrument; this fluid is termed the effluent. It should be remembered that the serial numbers of the tubes start with zero so that the total number of tubes is one greater than the serial number of the last tube,  $N$ . Consider the case when the solute maximum reaches the last tube, that is,

$$r_{\max,N} \equiv N = (n_{\max,N})p \quad (9)$$

where  $n_{\max,N}$  indicates the number of transfers which have been carried out to transport the solute maximum to the last tube in the distributor. To elute the solute maximum from the distributor requires one additional transfer, and this number of transfers is defined  $n_R$ , the retention number:

$$n_R = 1 + n_{\max,N} = 1 + \frac{N}{p} \quad (10)$$

and from equation 2,

$$n_R = 1 + \frac{N(Kv_S + v_M)}{v_M} \quad (11)$$

If  $n_R$  is large

$$n_R \cong \frac{N(Kv_S + v_M)}{v_M} \quad (12)$$

The retention volume is the volume of mobile phase which must pass through the distributor just to achieve a solute maximum in the effluent and is defined  $V_R$ . From equation 11,

$$V_R = n_R v_M = v_M + N(Kv_S + v_M)$$

Let  $V_M = v_M(N + 1)$ , which is the total volume of mobile phase in the distributor, and let  $V_S = v_S(N + 1)$ , the total volume of stationary phase in the distributor. If  $N$  is large,  $N + 1$  is not appreciably different from  $N$ , and thus

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$$V_R \cong KV_S + V_M \quad (13)$$

This is the most common equation in both countercurrent distribution and the analogous processes of chromatography. It predicts that solutes with different distribution coefficients will have different retention volumes.

#### b. PEAK WIDTHS

Differences in retention volumes are important but not sufficient for either preparative or analytical separations of solutes. The degree of overlap of two solute profiles also must be considered and is readily determined from graphs of solute fraction in the effluent. This can be handled most conveniently by utilizing the continuous Gaussian approximation to the binomial distribution. If equation 7 is used, it should be multiplied by  $p$  to determine the fraction of solute, in the mobile phase of the last tube  $N$ . Then,

$$F \equiv F_{n,N+1} = pF'_{n-1,N} = \frac{p}{\sqrt{2\pi npq}} \exp \left[ \frac{-(r_{\max} - N)^2}{2npq} \right] \quad (14)$$

in which  $F_{n,N+1}$  is the fraction of solute in the effluent after  $n$  transfers.

The distribution, as each solute in turn reaches a maximum in the last tube in an extraction train, is shown in Fig. 12.8. Figure 12.9 depicts the fraction of solute in upper and lower phases for the furthest transported solute within the distributor, and Fig. 12.10 represents the solute fraction in the effluent.

Effluent profiles are sometimes reported in terms of volume of effluent

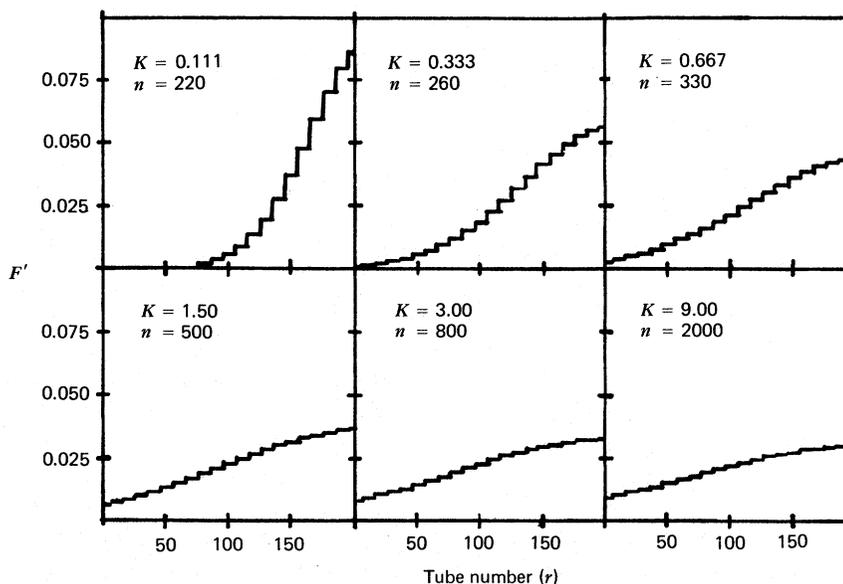


Fig. 12.8. Fraction of solute in the tubes of a distributor as solute maximum reaches the  $N$ th tube. Conditions are the same as in Fig. 12.4, except for the number of transfers,  $n$ .

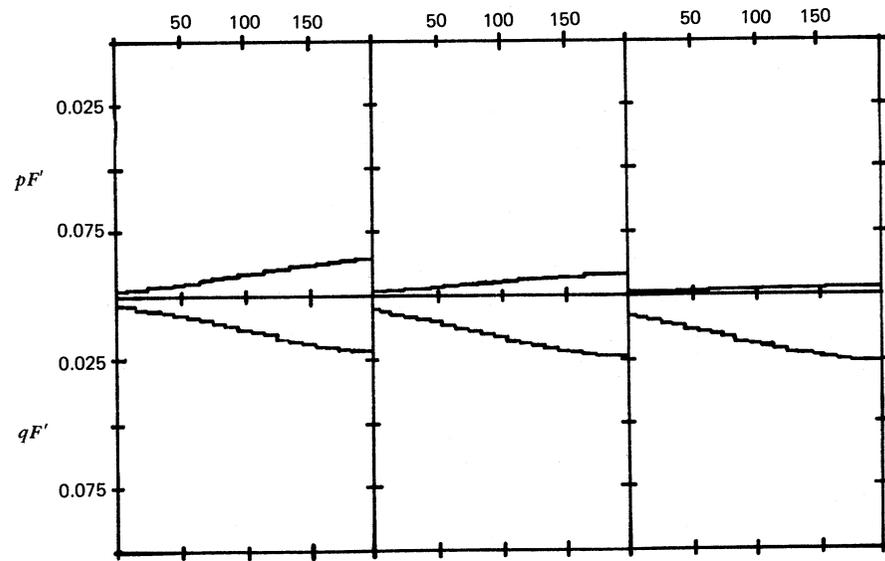
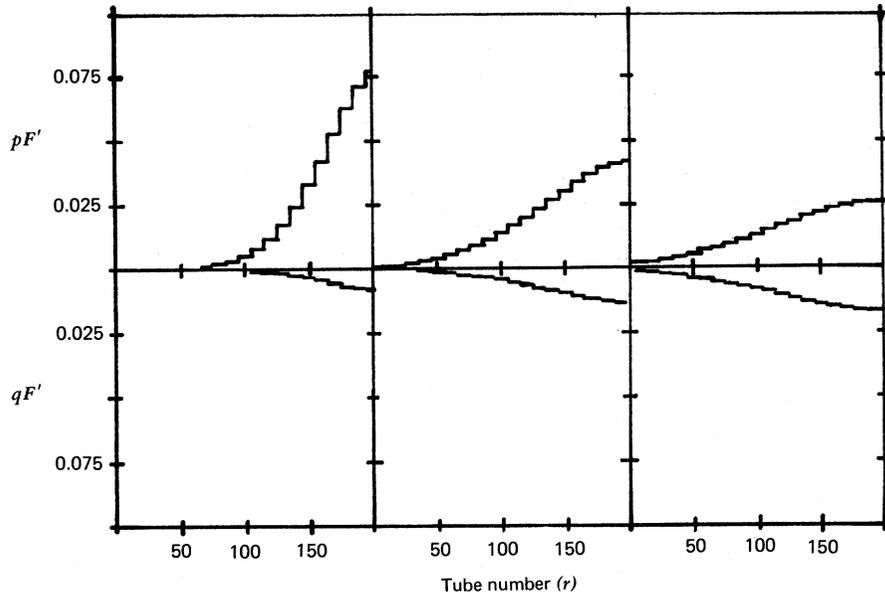


Fig. 12.9. Fraction of solute in the mobile phase (plotted upward) and in the stationary phase (plotted downward) as the solute maximum reaches the  $N$ th tube. Conditions as in Fig. 12.8.

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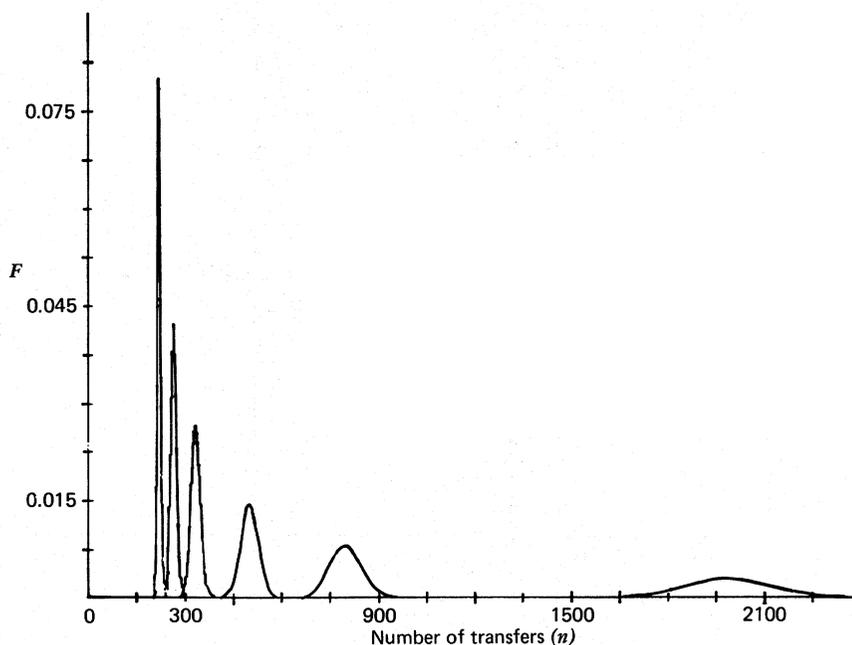


Fig. 12.10. Fraction of solute in effluent plotted vs. number of transfers,  $n$ , for solutes described in Fig. 12.9.

rather than number of transfers. Equation 14 then may be transformed to a more useful and familiar form by substitution:

$$npq = \frac{nv_M}{Kv_S + v_M} \frac{Kv_S}{Kv_S + v_M} = \frac{Nnv_M}{N(Kv_S + v_M)} \frac{NKv_S}{N(Kv_S + v_M)} \quad (15)$$

Here,  $N(Kv_S + v_M) = V_R$ ,  $NKv_S = V_R - V_M$ , and  $V = nv_M =$  the total volume of mobile phase which has passed through the zeroth tube. And, if the distributor had been appropriately filled with both phases prior to the start,  $V$  is also the volume of mobile phase which has passed through tube  $N$ . Then,

$$npq = \frac{NV(V_R - V_M)}{V_R^2} \quad (16)$$

When this expression is substituted into the exponential term in equation 14 and  $r_{\max}$  is replaced with  $nv_M/(Kv_S + v_M)$ , after simplification,

$$F_{n,N+1} \equiv F = \frac{p}{\sqrt{2\pi npq}} \exp \left[ \frac{-N(V - V_R)^2}{2V(V_R - V_M)} \right] \quad (17)$$

At the maximum of the peak eluted from the distributor,  $V = V_R$ , and the value of the exponential term is 1. The fraction of solute at the peak maximum,  $F_R$ , is the preexponential term  $p/\sqrt{2\pi npq}$ . Then,

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$$F = F_R \exp \left[ \frac{-N(V - V_R)^2}{2V(V_R - V_M)} \right] \quad (18)$$

Or, if molar concentrations are used rather than fraction of initial solute,

$$C = C_R \exp \left[ \frac{-N(V - V_R)^2}{2V(V_R - V_M)} \right] \quad (19)$$

### c. EVALUATION OF NUMBER OF STAGES FROM SOLUTE PROFILES

Consider the points in a solute profile where  $F = F_R/e$  or  $C = C_R/e$ . Let  $V = V_a$  at these points. Consultation of Fig. 12.11 indicates that there are two points where  $V_a$  may be evaluated:

$$C = \frac{C_R}{e} = C_R \exp \left[ \frac{-N(V_a - V_R)^2}{2V_a(V_R - V_M)} \right] \quad (20)$$

The natural logarithms of both sides may be taken and after cancellation of similar terms:

$$N = \frac{2V_a(V_R - V_M)}{(V_a - V_R)^2} \quad (21)$$

In this manner, the number of tubes in the instrument may be calculated from the output profile.

The width of the Gaussian curve may be defined to cover any appropriate percentage of the area. For example, if the width is considered from the peak maximum  $\pm 2\sigma$ , 95% of the area of the curve falls between these limits. This situation is depicted in Fig. 12.11. This means that 2.5% of the area lies

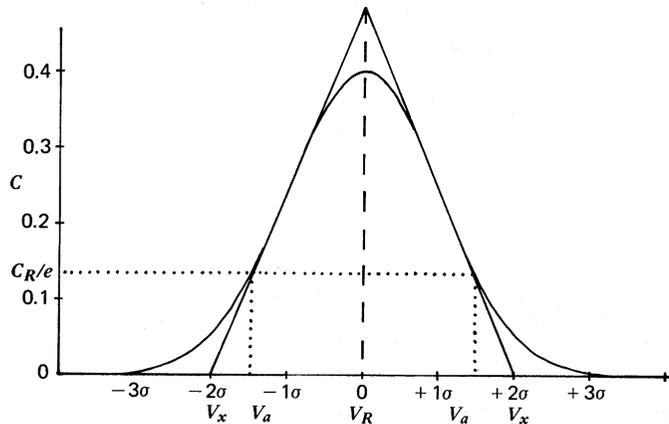


Fig. 12.11. Properties of a Gaussian approximation useful for evaluation of CCD profiles. Tangents to the curve intersect the baseline at  $\pm 2\sigma$ , which corresponds to  $V_x$ . Perpendiculars dropped from the curve intersect the baseline at  $V_a$  when  $C = C_R/e$ .

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outside the higher limit ( $V_R + 2\sigma$ ) and another 2.5% lies outside of the lower limit. These limits may also be used for evaluation of the number of tubes in the distributor. Let  $V_X = V_R + 2\sigma$  or  $V_X = V_R - 2\sigma$ , since the curve is symmetrical about the mean. For Gaussian curves at  $V_X$ ,  $C = C_R$  (0.13533). If this is substituted into equation 19 and natural logarithms of both sides are taken, after rearrangement,

$$N = \frac{4V_X(V_R - V_M)}{(V_X - V_R)^2}$$

There is a relatively convenient way to find  $V_X - V_R$ . As is noted in Fig. 12.11, tangents drawn to the Gaussian curve intersect the baseline at  $V_X$ . The total distance from  $V_X$  below  $V_R$  to  $V_X$  above  $V_R$  is usually denoted  $w$ , and

$$w = 2 |V_X - V_R|$$

Thus,

$$N = \frac{16V_X(V_R - V_M)}{w^2} \quad (22)$$

At large values of  $V_R$ ,  $V_R$  may be substituted for  $V_X$  in equation 22 and

$$V_R - V_M \cong V_R$$

Under the conditions where the approximations are valid, equation 22 may be rewritten as

$$N \cong \frac{16V_R^2}{w^2} \quad (23)$$

This form of the equation for calculation of number of plates is commonly used in chromatography but may cause significant errors, particularly when  $V_R \leq 2V_M$ , as will be demonstrated in a later section.

## C. MULTIPLE INPUTS

### 1. Elution Profiles

#### a. STEP FUNCTIONS

Countercurrent distribution instruments often have relatively large tube volumes and many stages which make them particularly useful for preparative separations. In the approach described previously, a single input of solute was added to the zeroth tube, and successive inputs to this tube, after each transfer, were devoid of solute. An alternate to this is the addition of successive samples of solute to the zeroth tube which leads to throughput of large amounts of material. The solute profile can be visualized as the resultant profile from a series of individual solute input profiles, each displaced by one tube (or transfer number). The total amount of solute in any tube is the

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sum of the amounts of solute in that tube resulting from each input. If a large number of solute inputs are successively introduced into a distributor, a steady-state condition will be achieved in which the concentration of solute in the effluent is equal to its input concentration. This situation can be expressed exactly as a sum for discrete inputs or, alternatively, approximated as the integral of the continuous Gaussian distribution:

$$Y = \frac{C}{C^*} = \frac{1}{\sqrt{2\pi}} \int_{v=0}^v \exp \left[ \frac{-N(V - V_R)^2}{2V(V_R - V_M)} \right] dV \quad (24)$$

In this expression,  $C^*$  is the concentration of solute in the input and  $C$  is the concentration in the effluent. Thus,  $0 \leq Y \leq 1$  in the ideal case. The integral is usually written as

$$Y = \operatorname{erf} \left[ \frac{(V - V_R)}{\sigma} \right] \quad (25)$$

The expression is of the form

$$Y = \frac{1}{\sqrt{2\pi}} \int_{v=0}^v \exp \left( \frac{-t^2}{2} \right) dt \quad (26)$$

which is readily found in tables of integrals of the normal curve of error. The expression describes the front edge of the curve in Fig. 12.12. It is usually inconvenient to specify  $Y = 1.000$  as the steady-state condition, since the properties of the integral Gaussian are such that the value of 1.000 is reached only as a limit. Typically, some value within experimental error is accepted as the steady-state value, for example,  $Y = 0.99$ , which occurs at  $V_2$ , as depicted in Fig. 12.12. This means that there is only a 1% difference between the concentration of solute in the input and output solutions. Similarly, some convenient concentration is usually chosen to denote first detectable emergence of solute from the distributor, and  $Y = 0.01$ , which occurs at  $V_1$ , was chosen in Fig. 12.12. The retention volume  $V_R$ , which corresponds to the value for  $V_R$  if only a single input of solute had been used, occurs at  $Y = 0.500$  if the elution curve is symmetrical. In any case,  $V_R$  for a step function (the type of curve shown in Fig. 12.12) occurs at the inflection point of the output curve, as can be seen by differentiation of equation 24. If input of solute is discontinued, the concentration of solute in the output falls as depicted between  $V_3$  and  $V_4$  in the figure. Values of  $Y$  may be calculated in a manner analogous to that described for the leading edge of the curve.

If a solution containing two relatively well-separated solutes is fed into the distributor until steady state is reached, and then the feed solution is changed to the original mobile phase, an output profile such as that in Fig. 12.13 is observed. The concentrations of the solutes in the feed may be determined by measurement of the heights of the experimental output curves in the regions where overlap of the two solutes is not important. The retention volumes for the two solutes may be determined from the points of maximum slope of the leading edges of the two "steps." Purified solute 1 is

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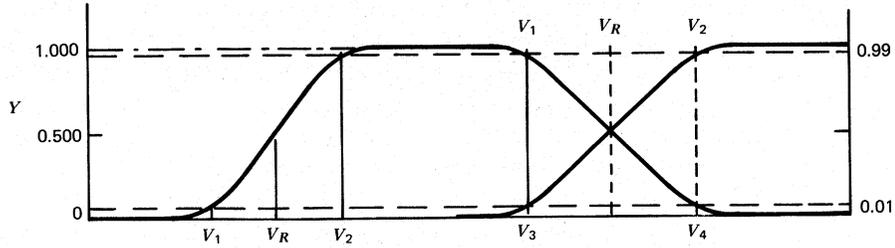


Fig. 12.12. Integral Gaussian or step function resulting from multiple inputs of solute.  $Y = 0.99$  at  $V_2$ , which is considered to be at the plateau within experimental error.  $V_R$  corresponds to  $Y = 0.500$  for symmetrical elution curves.

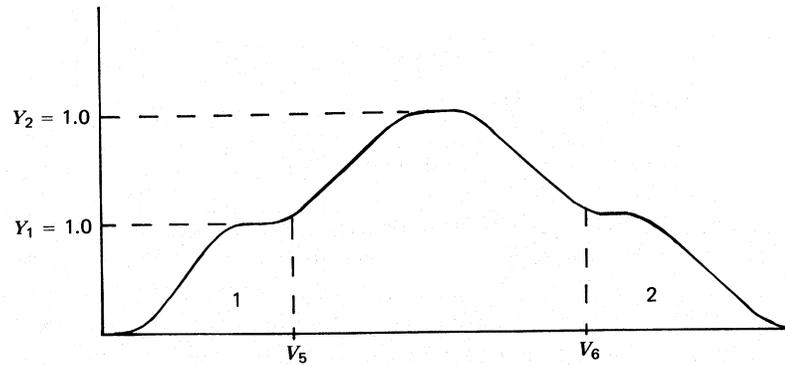


Fig. 12.13. Overlapping integral Gaussian output profiles. Solute 1 may be collected up to  $V_5$ , and solute 2 may be collected after  $V_6$ . The solute in the region of overlap,  $V_6 - V_5$ , may be recycled. The concentration of each solute in the output reaches its concentration in the feed ( $Y_1 = Y_2 \sim 1$ ).

found in the first region, and purified solute 2 is found in the last region. The solute in the region from  $V_5$  to  $V_6$  may be recycled to provide more feed solution. For preparative purposes, it is usually wasteful of time and materials to have a solute profile as in Fig. 12.13.

An alternative approach is represented in Fig. 12.14. Solute may be fed into the distributor for enough transfers so that one of the output curves just barely reaches the specified steady state value, for example,  $Y = 0.99$ . Any solute emerging prior to the solute under consideration will have reached  $Y = 0.99$  and for all intents and purposes will appear to have a flat portion or plateau at the top. Any solute emerging after the solute under consideration will not have reached  $Y = 0.99$  (equation 27) and will appear as a broad, almost Gaussian curve.

The number of inputs, or volume of solute, required to result in an output curve in which  $Y = 0.99$  may be calculated in a straightforward manner. In Fig. 12.12, the region from  $V_2$  to  $V_3$  adds to the length of the plateau but is in excess over the amount required to just achieve  $Y = 0.99$ , that is, in excess

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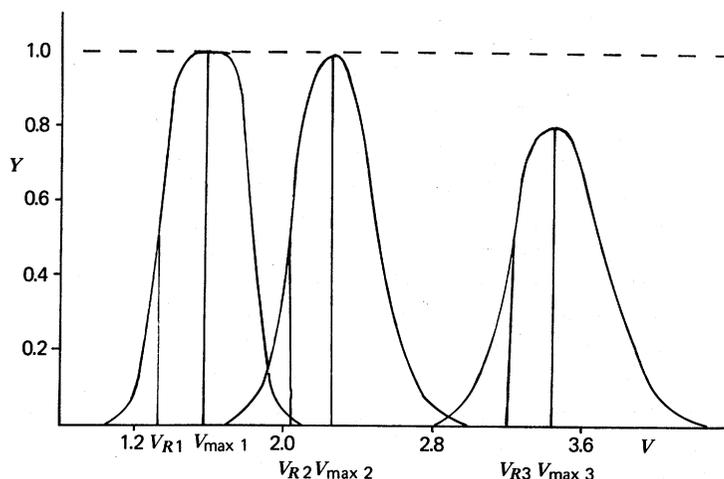


Fig. 12.14. Schematic representation of output curves for solutes with  $K$  values of 0.667, 1.50, and 3.00 ( $Y$  plotted vs. effluent volume  $V$ , in liters). Conditions are the same as in Fig. 12.10, except that a mixture of the solutes was added to the zeroth tube sequentially for 128 transfers. The solute with  $K = 1.50$  reached  $Y = 0.99$ . If only one input had been used,  $V_{\max}$  would have coincided with  $V_R$ . Any solute of higher  $K$  has  $Y_{\max}$  less than 0.99.

over the amount required to reach a step function or frontal output. The amount required is that to get a solute output from  $V_1$  to  $V_2$  plus that from  $V_3$  to  $V_4$ . If the leading edge of the output curve is transposed and  $V_1$  is overlapped with  $V_3$  and  $V_2$  is overlapped with  $V_4$ , and then the overlapped  $Y$  values are summed over this interval, it is apparent that  $Y$  at:  $(V_1 = 0.01) + (V_3 = 0.99) = 1$ ;  $(V_R = 0.50) + (V_R = 0.50) = 1$ ;  $(V_2 = 0.99) + (V_4 = 0.01) = 1$ ; and so on. The sums are always equal to  $Y = 1.0$ . Thus, the amount of solute in the rectangle  $V_1, V_2, V_4, V_3$  equals the amount of solute required to just achieve a frontal output. The distance from  $V_3$  to  $V_4$  (or  $V_1$  to  $V_2$ ) can be evaluated from the Gaussian approximation to the binomial distribution. The distance in  $\sigma$  units, evaluated from tables of Gaussian integrals (49), is  $4.66\sigma$  for the distance from  $Y = 0.01$  to  $Y = 0.99$ . The volume of solute input required to just reach the frontal output,  $V_F$ , can be calculated from  $\sigma$  as defined in equations 24–26. Then,

$$V_F = 4.66\sigma = 4.66 \left[ \frac{V(V_R - V_M)}{N} \right]^{1/2} \quad (27)$$

Since  $V \cong V_R$ ,

$$V_F \cong 4.66 \left[ \frac{V_R(V_R - V_M)}{N} \right]^{1/2} \quad (28)$$

and  $n_F$ , the number of inputs to just achieve a frontal output, may be expressed as

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$$n_F = \frac{V_F}{v_M} \quad (29)$$

### b. PEAK POSITION AND PEAK BROADENING

Examination of output profiles resulting from multiple inputs reveals changes in position of the peak maximum and the peak width. When a single input is applied, the peak maximum occurs at  $V_R$ ; but when multiple inputs are applied, the peak maximum occurs at volumes greater than  $V_R$ . For example, when the appropriate number of inputs, to just achieve  $Y = 0.99$  in the output, are applied, the maximum occurs at  $Y = 0.99$ . However,  $V_R$  coincides with  $Y = 0.5$  for this type of output. Although of little consequence in some separations, if the position of the maximum is used to determine  $V_R$  and thermodynamic parameters through equation 13, errors would occur. Chromatographers are particularly concerned about this source of error and have demonstrated that the retention volume can be determined if the input volume  $V_Z$  is known through equation 30 in which  $V_{\max}$  is the effluent volume where the output concentration is at a maximum:

$$V_{\max} = V_R + \frac{V_Z}{Z} \quad (30)$$

This equation pertains in CCD, and, since each input and transfer is of volume  $v_M$ , when  $Z$  inputs are added the number of transfers required to reach solute maximum in the output may be calculated as follows:

$$\begin{aligned} V_Z &= Zv_M \\ n_{\max} &= n_R + \frac{Z}{2} \end{aligned} \quad (31)$$

Use of multiple inputs results in peak broadening. For a single input eluted as a nearly Gaussian curve, 98% of the elution curve would fall under the region from  $V_1$  to  $V_2$  ( $Y = 0.01$  to  $Y = 0.99$ ) in Fig. 12.12. Yet for the curve in Fig. 12.14 which just reaches  $Y = 0.99$ , only the leading edge of the output curve falls in this region. The trailing edge requires, for symmetrical curves, a similar distance along the output axis. Thus, this frontal output covers approximately twice the output volume that a single input would cover. For multiple inputs, less than the number required to achieve a frontal, the curves would be broader than the distance from  $V_1$  to  $V_2$  in Fig. 12.10, the ascending part of the frontal output. For multiple inputs greater in number than  $n_F$ , the predominant effect is lengthening of the plateau and thus further broadening. As a result, when multiple inputs are used in preparative work, the degree of overlap of two slightly separated peaks would be increased and poorer separations would result. This can be acceptable if conditions are adjusted in order to improve the separation.

Solute profiles eluted from the distributor can be described mathe-

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matically for  $Z$  inputs when  $n_F > Z > 1$  according to

$$Y = \operatorname{erf} \left[ \frac{(V - V_R)}{\sigma} \right] - \operatorname{erf} \left[ \frac{(V - V_Z - V_R)}{\sigma} \right] \quad (32)$$

In this expression, the value of  $\sigma$  may be determined from the solute profile resulting from a single solute input or enough solute inputs to achieve a step function. The value of  $Y$  at the maximum ( $Y_{\max}$ ) may be evaluated from the derivative of equation 32:

$$Y_{\max} = 2 \left[ \operatorname{erf} \left( \frac{V_Z}{2\sigma} \right) \right] - 1 \quad (33)$$

An appropriate representation of the curve resulting from multiple inputs can be readily constructed from equation 30, which gives the position of the maximum, and equation 33, which gives the curve height at the maximum, if the width of the curve is known. This width can be approximated by consideration of the width of the Gaussian approximation (equations 21 through 22) and the observation that the width ( $w_Z$ ) of a curve when  $Z$  inputs are used is

$$w_Z \cong w + V_Z = 4\sigma + V_Z \quad (34)$$

The use of multiple inputs in CCD is extremely common. For example, in Fig. 12.7 solute was loaded into the first 10 tubes of the distributor in a procedure called batch loading. This technique will be compared to the stepwise solute-loading approach in a later section. The purpose of multiple inputs is to provide separations of relatively large quantities of solutes while avoiding significant nonideal effects. These effects are considered further in a subsequent section of this chapter.

### D. CHROMATOGRAPHIC ANALOGY

#### 1. Comparison of Bands Retained Within Extraction Trains and Chromatographic Devices

It is possible to distribute solutes in chromatographic columns, halt the flow of eluent, and then section the columns to determine the transport of solutes through the columns. However, elution of solute from the end of the column is much more common. There are two major chromatographic forms, thin-layer and paper chromatography, in which solutes are not eluted from flat beds that are equivalent to columns. This may be compared with the situation when solute is not eluted from the instrument in countercurrent distribution.

In addition to significant differences due to the continuous nature of the chromatographic devices and discontinuous CCD, it should be noted that the volume ratio of stationary to mobile phase may vary during the course of flat

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bed chromatography along the direction of migration and with time of elution. Time in these systems may be analogous to the number of transfers in CCD.

### a. $R_F$ CONCEPT

In flat-bed chromatography, the ratio of the distance the solute band center has traveled divided by the distance the solvent front has traveled is defined as  $R_F$ . This is readily determined in CCD since the solute band center is  $r_{\max}$  and the front has traveled  $n$  transfers. Therefore,

$$R_F = \frac{np}{n} = p = \frac{v_M}{Kv_S + v_M} = \frac{1}{\left(\frac{Kv_S}{v_M}\right) + 1} \quad (35)$$

Thus,  $R_F$  is related to solution volumes and the equilibrium constant. Solutes of differing  $K$  can be separated; or if the appropriate column parameters are known (usually they are not),  $K$  may be determined. Extensive tabulations of  $R_F$  values in chromatography are available, but there is much less information available for CCD (33).

### b. BAND SPREADING

#### (1) Plate Number

Earlier, it was demonstrated that the fraction of solute in any tube in a distributor could be approximated by equation 7:

$$F'_{n,r} \cong \frac{1}{\sqrt{2\pi npq}} \exp \left[ -\left(\frac{r_{\max} - r}{2npq}\right)^2 \right]$$

After a solute has been distributed, at  $r_{\max}$ ,

$$F'_{n,r_{\max}} = \frac{1}{\sqrt{2\pi npq}} = \frac{1/\sqrt{q}}{\sqrt{2\pi r_{\max}}}$$

since at the maximum  $r = r_{\max}$  and the postexponential term is zero. It is often thought that  $F'_{n,r_{\max}} \cong 1/\sqrt{2\pi r_{\max}}$ , but this approximation is reasonable only when  $q > 0.6$  and  $1/\sqrt{q}$  approaches 1. In the region of  $1 \geq q > 0.6$ , all solutes reaching a particular  $r_{\max}$  have nearly identical values of  $F'_{n,r_{\max}}$ . Similarly, since  $\sigma' = \sqrt{npq} = \sqrt{q} \sqrt{r_{\max}}$ , when  $q > 0.6$  or  $\sqrt{q}$  approaches 1,  $\sigma' \approx \sqrt{r_{\max}}$ . Hence, the distribution profile of any solute reaching a particular  $r_{\max}$  will be essentially identical to all others that have reached that value. The further a solute in this  $q$  range has passed within the distributor, the broader and lower is its distribution profile. These phenomena are readily discernible in Figs. 12.4 and 12.5. The three furthest transported solutes, those with lowest values of  $K$ , have  $q$  values less than 0.6.

The fourth most transported solute with a  $K$  of 1.50 ( $q = 0.6$ ) is the first of the solutes (Fig. 12.5) which demonstrates the aforementioned behavior. In Fig. 12.8, the fraction of solute remaining within the distributor is depicted at the transfer in which the solute maximum has reached the last tube in the

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instrument. The values of  $F'_{n,r}$  for the last three solutes eluted are close to one another, although the number of transfers required to move the solutes to the end of the distributor varies significantly. Similarly, the profile after 100 transfers for the solute with  $K$  of 3.00 (Fig. 12.4) has an  $F'_{n,r_{\max}}$  of about 0.09, and the solute with  $K$  of 9.00 has a similar profile after 200 transfers (Fig. 12.5). In the range  $0.8 > q > 0.2$ ,  $\sqrt{pq}$  varies little so that for a given  $n$ , the widths of such bands inside the distributor will be similar. Four bands in Figs. 12.4 and 12.5 show this behavior.

### (2) Transfer Number

For a series of well-separated solutes after  $n$  transfers, the process may be halted and the distribution examined. The relative maximum for each solute is contained in a different tube and each "solute maximum has experienced a differing number of tubes." The furthest transported solute maxima have been through all the tubes up to their particular  $r_{\max}$  values, although the least mobile solute has been at a maximum in many fewer tubes. All of the solutes have, however, experienced the same number of transfers,  $n$  (Fig. 12.3). This transfer number may be determined readily from the distribution profiles. For example, if  $p$  is known,  $r_{\max}$  may be determined and  $n$  calculated directly. Unfortunately,  $p$  is usually not well known. However, since

$$r_{\max} = np \quad \text{and} \quad \sigma' = \sqrt{npq}$$

Then,

$$\frac{r_{\max}^2}{[r_{\max} - (\sigma')^2]} = n \quad (36)$$

This is a straightforward calculation for CCD but is not usually necessary, since modern distributors contain automatic transfer counters. In flat-bed chromatography, solute concentration profiles may be estimated visually and/or determined spectrometrically. A parameter theoretically equivalent to  $n$  could be evaluated in the following manner. The distance a zone center has traveled from the point of application must be measured and is denoted  $X_{\max}$ . The height equivalent to a theoretical plate,  $H$ , must also be evaluated and is generally determined in flat-bed chromatography by dividing the distance a solute band has traveled by the number of plates,  $N'$ , the solute has traversed in that distance ( $H = X_{\max}/N'$ ). If the solute band has not traversed a sufficient number of plates, there may be significant differences in the plate numbers over the range of the solute. Since the number of plates is evaluated over some range of the solute profile, as in equation 21, the average number of plates may be used and should correspond to the number of plates at the solute maximum. A more significant source of error results from the use of Gaussian approximations which may not apply, particularly when the solute has not migrated far from the origin and the process has proceeded for a short time. This corresponds to failure of the requirement in CCD  $n \gg r \gg 1$ . Smooth curves which appear to be Gaussian may be used

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to approximate the data such as that in Fig. 12.5, but if the equations such as equation 23 are used improperly for CCD, misleading values are obtained. Craig (23) noted that CCD of human serum albumin in a butanol-water-ethanol system had a much narrower band distribution than would be predicted by chromatographic equations.

If a spectrometric evaluation is made of the solute profile, the distance from the zone center to each inflection point, corresponding to the standard deviation  $d_{\sigma'}$ , may be evaluated by previously described techniques. From equation 36,

$$n = \frac{r_{\max}^2}{[r_{\max} - (\sigma')^2]} \approx \frac{d_{\max}^2/H^2}{(d_{\max}/H) - (d_{\sigma'}^2/H^2)} = n' \quad (37)$$

The number  $n'$  thus calculated for flat-bed chromatography would be "equivalent to the number of theoretical transfers" in CCD. At any point in a countercurrent distribution (that is, after any number of transfers),  $n$  calculated from all solute profiles should be the same within experimental error. The situation in flat-bed chromatography may not be so straightforward due to variations in amount and composition of mobile and stationary phases with position along the bed and a variety of kinetic processes which influence the width of the solute distributions and, hence,  $d_{\sigma'}$  and  $H$ . Guiochon has observed that peak widths are similar to one another in flat-bed chromatography except when  $R_F$  is near zero or 1 (39). Although kinetic and other factors are important in such chromatography, it is interesting to note that the CCD model predicts the observed trend.

### 2. Comparison of Peaks Eluted from Extraction Trains and Chromatographic Columns

#### a. RETENTION VOLUME

The retention equation in volume units (equation 13) has been shown to be essentially identical for both countercurrent distribution and chromatography in ideal cases.

#### b. PLATE NUMBER

In an earlier section it was demonstrated that for CCD,

$$N = \frac{16V_x(V_R - V_M)}{w^2} \quad (22)$$

and in chromatography,

$$N \cong \frac{16V_R^2}{w^2} \quad (23)$$

Figure 12.15 depicts the use of approximate equation 23 for the calculation of the number of tubes in an extraction train. The hypothetical solutes eluted under the conditions in Fig. 12.10 provided the data for the calculation.

## 12. COUNTERCURRENT DISTRIBUTION

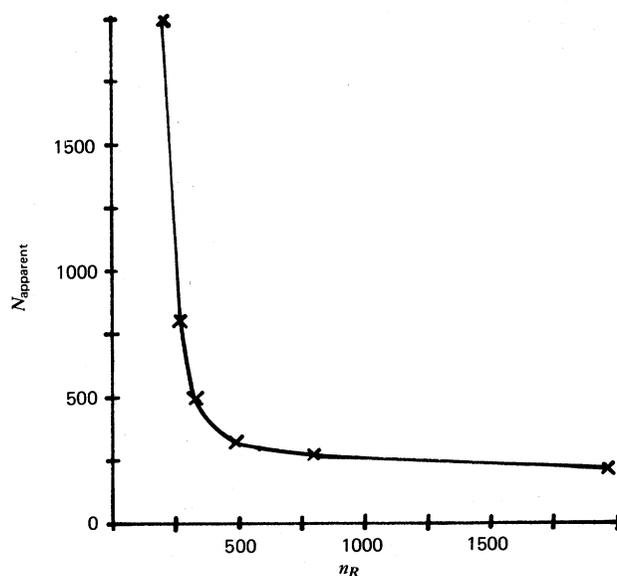


Fig. 12.15. Apparent number of plates ( $N_{\text{apparent}}$ ) calculated from equation 23 vs.  $n_R$  for a variety of solutes corresponding to those in Figs. 12.4 and 12.5. The actual value of  $N$  is 200.

Since the extraction train contained 200 tubes (and use of equation 22 gave this value consistently), it is apparent that the approximate equation gives seriously inflated plate numbers at low values of  $V_R$ . It is also apparent that when  $V_R \cong V_M$ , none of these equations should be used, since the Gaussian approximation is invalid in this region in the case of CCD. There are a number of situations in chromatography where retention volumes are not much greater than the mobile phase volumes. For example, in chromatography when  $V_M \cong V_R \cong 2V_M$  the resulting calculations of  $N$  from the approximate equation may be greatly in error (50,84).

### 3. Limitations of the Plate Theory as Applied to Chromatography

#### a. DISCONTINUOUS VERSUS CONTINUOUS PROCESSES

It has been demonstrated in preceding sections that there are similarities in output profiles for countercurrent distribution and elution chromatography. Cross sections of solute profiles in flat-bed chromatography are often similar to solute profiles within extraction trains. Mathematical representations of both classes of separation also have significant similarities. In part, this is due to the use of Gaussian or integral Gaussian approximations to describe chromatographic solute profiles. Phenomenologically, there are obvious problems in the use of the countercurrent model for chromatography. Column packings are usually discontinuous in the stationary phase, yet there are no clear-cut boundaries that result in discrete equilibrium

## G. SEPARATION TECHNIQUES

plates. The mobile phase is continuous in chromatography, although in CCD it is discontinuous. In Martin's approach (56) to chromatography, infinitesimal increments with volume  $\partial V$  were considered to be transferred from one plate to another. Hynninen has made a careful comparison of CCD and the Martin and Synge approach (45,46). In the approach by Mayer and Tompkins (58), finite increments were considered to be transferred. Gluekauf has criticized this approach and pointed out that impurity ratios calculated by Mayer and Tompkins could seriously underestimate the degree of impurity (35). Gluekauf developed a continuous-flow model of plate theory based upon conservation of mass in segments of the column. The approach used a Taylor's expansion in which terms greater than second order were dropped and resulted in equations that could predict the position and shape of the curves.

### b. KINETIC PERTURBATIONS

The continuous and flowing nature of chromatography leads to deviations from ideality which result in band and peak spreading, a situation differing from that in CCD. The model of Gluekauf could, in chromatography, be used to account for diffusional spreading of the bands. This continuous-flow plate model is significant since nonideal kinetic phenomena, such as diffusion along the length of the column, flow inhomogeneities across the column and near the walls, and nonequilibria perturb the shape of bands passing through the column.

This does not imply that CCD is ideal, as will be demonstrated in succeeding sections; nonequilibrium, nonideal phase equilibria, and wall effects do occur. There are a number of approaches to the attack of such nonideal phenomena. They could be handled in a stochastic manner in which the square of the standard deviation for the ideal distribution is added to the sum of squares for the various processes which tend to broaden the distribution profile. Then,

$$\sigma_{\text{overall}}^2 = \sigma_1^2 + \sigma_2^2 + \dots$$

In this chapter, we have chosen to use alternative approaches to describe nonideal effects, for we have been interested in phenomena in addition to band broadening. In this approach, the processes which lead to nonideality are either evaluated mathematically or are simulated in terms of the counter-current model with the aid of a computer. In each case, the validity was checked by experiment as will be seen in succeeding sections. The significance of such an approach is that variations, including those in equilibrium constants or volume reorganizations, may be evaluated and extrapolated to the analogous processes of chromatography. Although the plate concept is of fundamental significance in CCD, it is a convenient fiction, a curve-fitting parameter for the Gaussian approximation when applied to chromatography. Detailed considerations of rate processes in chromatog-

## 12. COUNTERCURRENT DISTRIBUTION

raphy have been documented by Giddings (34) and will be discussed in succeeding chapters.

### E. NONIDEAL SYSTEMS

#### 1. Countercurrent Distribution Transfer Devices

##### a. EQUIPMENT

For fractionations where only a small number of equilibrations are needed, the distributions may conveniently be carried out with a series of separatory funnels. Pesticide mixtures free of interfering contaminants have been isolated from plants and foods using a relatively simple assembly (11).

But for separation of more closely related solutes, more elaborate equipment capable of performing hundreds of equilibrations and transfers automatically is required (89). A number of tube assemblies have been described for such operations (3,27,82,94). One widely used type, the Craig design, is shown in Fig. 12.16. A robot controls the position of the tubes which allows mixing, settling, and decantation to take place as shown. Transfer and addition of mobile phase takes place on return to the equilibra-

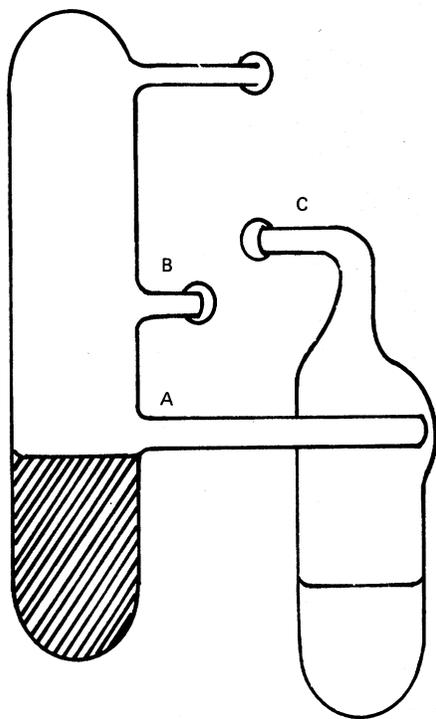


Fig. 12.16. Countercurrent distribution stage according to Post and Craig (66) in the decantation step. Solute is equilibrated by shaking in the long tube. After settling, the CCD tube is moved to this position, and mobile phase runs out of the lowest cross tube A. After counterclockwise rotation, mobile phase runs out into the next CCD stage through tube C, and new solvent enters this stage through tube B. The top tube is closed.

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tion position. The length of the mixing cycle and the length of settling time may be controlled to assure attainment of equilibrium and complete separation of phases. A completely different approach is demonstrated schematically in Fig. 12.17. As the extractor is rotated, the two phases are gently mixed. When the eccentric holes in the compartment dividers reach their low point, transfer to the next stage occurs. Automated extractors also frequently control fraction collectors and refractive index (16) or spectral devices for obtaining output profiles.

Another device, invented by Hietala (41), is shown in Fig. 12.18. The beauty of this device is that either upper or lower phases may be transported, depending upon angle of tilting. It is also possible to transfer both phases, each of which moves in the opposite direction (CDCD). The result of

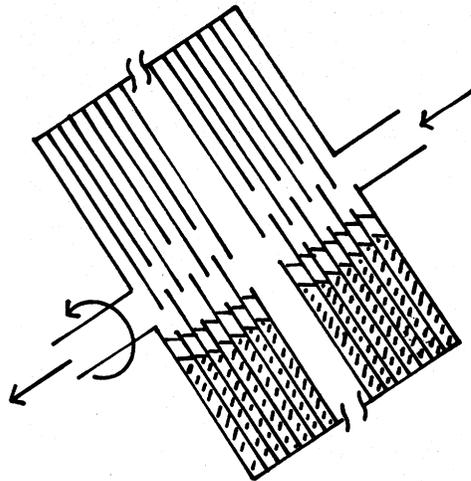


Fig. 12.17. Schematic diagram of rotating distributor. When material is above the lowest holes in the divider, transfer to the next stage occurs. Stationary phase is crosshatched (82).

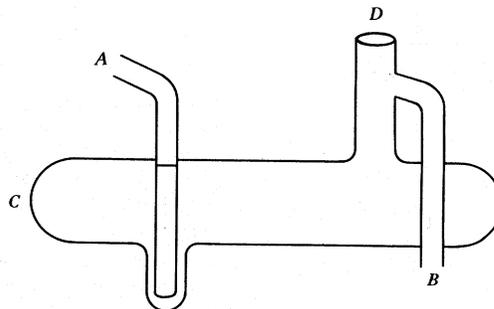


Fig. 12.18. Countercurrent stage of Hietala (41). Phases flow through tubes A and B. Tubes are filled through D, which is stoppered prior to shaking.

## 12. COUNTERCURRENT DISTRIBUTION

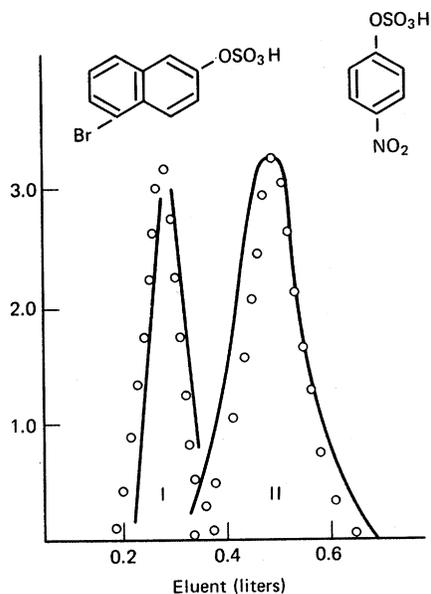


Fig. 12.19. Separation of phenolic sulfate esters using a continuously flowing mobile phase. A total of 25 mg of each compound was loaded into the first four tubes of the 50-tube distributor and eluent absorbance was measured. Smooth curves are experimental; circles are theoretical values;  $v_M = 4.0$  ml,  $v_S = 9.5$  ml. Solvent system was H<sub>2</sub>O/formamide/diisopropyl ether/benzene (2.5/2.5/3.7/1.0; v/v) (5).

an additional option is shown in Fig. 12.19 (5). Here, one of the phases flowed continuously through the apparatus. The Martin and Synge distribution as described by Hynninen (46) was used to calculate the output curve.

### b. CONSEQUENCES OF NONIDEAL TRANSFERS

Regardless of the design, imperfect transfers may take place if some of the stationary phase is transferred or some of the mobile phase is not transferred. In the use of the Craig and Post system, if the volume of lower or stationary phase falls below the opening leading to the next stage, some upper phase and dissolved solute will not be transferred. Alternatively, the lower-phase volume may be higher than the opening leading to the next stage, causing some stationary phase and dissolved solute to be transferred. In addition, upper phase may be adsorbed on the walls of the equilibration section and lower phase adsorbed at the upper phase-air interface. In the Craig and Post machine, a constant amount of lower phase is added on each cycle to reduce the amount of upper phase retained. But this is often in excess of what is needed so that a portion is transferred. Figure 12.20 compares the output profile obtained when some of these conditions resulted in imperfect transfers with the profile predicted using the ideal equations developed in Section II. In the example, the middle peak approximates that predicted, but the lower  $K$  solute emerges later than predicted while the higher emerges earlier. Considerably less pure material with  $K = 0.5$  would result from this situation, but it can be explained and corrected if the fundamental model is followed.

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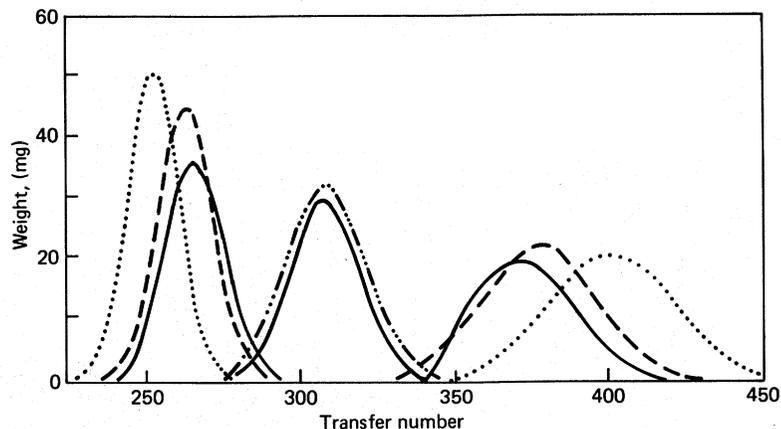


Fig. 12.20. Effect of imperfect transfers: (—) experimental; (····) ideal equation 14,  $a = 0$ ,  $b = 0$ ; (---) calculated from equation 41,  $a = 0.14$ ,  $b = 0.08$ ,  $N = 200$ ,  $K_1 = 0.062$ ,  $K_2 = 0.13$ ,  $K_3 = 0.24$ .

### C. CORRECTIONS TO THE IDEAL EQUATIONS

Consider the treatment in Table 12.I, but where a fraction  $a$  of  $v_s$  is transferred and a fraction  $b$  of  $v_M$  is retained. Then the fraction of  $p$  transported,  $p''$ , and the fraction of  $q$  retained,  $q''$ , become

$$p'' = p - bp + a(1 - p) = p(1 - b - a) + a \quad (38)$$

$$q'' = (1 - p) + b - a(1 - p) = (1 - p)(1 - b - a) + b \quad (39)$$

Proceeding stepwise as in the earlier section, a pattern evolves which is again on the form of the binomial,  $(p'' + q'')^n = 1$ . If  $s = V_M/V_S$ , then the elution peak maximum is given by

$$n_R = \frac{N}{p''} = \frac{N}{[(1 - b - a)/1 + (K/s)] + a} = \frac{N[1 + (K/s)]}{(1 - b) + (aK/s)} \quad (40)$$

When  $b = a = 0$ , then equation 40 reduces to the ideal expression (equation 10). Examination of these expressions indicate that for reasonable solvent ratios when  $b > aK/s$ , the peak elutes later than predicted by the ideal equations and when  $b < aK/s$ , the peak elutes earlier than predicted. If the same number of millimoles retained in untransferred upper phase is transferred to lower phase, no effect is seen. This condition was set for calculation of the middle peak in the experiment shown in Fig. 12.20. For lower values of  $K$ ,  $n_R$  is increased over the ideal values. The reverse is true for higher  $K$  values.

The Gaussian expressions may also be modified to include corrections for two-phase flow. For a single input, equation 14 becomes

$$F = \frac{1}{n_R} \sqrt{\frac{N}{2\pi q''}} \exp \left[ \frac{-(p'')^2}{2q''N} (n - n_R)^2 \right] \quad (41)$$

and for frontal output profiles,

$$Y = \sqrt{\frac{(p'')^2}{N2\pi q''}} \int_0^n \exp \left[ \frac{-(p'')^2}{2q''N} (n - n_R)^2 \right] \quad (42)$$

while the corrected version of equation 28 is

$$n_F = \frac{4.66 \sqrt{Nq''}}{p''} \quad (43)$$

Since  $\sigma = (Nq'')^{1/2}(p'')^{-1}$ , it is apparent that not only the peak position but also its shape is influenced by two-phase flow. An example from an in-depth study of nonideal effects in CCD (76) is given in Table 12.II; it shows the applicability of these equations. In this treatment,  $a$  and  $b$  were constant throughout the distribution. It will be demonstrated in Section II.E.3 that, in practice, even these corrections may not be sufficient to explain all deviations from ideality.

## 2. Rate Phenomena

### a. NONEQUILIBRIUM

When two phases that are not at equilibrium are mixed, the rate of transfer of solute according to the two-film theory is given by

$$\text{rate} = k_M A (C_M^* - C_M) = k_S A (C_S - C_S^*) \quad (44)$$

where  $k_M$  and  $k_S$  are the mass transfer coefficients of the mobile and stationary phases, respectively;  $A$  is the interfacial area;  $C_M$  and  $C_S$  are the concentrations in the bulk phases; and  $C_M^*$  and  $C_S^*$  are the concentrations near the interface. In this model, it is assumed that  $K = C_S^*/C_M^*$ . It is apparent that transfer is accelerated by increasing  $A$  through agitation to decrease the

TABLE 12.II  
Number of Inputs Required to Achieve a Frontal Output<sup>a</sup>

System	$V_{MT}$	$V_T$	$v_M$	$v_S$	$N$	$n_R$	$n_F$ from		
							Data	Equation 28 <sup>b</sup>	Equation 43
$K = 0.26$	23.4	26.6	25.0	40.0	100	145	38	35	38
$K = 0.50$	23.4	26.6	25.0	40.0	100	180	56	56	56
$K = 0.88$	23.4	26.6	25.0	40.0	100	228	80	85	80

<sup>a</sup>  $V_{MT}$  = Volume of mobile phase transferred;  $V_T$  = total volume of both phases transferred.

<sup>b</sup>  $v_{M^*}$  and  $v_S$  used for calculation in equation 28.

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droplet size. However, solute transfer is impeded if the size is too small because the droplets behave as rigid spheres so there is no agitation within the drops (40), and only diffusion occurs. The interfacial area also depends on the composition of the system as this affects interfacial tension, as does temperature fluctuation. When appreciable mass transfer takes place across the phase interface, violent surface pulsations often occur (53). They are set up by gradients in the transfer rate across the interface and greatly enhance mass transfer. There appears to be a range of volume ratios over which either phase would disperse when two immiscible phases are mixed (81). In general, the more viscous phase tends to disperse unless  $s$  is either very small or very large, when the smaller phase is dispersed.

If  $K$  is high, the principal resistance to mass transfer will be in the mobile phase (92), and the solute will elute later than predicted if equilibrium is not reached. Solutes which strongly favor the mobile phase would elute earlier. This would appear to enhance the separation. However, for closely related solutes, this resistance to mass transfer reduces the probability of a solute molecule interacting with the solvent molecules in the other phase, thereby diminishing the separation obtained with a given number of stages.

In countercurrent distribution, the agitation is relatively mild so that the equilibration part of the cycle must be sufficiently long.

### b. SETTLING TIME

If the densities of the phases are too close, their viscosities too high, or their interfacial tensions too low, phase separations may be slow. The settling time is adjustable in the Craig-Post and other similar distributors, although in the rotating distributor (Fig. 12.17) there is no discrete settling step so that settling time is controllable only so far as the total time of rotation is controllable. It is conceivable that in order to reduce analysis time, some incomplete phase separation will be tolerated. If the degree of intermixing at the time of transfer is constant during a separation, the perturbation of the distribution would be described by the two-phase equations derived in the previous section.

## 3. Phase Equilibria

### a. VOLUME REORGANIZATIONS

Solvents used in countercurrent distribution are usually not completely immiscible. In order to minimize nonideal transfer effects, each solvent is preequilibrated with the other at the temperature of operation. However, even at low solute inputs, the intersolubilities of the solvents change as the solute level varies during the distribution.

The effect of these changes on a CCD frontal output profile is shown in Fig. 12.21. The ternary diagram describing this system is shown in Fig. 12.22. Variations in the first tube of the distributor as inputs are made as shown in Fig. 12.23a. A decrease of the preequilibrated phase volume ratio

## 12. COUNTERCURRENT DISTRIBUTION

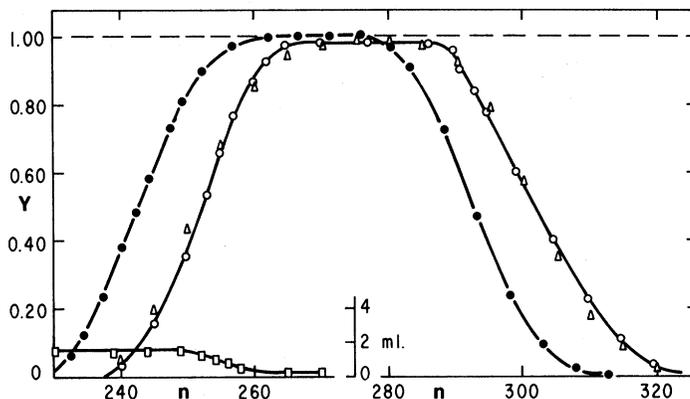


Fig. 12.21. Countercurrent distribution of methyl hexadecanoate (palmitate) in hexane-acetonitrile: (●) output profile calculated from equation 24,  $K = 0.112$ ,  $v_M = 20$ ,  $v_S = 39.2$ ,  $N = 200$ ; (○) experimental output; (△) computer simulated output; (□) volume of lower phase in output (76).

occurred with the initial inputs. This “extra” lower phase is transferred, since it is above the transfer arm of the tube. As more inputs are made and transfers carried out, the phase ratio increases, even though the solute level does not exceed 4%. A quantitative description is given in the next section. As the mobile phases move along the distributor, the solute level decreases and lower-phase solvent comes out of solution and is transported as a pulse of stationary phase out of the distributor. This is shown by the lower curve

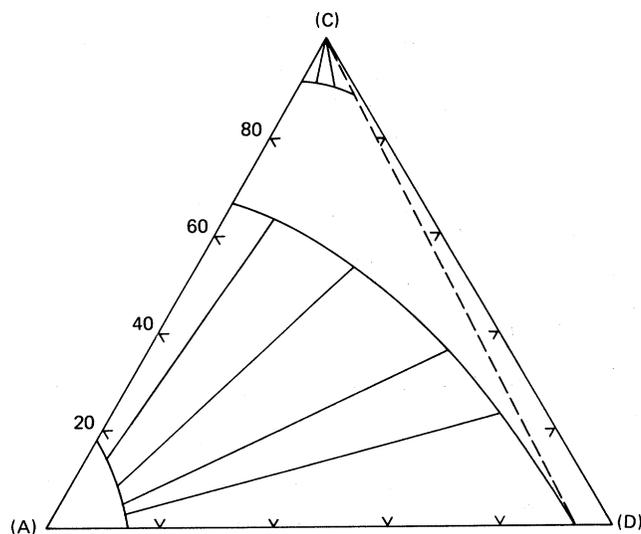


Fig. 12.22. Ternary phase diagram at 25°C. Hexane (D), acetonitrile (A), and methyl palmitate (C) (76).

## G. SEPARATION TECHNIQUES

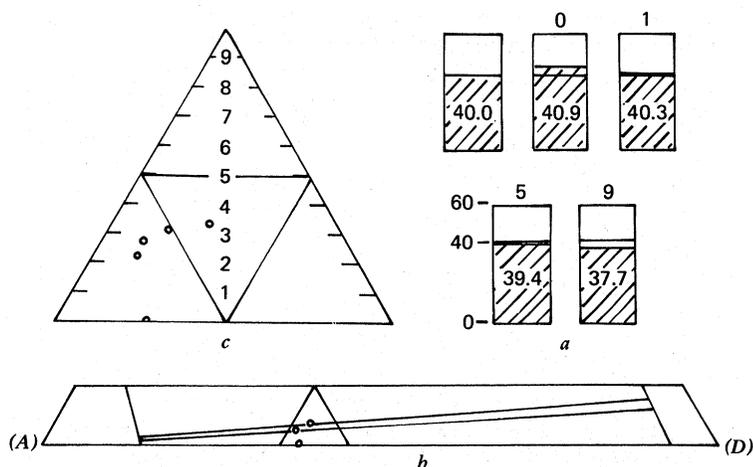


Fig. 12.23. (a) Representation of phase volumes in tube  $r = 0$  for  $n = 0, 1, 5$ , and  $9$  for the conditions of the experiment shown in Fig. 12.21. Striped area is stationary phase with volume noted. (b) Lower portion of phase diagram in Fig. 12.22. (c) Blowup of triangle in (b) with percent methyl palmitate listed vertically. Circles represent the conditions in (a) starting at the baseline.

which precedes the experimental frontal profile in Fig. 12.21 (76). These effects could be significant in chromatography. Changes in the volumes of intracolumn stationary and mobile phases may lead to changes in paths of flow and flow rates within the column. Loss of stationary phase by solubilization in the mobile phase caused by the presence of the solute could be important. Pulses of stationary phase could give responses in refractive index monitoring devices which are not easily related to solute concentration. When mixed-solvent mobile phases are used with adsorbents or bound phases, the solute related changes in the intersolubilities may alter the environment at the support surface, which in turn changes the mobile-phase composition to produce anomalous detector responses. Precolumns cannot completely eliminate these effects, since they compensate for phase solubility in the absence of solute. Problems resulting from these phenomena have led to the use of stationary phases bonded to the surfaces of "inert" matrices for chromatography (50).

### b. TERNARY AND QUATERNARY SYSTEMS

Solute-solvent interactions may be expressed quantitatively using the Gibbs triangle such as that shown in Fig. 12.22. Perpendiculars from a point to the sides of the triangle designate the composition at that point (90). The curves inside the triangles define the immiscible region, while the lines between these solubility curves connect compositions which can exist in equilibrium (tie lines). Alternatively, right triangular coordinates may be used, since stating two of the compositions defines the third. According to

## 12. COUNTERCURRENT DISTRIBUTION

the lever rule, the mass of each phase of a two-phase system is inversely proportional to the length of the tie line segments connecting the original composition point to the equilibrium compositions. The system shown contains a solid-liquid immiscible region.

The low-solute portion of Fig. 12.22 is amplified in Fig. 12.23*b* to explain the phenomena discussed qualitatively in the previous section. The solute level of the first input is about 2% (Fig. 12.23*c*). The lower-phase (MeCN-rich) tie line segment is shorter than the segment for the solute-free solvents at the given phase ratio. From the lever rule, decrease in phase ratio is predicted. Since this diagram is in weight percent units, densities are used to calculate volume changes. Subsequent inputs increase the segment length explaining the changes in tube volumes as shown. Results of computer simulation of the countercurrent process (Fig. 12.21) which incorporated phase-equilibria data accurately predicted the position and shape of the output profile as well as stripping of lower phase. The phase diagram also explains why the plateau in this frontal experiment is at  $Y = 0.96$  rather than at  $Y = 0.99$  as equation 26 dictates. Solute is usually added in mobile phase which has the composition given by the intersection of the solubility curve of the mobile phase (Hex) and the base of the triangle. Adding solute to this solution is the equivalent of moving along a line from this point to the apex denoting 100% solute. But points along this line cannot exist in equilibrium with MeCN-rich solvent, since the points fall in the miscible region. The maximum solute composition must lie on the solubility curve, and it is this composition which determines the plateau level. Thus, the feed composition is approached but cannot be reached.

The system shown contains an isopycnic line, that is, a tie line which connects two phases of equal density. If solute compositions along this line were exceeded during a distribution experiment, phase inversion would occur and the previously stationary phase would be transferred instead of the previously mobile phase. As compositions approach this line, settling time becomes extremely long. This is also true when any critical point is approached.

The ternary diagrams show that stating only solute concentration does not uniquely define  $K$ . This is further demonstrated in Fig. 12.24 (76). At concentrations of 1% methyl oleate, the percentages of two of the three components must be stated to designate  $K$ . The limits of the immiscible region are denoted by the dashed lines.

The effect of variable  $K$  on the position of CCD profiles and their shape as judged by the number of inputs to achieve a frontal is indicated in Table 12.III. When  $K$  decreases as solute concentration increases, little overall effect is observed. However, when  $K$  increases,  $n_r$  occurs later and the peak is more spread out than if only the initial value was considered.

The only reasonable way to deal with all of these factors in a predictive manner is by computer simulation. This was done for the data of Figs. 12.22 and 12.23 and the results of the simulation, in which no assumptions con-

## G. SEPARATION TECHNIQUES

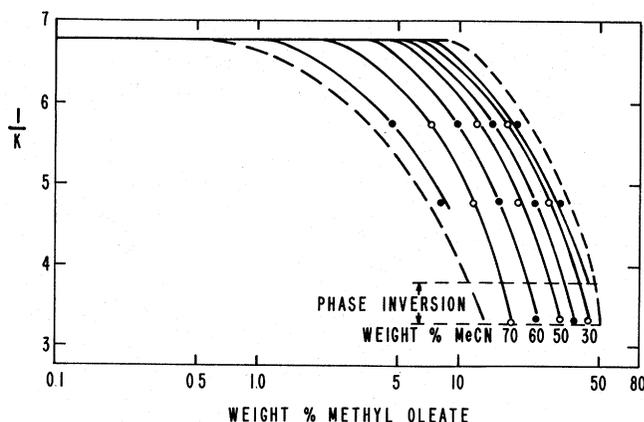


Fig. 12.24. Data of Fig. 12.25 plotted as  $1/K$  for the distribution of methyl oleate vs. wt-% methyl oleate at 25°C. Two of the three compositions must be given to define  $K$  in this system.

cerning profile shape or position were made. The results of the simulation are given in Fig. 12.21 (57). As seen, the simulation accurately predicts the position, shape, and height of the profile which deviated greatly from those calculated using the ideal equations. Even the stripping and “pulsing” of lower phase (as a function of transfer number), which is a result of solute dependent volume reorganizations, was described.

Temperature influences the phase equilibria and therefore the behavior of a system in CCD. The contraction of the immiscible region with increasing temperature for a ternary system (6,7) is shown in Fig. 12.25, while variation of  $K$  values for two solutes at constant solvent ratio is given in Fig. 12.26 (7). Peak maxima and solute separability would be affected by such variation.

Ternary diagrams give useful information about solute-solvent interac-

TABLE 12.III  
Effect of Variable  $K$  on the Position of CCD Profiles and Their Shape

System	$N$	$n_R$	$n_F$ from		
			Data	Equation 28	Equation 43
$K = 0.108 - 0.125\tau^a$	200	249	35	34	36
$K = 0.108$	200	249	36	35	37
$K = 0.108 + 0.125\tau$	200	250	36	35	37
$K = 0.500 - 0.215\tau$	20	37	25	26	26
$K = 0.500$	20	40	29	29	29
$K = 0.500 + 0.215\tau$	20	46	45	35	35
$K = 9.29 - 0.0007\tau$	200	3610	1140	1160	1160
$K = 9.29$	200	3910	1270	1270	1270
$K = 9.29 + 0.0007\tau$	200	4620	1970	1490	1490

<sup>a</sup> 0.5 ml retained in this case,  $v_M = 20.0$ ,  $v_S = 40.0$  in all cases,  $\tau$  = total amount of solute in tube.

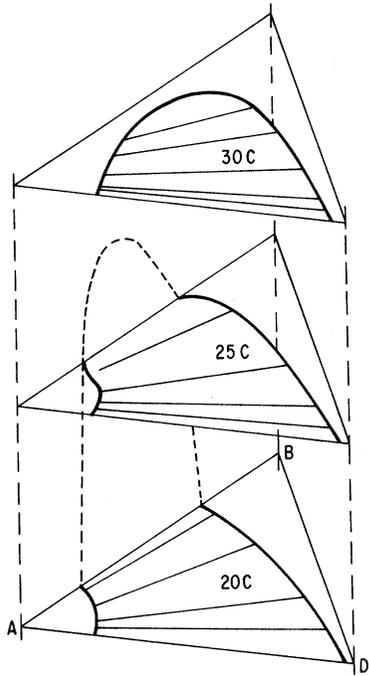


Fig. 12.25. Influence of temperature on phase equilibria: A, Acetonitrile; B, methyl oleate; D, hexane (16).

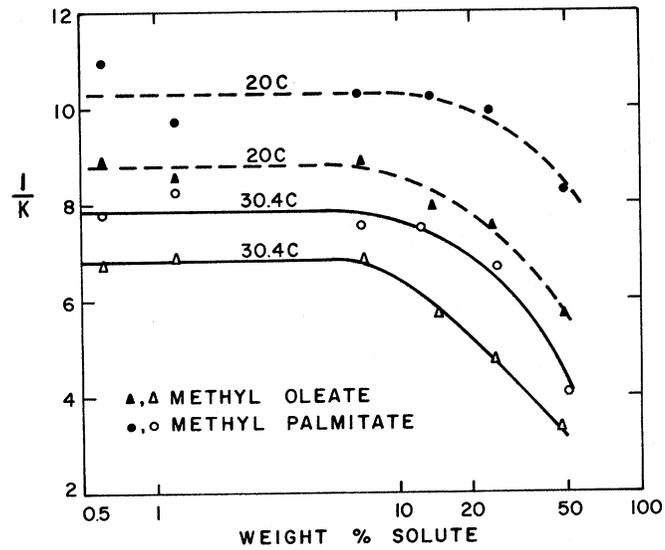


Fig. 12.26. Variation of  $K$  for two solutes as temperature changes: (●, ○) methyl palmitate; (▲, △) methyl oleate; hexane/acetonitrile = 1.13 (7).

## G. SEPARATION TECHNIQUES

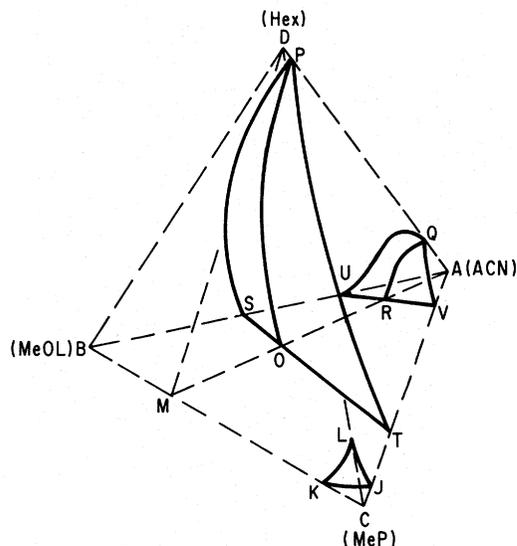


Fig. 12.27. Quaternary diagram: A, Acetonitrile; B, methyl oleate; C, methyl palmitate; D, hexane. 25°C (77). Immiscible region falls between the surfaces PSOT and QURV. Methyl palmitate is a solid at this temperature, as denoted by the solubility surface LJK.

tions, but for separations solute–solute and competitive solute–solvent interactions also need to be considered. Distribution coefficients are often closer to one another in multicomponent systems than in single solute–two solvent ternary systems (77). In addition, as purification of one of the components of a mixture progresses, the miscibility of the phases may increase, causing coalescence of the system as the contaminants are removed (6).

Visual representation of four-component systems is complicated (Fig. 12.27). Beyond four components, representation in two dimensions becomes virtually impossible.

Palatnik and Landau (63) have applied the technique of vector notation, linear algebra, and topology to the description of multicomponent systems. For a two-phase system, a generalized lever rule was developed:

$$\frac{\text{mass of phase 2}}{\text{mass of phase 1}} = \frac{w_{i1} - W_i}{W_i - w_{i2}} \quad (45)$$

where  $W_i$  is the weight fraction component  $i$  in the system and  $w_{i1}$  and  $w_{i2}$  are its weight fractions in phases 1 and 2, respectively. Some applications of this approach in a quaternary system have been described (77).

The construction of ternary and multicomponent phase diagrams requires a large number of data points. For many CCD applications, such detail is not as necessary as in the studies referred to here. These were performed to better understand the countercurrent process. However, phase diagrams can be roughed in with a few points as a guide for separation design.

## 12. COUNTERCURRENT DISTRIBUTION

### F. OPTIMIZATION

#### 1. Standards of Degree of Separation

Separations are carried out to achieve some predefined goal. In counter-current distribution, that goal might be to resolve the components of a mixture to such a degree that a plot of concentration versus transfer number can be constructed in order to determine the composition of a mixture. This may be done manually by collecting fractions, evaporating solvents, and weighing the solute or, more conveniently, traces of output profiles obtained using a recording differential refractometer (16), spectrophotometer, or other suitable monitor. The transport of lower phase out of the apparatus, described earlier, may cause anomalous peaks, particularly when refractive index monitoring is used.

Another goal might be to utilize the shapes of CCD bands and peaks as criteria of purity (Fig. 12.7) (67,69) or to study interactions between species (5a). Again, nonideal effects must be eliminated or compensated for in any approach with that goal.

But most commonly at present, CCD is used to isolate enough material so that other methods can be employed for structure determination and component identification or for product purification. In these cases, it may be desired to recover all of the components in high yield and purity; the components in high purity regardless of yield; or one of the components in high yield and/or purity at the expense of the others. High solute throughput may or may not be important.

Although the goals of the distribution will influence the operational parameters, some yardstick of degree of separation must be chosen to evaluate parameters on a rational basis. A number of concepts have been proposed. Usually, two difficultly separable components are focused upon and the regions occupied by the components and the boundary between them, the "cutpoint," considered in terms of the amount of each component in its respective region and its purity. Let

$X_{ij}$  = the number of moles of component  $i$  in region  $j$        $i = 1,2; j = 1,2$   
 $X_{ir}$  = total number of moles of component  $i$   
 $X_{rj}$  = total number of moles of components in region  $j$   
 $X_{rr}$  = total number of moles of both components

The total amount of solutes partitioned into regions in which they are at their highest concentrations or purity may be represented by the "quantity factor" ( $QF$ ) (59) so that  $QF = X_{11} + X_{22}$ . The impurity of region 1 is defined as  $X_{21}/X_{r1}$ . A measure of quality of separation can then be written as "total percent impurity" (TPI):

$$\text{TPI} = 100 \left( \frac{X_{21}}{X_{r1}} + \frac{X_{12}}{X_{r2}} \right)$$

## G. SEPARATION TECHNIQUES

The goal of many separations is the maximization of  $QF$  and the minimization of TPI. If only one cutpoint between the regions is utilized, no cutpoint is known which meets both of these goals.

Table 12.IV (59) lists the results of computer simulation of CCD and demonstrates the interrelationships between several possible cutpoints with varying component ratios and between some proposed concepts for evaluating separations when some overlap occurs. This is not an uncommon situation in CCD or chromatography.

Gluekauf (35) proposed that the fractional impurity be used as a standard of resolution in chromatography. His well-known, often reproduced nomograph related relative retention to the number of theoretical plates required to achieve a separation where the fractional impurities in each region are equal. One conclusion of Gluekauf's approach is that it is easier to achieve a given separation the more unequal the amounts of the components are. As Boyde (13) pointed out, the derivation is limited to cases where the fractional impurity is very much smaller than the ratio of component amounts. When equal amounts are fractionated, the impurities-equal cutpoint of Gaussian profiles is found at the geometric mean of the retention volumes or in CCD retention number. Said (78) observed that no analytic expression could be derived to predict the location of this "optimum" cutpoint for unequal solute amounts. This limits its utility for calculating optimization parameters.

The extent of separation  $\xi$  proposed by Rony (74,75) as a universal index for evaluating separations is similar to efficiency number  $E$ , which had been described previously (59). For a two-component, two-region separation,

$$\xi = \left| \frac{X_{11}}{X_{1r}} + \frac{X_{22}}{X_{2r}} - 1 \right| = E = \left| \frac{X_{11}}{X_{1r}} - \frac{X_{21}}{X_{2r}} \right|$$

It was proposed that the optimum cutpoint is that which maximizes  $\xi$ . For Gaussian profiles, this corresponds to the intersection of the normalized profiles. Table 12.IV clearly shows, however, that although large and similar  $\xi$  values are obtained in the three cases, different quantities are recovered and the purities of each component are different. The total percent impurities values are the highest at this cutpoint.

The intersection of the molar profiles maximizes  $QF$  because it assigns to region 1 all fractions containing the major portion of component 1 and to region 2 all fractions containing the major portion of component 2. Expressions, though not simple, exist for calculating the intersection of ideal CCD elution (59) and frontal curves (9). When curves are observed which depict the total amount of solute in a tube or in each portion of the effluent, curve resolution methods must be employed. Parameters may be evaluated to provide the lowest TPI at this cutpoint.

The concept of resolution  $R_s$  is also employed widely to evaluate chromatographic (4) and CCD separations (36). For CCD bands inside the distributor,

TABLE 12.IV  
Comparison of Cutpoints

$X_{1r}/X_{2r}$	Description of cutpoint	Cutpoint number	TPI	$X_{11}$	$X_{21}$	$X_{12}$	$X_{22}$	$\xi$	$QF$
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
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
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

## G. SEPARATION TECHNIQUES

$$R'_S = \frac{r_{\max 1} - r_{\max 2}}{2(\sigma'_1 + \sigma'_2)} = \frac{0.5 \sqrt{ns} (K_2 - K_1)}{\sqrt{K_2}(s + K_1) + \sqrt{K_1}(s + K_2)} \quad (46)$$

and for output profiles,

$$R_S = \frac{n_{R_2} - n_{R_1}}{2(\sigma_2 + \sigma_1)} = \frac{0.5 \sqrt{N} (K_2 - K_1)}{\sqrt{K_1}(s + K_1) + \sqrt{K_2}(s + K_2)} \quad (47)$$

Resolution as defined is independent of relative solute amounts. The same value would be calculated for unequal amounts, but the intersection point of overlapping Gaussian profiles would shift toward the smaller band or peak (9).  $QF$  and TPI would differ, so for some purposes the resolution concept is ambiguous.

Thus, no single concept seems to exist that unambiguously provides a tool for evaluating separations and that leads to calculation of optimization parameters. These and others that have been proposed have utility only so far as their limitations are recognized.

### 2. Solvent Systems

Solvent properties are the most important variables in sequential extraction since they determine  $K$  for the solutes to be separated. Chapter 11 in this volume and others by Irving (47) have documented many solvent systems useful for the separation of particular solutes. A compilation of CCD separations has been published (26), and Francis has studied phase equilibria in over 1000 ternary systems for about 300 components (31). Many of these documented systems could serve to provide insights for the design of specific separations. Although these will not be repeated here to any great extent, a number of examples are provided to illustrate the general approach. Equations 46 and 47 reveal that the differences between the band or peak centers are related to thermodynamic properties of the system which may be determined experimentally (77). If a solvent system will not provide an adequate separation, another solvent system may be chosen (6,47,90). Alternatively, the solutes may be reacted differentially with other soluble components to provide the appropriate "leverage" for the separation.

#### a. MULTIPLE EQUILIBRIA

Use of complexing agents, the manipulation of pH, and ionic strength are among the most common approaches in countercurrent distribution (85). An example is given in Fig. 12.28 in which the interaction of silver ion with unsaturated compounds was utilized for the difficult separation of cis from trans isomers of methyl  $\Delta^9$ -octadecenoate (80). This may be compared with better separations by high-performance liquid chromatography (12).

A series of alkaloids of *Strychnos nux-vomica* was separated by CCD using chloroform as the stationary phase and aqueous buffer as the mobile phase. Stepwise changes in pH of the mobile phase were used to separate the

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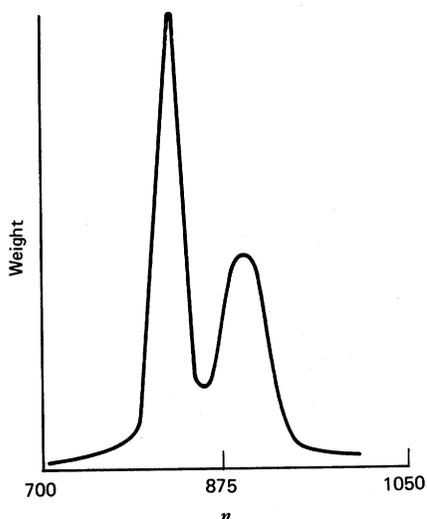


Fig. 12.28. Separation of *cis*-methyl  $\Delta^9$ -octadecenoate (second peak) and *trans*-methyl  $\Delta^9$ -octadecenoate. Solvents: hexane/0.2 *M*  $\text{AgNO}_3$  in 90% methanol (0.25), with recycling (80).

nine known bases and four additional bases that were discovered (32). This study provides a useful example of the approach. Equation 1 defined  $K$  in terms of the number of millimoles (mm) of solute in each phase. Here,

$$X_S = \text{mmB}_S + \text{mmBH}_S^+$$

$$X_M = \text{mmB}_M + \text{mmBH}_M^+$$

and

$$K_a = \frac{[\text{B}]_M[\text{H}^+]_M}{[\text{BH}^+]_M}$$

where  $\text{B}$  is the basic alkaloid and  $\text{BH}^+$  represents its protonated form. The uncharged alkaloid may be distributed between the mobile and stationary phase as represented by  $D$ :

$$D = \frac{[\text{B}]_S}{[\text{B}]_M} \quad (48)$$

The pH range was restricted to 3–7 to limit decomposition of the alkaloid bases and to guarantee the absence of emulsions. In this pH range,

$$[\text{BH}^+]_M \gg [\text{B}]_M$$

for example, for strychnine,  $K_a \cong 1.0 \times 10^{-8}$  and for brucine,  $K_a \cong 1.1 \times 10^{-8}$ . In this solvent system for strychnine,  $D = 1.2 \times 10^3$  and for brucine,  $D = 1.7 \times 10^3$ . With this information and the additional simplifying approximation,  $\text{mmB}_S \gg \text{mmBH}_S^+$ , equation 1 may be rewritten for these alkaloids as

$$K \cong \frac{[B]_S}{[BH^+]_M} = \frac{K_a D}{[H^+]_M} \quad (49)$$

and

$$\log K \cong \log K_a D + \text{pH} \quad (50)$$

It is apparent that  $\log K$  and hence retention volume varies directly with pH for this set of assumptions. Similarly, the greater the acid dissociation constant, the more uncharged base B will be present, and it will be retained by the chloroform stationary phase. Resolution, as defined in equations 46 and 47, is directly proportional to differences in the peak centers, and for the pair of alkaloids, the values of  $K_a$  and  $D$  both lead to lower retention of strychnine relative to brucine. The elution of alkaloids is summarized in Fig. 12.29.

#### b. PREDICTIVE EQUATIONS

The number and complexity of potential systems for CCD is vast. This has led to many attempts to organize large quantities of data in forms which could also be used to predict the applicability of solvent systems. One of the best known of these approaches is the "regular solution theory" of Hildebrand and Scott (42,43) and their use of the "solubility parameter." This has been considered in another chapter. For those molecules whose interaction

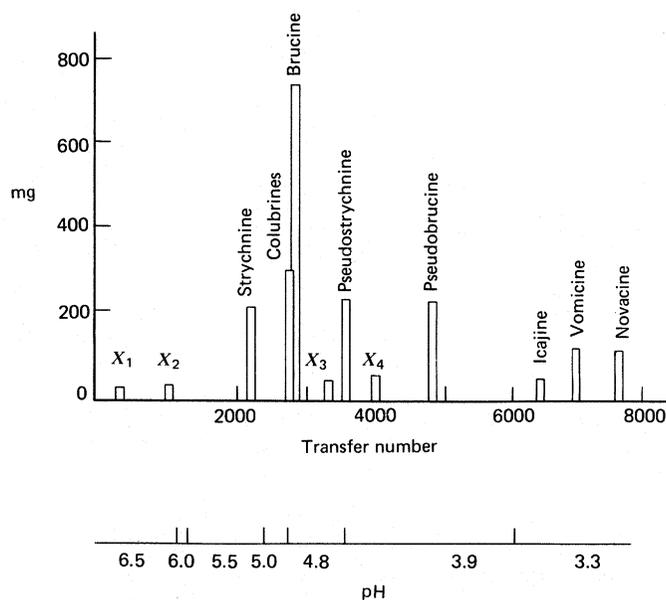


Fig. 12.29. Separation of alkaloids from *Strychnos nux-vomica* by CCD. Stationary phase was  $\text{CHCl}_3$ ; mobile phase was 0.2 M phosphate buffer, the pH of which was changed in steps;  $N = 200$ ;  $v_M = v_S = 10$  ml. Solute in mg in effluent plotted vs. transfer number (32).

## 12. COUNTERCURRENT DISTRIBUTION

is determined primarily by London dispersion forces, the regular solution theory provides a useful approach. This rules out electrolytes, solutes, and solvents which hydrogen bond or have a net dipole moment.

Hansch (52) has proposed a completely different approach for the organization and, in some cases, the prediction of distribution equilibria. He used an expression of the form

$$\log K_{\text{solv}} = a \log K_{\text{octanol}} + b \quad (51)$$

In this "linear free-energy" approach, the factors which contribute to  $\Delta G^0$  and, hence,  $\log K$  are considered to be the sum of the functional group values. There are corrections for inductive, resonance, steric, and conformational effects as well as branching and chain length. Data are related to distribution of classes of solutes between octanol and water.

The concentration of solute in the organic phase is always divided by its concentration in the aqueous phase at equilibrium in the calculation of either  $K_{\text{solv}}$  or  $K_{\text{octanol}}$ . This is then related to other solvent-aqueous systems through equation 51. The terms  $a$  and  $b$  in equation 51 are obtained experimentally by fitting data through regression analyses. Hansch provides information on the number of molecular species studied, the correlation coefficient, the standard deviation from the regression, and 95% confidence intervals for the  $a$  and  $b$  terms. In a recent review, he has provided about 6000 entries, which is a valuable resource for consideration prior to designing a separation system. The additive nature of the factors has allowed Hansch to relate structure to a variety of biological processes.

We have shown that in CCD separations, ternary and quaternary diagrams are needed to specify completely the system, unless the solutes are at very low concentrations. This may be one of the limitations of most listings of  $K$ . Many workers have attempted to provide other predictive approaches to phase equilibria through statistical thermodynamics. An important type of approach is based upon determining the deviation from Raoult's law in terms of the relationship of the partial molar excess Gibbs free energy ( $g^E$ ) and the activity coefficient ( $\gamma$ ) of a nonelectrolyte. Equation 52 defines the relationships through the usual partial derivative in

$$RT \ln \gamma_i = \left( \frac{\partial X_\tau g^E}{\partial X_i} \right)_{T,P,X_j(j \neq i)} \quad (52)$$

where  $T$ ,  $P$ , and  $R$  are the temperature, pressure, and gas constant;  $X_\tau$  is the total number of millimoles (or moles); and  $X_i$  is the number of millimoles (or moles) of component  $i$ . Prausnitz (1,73) has made use of Guggenheim's view of solutions (38) and has derived an equation to calculate the activity coefficient. Binary vapor-liquid equilibrium data are needed, and two adjustable parameters per binary must be determined usually by iterative processes. The treatment gives a good representation of vapor-liquid equilibria and reduces to the well known Wilson equations (96). For partially miscible systems, the mutual solubility data uniquely fix the two

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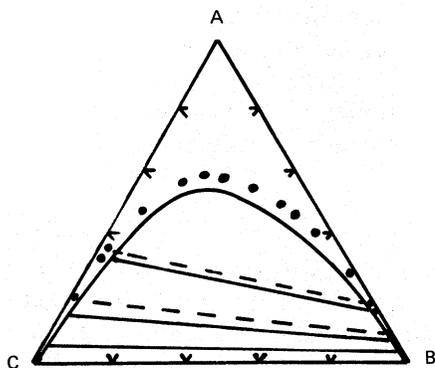


Fig. 12.30. Ternary diagram for the system chloroform (C), water (B), and acetone (A) at 60°C. Solid lines represent predicted values; dots and broken lines represent experimental values (1).

parameters needed for that particular binary, and the information may be used to sketch a ternary diagram. The latest form of equation developed by Prausnitz requires fewer parameters than an earlier version (73), and these parameters may be less arbitrary than before. Figure 12.30 shows good, though not perfect, agreement between "predicted" and experimental liquid-liquid data. There is a plait point in the diagram, that is, only one of the binary mixtures displays a region of immiscibility, and the predictions are not as good as in those cases without plait points. The predictions were improved by use of a limiting distribution coefficient as a parameter in the equations. The limiting distribution coefficient is obtained at very low solute concentrations in both phases.

A limited amount of ternary liquid-liquid equilibria may be used to develop parameters to predict the rest of the ternary diagram. The results as expected were very good for the system water-ethanol-ethyl acetate; yet when this information was used to predict binary vapor-liquid equilibria, the results were poor. Extension of the approach to quaternary systems, the absolute minimum for real CCD separations on the preparative scale, was not achievable (1).

These theoretical approaches bear further investigation and may help in selecting potential extraction systems, but careful experimental evaluation is still a necessary step in utilization of a CCD solvent system. Discussions of the current status of these approaches have been published (59a,66a).

### 3. Operating Parameters

#### a. NUMBER OF STAGES

Once a suitable solvent has been selected, resolution may be further improved by increasing the number of transfers (95). This may be accomplished by increasing the number of tubes, recycling (27), or, in effect, by decreasing the ratio of mobile-to-stationary phases when the number of tubes is fixed. Rearrangement of equations 46 and 47 gives the required

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number of transfers or tubes needed to achieve a specified resolution of two solutes for fixed  $s$ ,  $K_1$ , and  $K_2$ .

### b. SOLVENT RATIO (EXCEPTIONS TO THE BUSH AND DENSEN RATIO)

From equation 46,  $R'_S = f(K_1, K_2, s, n)$ . It should be noted that specifying  $r$  is unnecessary, since inside the distributor  $r$  is dependent on the other variables. Differentiating that equation gives

$$dR'_S = \left( \frac{\partial R'_S}{\partial s} \right)_{n, K_1, K_2} ds + \left( \frac{\partial R'_S}{\partial n} \right)_{s, K_1, K_2} dn \quad (53)$$

If the first partial differential is considered,

$$\left( \frac{\partial R'_S}{\partial s} \right)_n = \frac{[(s + K_2) \sqrt{K_1} + (s + K_1) \sqrt{K_2}] 0.25 \sqrt{n} \left( \frac{K_2 - K_1}{\sqrt{s}} \right)}{[(s + K_2) \sqrt{K_1} + (s + K_1) \sqrt{K_2}]^2} - \frac{-0.5 \sqrt{ns} (K_2 - K_1) (\sqrt{K_1} + \sqrt{K_2})}{[(s + K_2) \sqrt{K_1} + (s + K_1) \sqrt{K_2}]^2}$$

Setting the result equal to zero, dividing by  $[0.5n^{1/2}(K_2 - K_1)s^{-1/2}]$ , and solving for the solvent ratio yields

$$s = \frac{K_2 \sqrt{K_1} + K_1 \sqrt{K_2}}{\sqrt{K_1} + \sqrt{K_2}}$$

Multiplying top and bottom by  $(K_1 K_2)^{1/2}$  gives the well-known Bush and Densen relationship (15,37)

$$s = \sqrt{K_1 K_2} \quad (54)$$

This relationship was originally developed empirically for a specified condition but has been generalized (60) and often considered as giving the best separation in all cases. However,  $n$  appears only in the numerator of equation 46, so that  $\partial R'_S / \partial n$  has no minimum. Thus, if the number of transfers is considered constant, the erroneous conclusion is reached that the solvent ratio given by equation 54 produces the optimum separation. The effect of lower solvent ratio on resolution is clear for the single withdrawal method (equation 47) since  $s$  appears only in the denominator.

A detailed mathematical and computer study of the solvent ratio (59) showed that making  $s$  small as possible gives the best separation (Table 12.V). An exception is when  $n < N$  and no solute leaves the distributor. In this case, equation 54 gives the best results for a given  $n$ , but not the best separation possible. The degree to which  $s$  can be reduced, however, is limited to a practical value in order to minimize the nonideal effects described earlier. A disadvantage of lower  $s$  or increased  $n$  is that separation time is increased.

Usually, a resolution of 1.0 will suffice. An approximate solution to equation 47 for this condition can be made:

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TABLE 12.V  
Effect of Solvent Volume Ratios<sup>a</sup>

Inside				Single withdrawal		
<i>s</i>	<i>n</i>	TPI	<i>R'<sub>s</sub></i>	<i>n</i>	TPI	<i>R<sub>s</sub></i>
0.8	72	39.0	0.41	141	30.8	0.51
0.5	89	35.4	0.44	193	26.0	0.54
0.125	229	29.8	0.54	602	18.3	0.65

<sup>a</sup> *N* = 50, *K*<sub>1</sub> = 1, *K*<sub>2</sub> = 0.67.

$$s = \frac{(N/4)[(K_2 - K_1)/(K_1 + K_2)]^2 - 1}{(K_1 K_2)^{-1/2}} \quad (55)$$

This equation can give negative results, which indicate that unit resolution cannot be obtained for the given *K*<sub>1</sub>, *K*<sub>2</sub>, and *N*. A lower bound of *N* can be calculated from equation 47 by setting *R<sub>s</sub>* = 1 and *s* = 0:

$$N = \frac{4(K_2 + K_1)^2}{(K_2 - K_1)^2}$$

More tubes would be actually needed in practice since *s* can not actually be zero.

### c. SOLUTE THROUGHPUT

When increased solute throughput is desired, the amount to be fractionated is divided into a number of increments in order to minimize nonideal effects. It has been suggested (51) that the increments be placed in the first few tubes of the distributor (batch loading). However, recent investigations have shown that better separation is obtained when the multiple input (sequential) approach described earlier was used (6). Batch loading, in a sense, shortens the length of the distributor. Data from computer simulated distributions is plotted in Fig. 12.31 for a system in which two solutes having partition coefficients of 1 and 0.67 were eluted from a 50-tube distributor using a solvent ratio of 0.5. The ordinate represents the number of tubes that would produce the same separation as the incremental methods if the solute were placed only in the first tube. The plots show strikingly how batch loading diminishes separation as compared to the sequential method. In fact, 25 inputs fed sequentially result in the same separation as five tubes batch-loaded. Thus, greater solute throughput can be achieved at the same separation efficiency when sequential loading is used.

The frontal approach was shown to be useful in obtaining sizable fractions in which minor components have been concentrated (9). In Fig. 12.32, fractions I, III, and IV were enriched 7-, 7-, and 38-fold, respectively; the last mentioned was originally present at only the 0.9% level.

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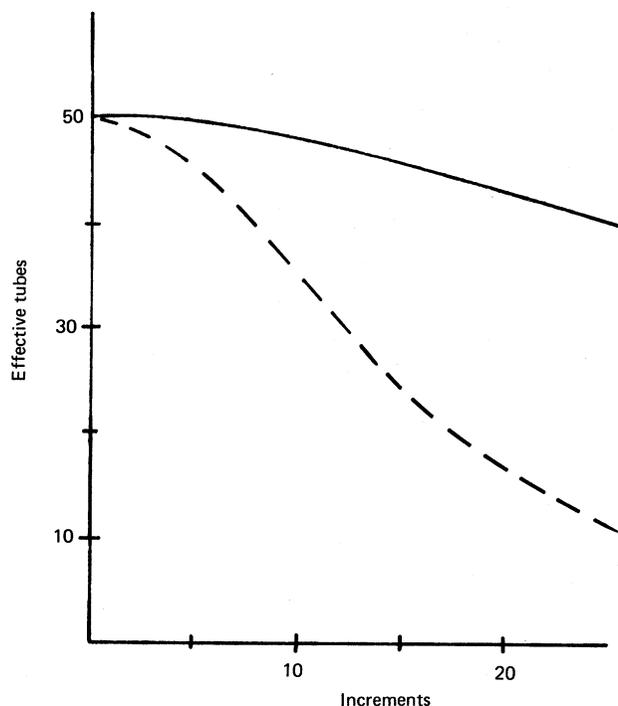


Fig. 12.31. Effect of method of introducing solute: Solid line represents sequential feed into  $r = 0$ ; broken line represents batch loading. States of tubes,  $N = 50$ ,  $s = 0.5$ ,  $K_1 = 0.67$ ,  $K_2 = 1$ .

In terms of the resolution concept for sequential feeding from equations 47, 30, and 34,

$$R_S = \frac{V_{\max,2} - V_{\max,1}}{\frac{1}{2}(w_{Z2} + w_{Z1})} = \frac{V_{R2} - V_{R1}}{2(\sigma_2 + \sigma_1 + V_Z)} \quad (56)$$

The reciprocal of this,

$$\frac{1}{R_S} = \frac{2(\sigma_2 + \sigma_1)}{V_{R2} - V_{R1}} + \frac{V_Z}{V_{R2} - V_{R1}}$$

represents a straight line with an intercept of the reciprocal of the resolution when  $V_Z = 0$ . This corresponds to the theoretical maximum in resolution. The slope of the line is the reciprocal of the difference in retention volumes. Thus, the deterioration in resolution for sequential feeding may be directly measured.

### G. EVALUATION OF THERMODYNAMIC PARAMETERS

Because the distribution of solutes in CCD is primarily dependent upon phase volumes and  $K$ , the distribution coefficient, it is a straightforward

## G. SEPARATION TECHNIQUES

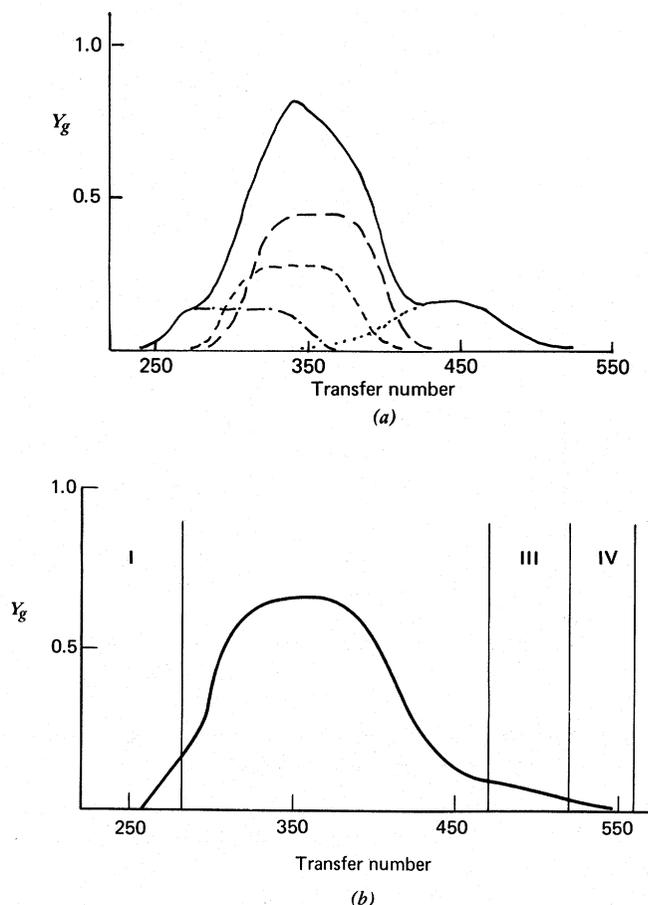


Fig. 12.32. Example of frontal fractionation for isolation of minor components: (a) Calculated output profile; (b) Experimental profile (9). Solute output in grams ( $Y_g$ ) plotted vs. transfer number. I, Methyl stearate; III, methyl linoleate; IV, methyl linolenate.

matter to evaluate  $K$  and  $\Delta G^0$  from a distribution profile (equation 13). Variation of the distribution profile and hence  $K$  with temperature may be used to calculate  $\Delta H^0$  and  $\Delta S^0$  through the usual techniques of thermodynamics. This case is almost trivial, since essentially the same information may be obtained from a single extraction stage without resorting to sequential extraction. If a mixture of solutes is to be studied, the thermodynamic information may be obtained as part of the separation process which is an advantage for sequential over a single extraction. The use of CCD for the study of equilibrium relationships in interacting systems provides a significant tool which has several distinct advantages over a variety of techniques, including electrophoresis, ultracentrifugation, chromatography (19), and spectroscopy (79).

## 12. COUNTERCURRENT DISTRIBUTION

Utilization of CCD for study of molecular interactions has been pioneered by Kegeles (49). The most sensitive of the methods he described involves fitting predictions from a model to an experimental distribution pattern. This is most conveniently done with the aid of a digital computer, and if nonideal effects are unavoidable, they may be included in the simulation. An example of one study is provided in Fig. 12.33. This is the study of the reaction



Estimates of the value of the formation constant of the mixed halide varied from 2 to 16 in methanol, 14 in water, to 100 in benzene. The distribution was carried out, and the circles represent experimental points for the distribution between benzene and water. The curves represent computer-calculated predictions assuming  $K$  values of about 0.95, 2.6, and 12 for  $\text{HgCl}_2$ ,  $\text{HgClBr}$ , and  $\text{HgBr}_2$ , respectively. The highest curve was drawn using the assumption that the formation constant for  $\text{HgClBr}$  in benzene was 100, the intermediate curve represents a formation constant of 15, and the bottom curve represents a formation constant of 2. It is clear that the data most nearly agree with the last value. Cann (19) has pointed out that the CCD approach is most satisfying since equilibration may be assured by control of shaking time and that CCD is not subject to other phenomenological uncertainties, such as diffusion and other rate dependent phenomena, that may occur in continuous systems.

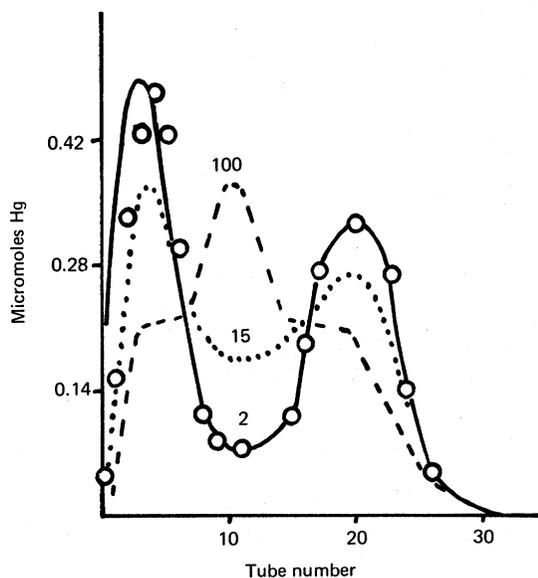


Fig. 12.33. Micromoles of mercury per tube plotted vs. tube number. Circles represent experimental points; curves represent theoretical calculations. Dashed line represents expected curve for a formation constant of 100. Dotted line represents expected curve for formation constant of 15. Solid line fits data best with formation constant of 2 (49,79).

### III. COUNTER-DOUBLE-CURRENT PROCESSES

#### A. IDEAL DISTRIBUTION WITHIN EXTRACTION TRAIN

Up to this point, the distribution scheme where only one phase is transferred has been described. However, the approach where each phase is transferred in opposing directions is also a useful separation tool. Although this is true countercurrent flow and a discrete stagewise analog of continuous extractors with extraction and stripping sections, Craig (66) named the approach "counter-double-current distribution" (CDCD) when he described an automated glass apparatus for carrying out the scheme, thereby differentiating it from CCD. The CDCD scheme is diagrammed in Fig. 12.34. If most solute remains inside the distributor and is introduced at the center tube, the distribution profile has the form of the binomial, although the abscissa is doubled. In reality, alternate tubes are not empty but contain solute because of imperfect transfer effects.

#### B. ELUTION FROM TRAIN

Although sundry mathematical approaches have been applied, no simple, closed-form expressions were developed that adequately describe the CDCD process once some solute has emerged. For example, Stene (86) found that three expressions were needed to describe the distribution of solute inside the distributor:

$$F'_{n,0} = q(F'_{n-1,r+1}) \quad (57)$$

$$F'_{n,N} = p(F'_{n-1,r-1}) \quad (58)$$

for the end tubes and

$$F'_{n,r} = \frac{n! [p]^{[(n+r)/2]} [(q)]^{[(n-r)/2]}}{[(n+r)/2]! [(n-r)/2]!} \quad (59)$$

for the other tubes. The fraction in both outputs at the  $n$ th transfer is computed by calculating the fraction in the lower phase of tube 0 and that in the upper phase of the highest-numbered tube at the  $(n-1)$ th transfer. The complexity of other expressions developed for output profiles is exemplified by the following:

$$F = \frac{2^\alpha}{N+1} p \exp\left(\frac{\alpha+r_e}{2}\right) q \exp\left(\frac{\alpha-r_e}{2}\right) \sum_{\gamma=1}^N \cos^{n-1} \cdot \frac{\pi-\gamma}{N+1} \sin \frac{\pi\gamma}{N+1} \sin \frac{\pi\gamma r_e}{N+1} \quad (60)$$

where  $r_e$  is the feed tube,  $m$  is the upper-phase product number,  $\alpha = r_e + 2(m-1)$ , and  $\gamma$  is the summation index (20). The use of computer simulation, however, conveniently enables useful insights to be gained.

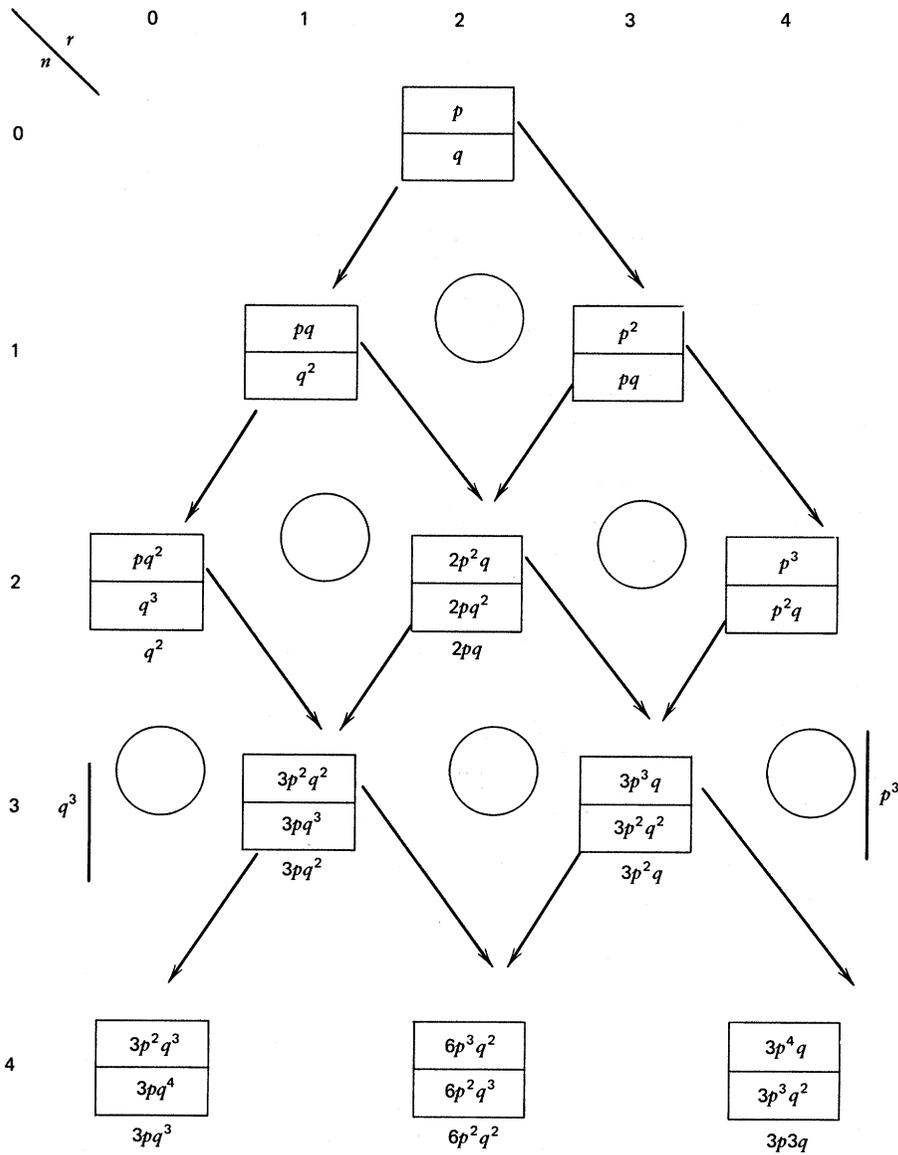


Fig. 12.34. Schematic of CDCD process for a single input of solute. Rectangles indicate fraction of solute in each phase in tube  $r$  at equilibrium after  $n$  transfers. Arrows denote the direction of transfer of the upper and lower phases. On alternate transfers, some tubes contain no solute. The totals in some tubes are shown underneath the rectangles. Upper and lower phases each containing solute emerged on the third transfer.

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### C. OPTIMIZATION

#### 1. Number of Stages, Solvent Ratio, and Feed Tube Position

Characteristics of CDCD are illustrated in Fig. 12.35. In this center-loaded case, solutes with  $K$  near 1 remained near the feed tube while solutes with lower  $K$  elute in the lower phase and those with higher  $K$  elute in the upper. A calculated CCD profile for the same solutes, transfer number, and number of tubes is also shown in Fig. 12.35 for comparison.

As in CCD, the degree of separation is related to the number of transfers. Shifting the feed tube off center increases the purity of one of the components at the expense of the others, while the yield of that component decreases. Increasing the number of tubes increases the resolution of all components. In contrast to CCD, decreasing the solvent ratio (volume of upper phase/volume of lower phase) does not increase resolution of all components because doing so, in effect, lessens the number of transfers to elute the solutes with  $K > 1$ . Here,  $K$  is defined as the concentration of solute in the

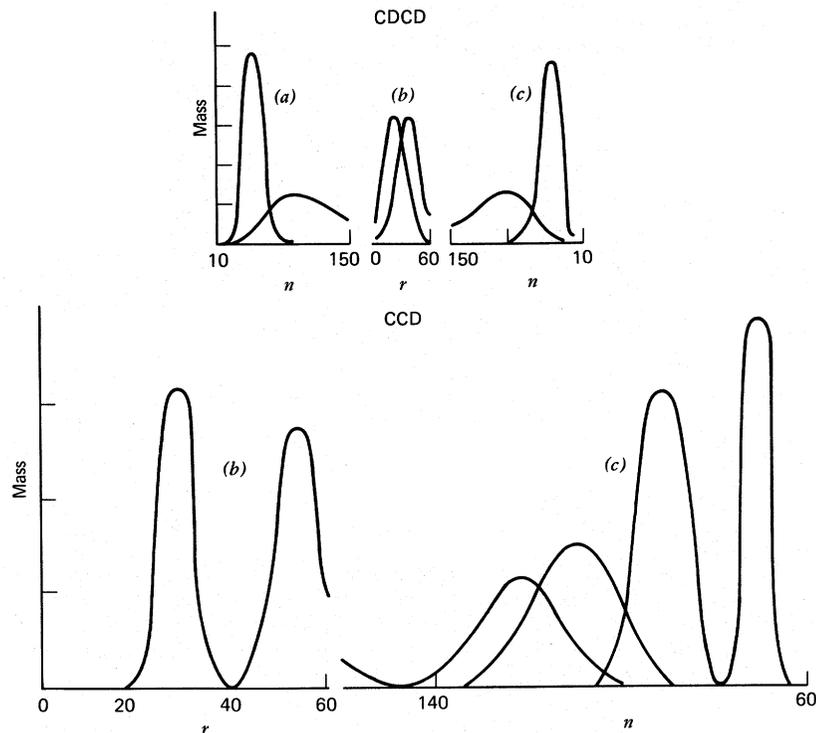


Fig. 12.35. CDCD and CCD profiles calculated using same number of tubes and transfers in each case;  $N = 58$ ,  $n = 150$ ,  $s = 1$ ,  $K_1 = 0.22$ ,  $K_2 = 0.58$ ,  $K_3 = 0.91$ ,  $K_4 = 1.10$ ,  $K_6 = 4.29$ . (a) Lower-phase effluent; (b) inside distributor; (c) upper-phase effluent (87).

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lower phase divided by its concentration in the upper phase at equilibrium. To purify highly a single component, selecting a solvent ratio such that  $K/s \sim 1$  produces the best result since that solute would essentially remain in the distributor and solutes with higher or lower  $K$  would be eluted.

### 2. Transfer Programming

An alternative to increasing  $n$  or changing the feed tube position is varying the ratio of the number of upper-to-lower phase transfers. An automatic device for carrying out such distributions has been described (3). The purification of alanine acceptor S-RNA is shown in Fig. 12.36. Elution CCD was applied for 230 transfers, at which point only solutes with high  $K$  remained in the distributor. After applying the appropriate transfer programs,

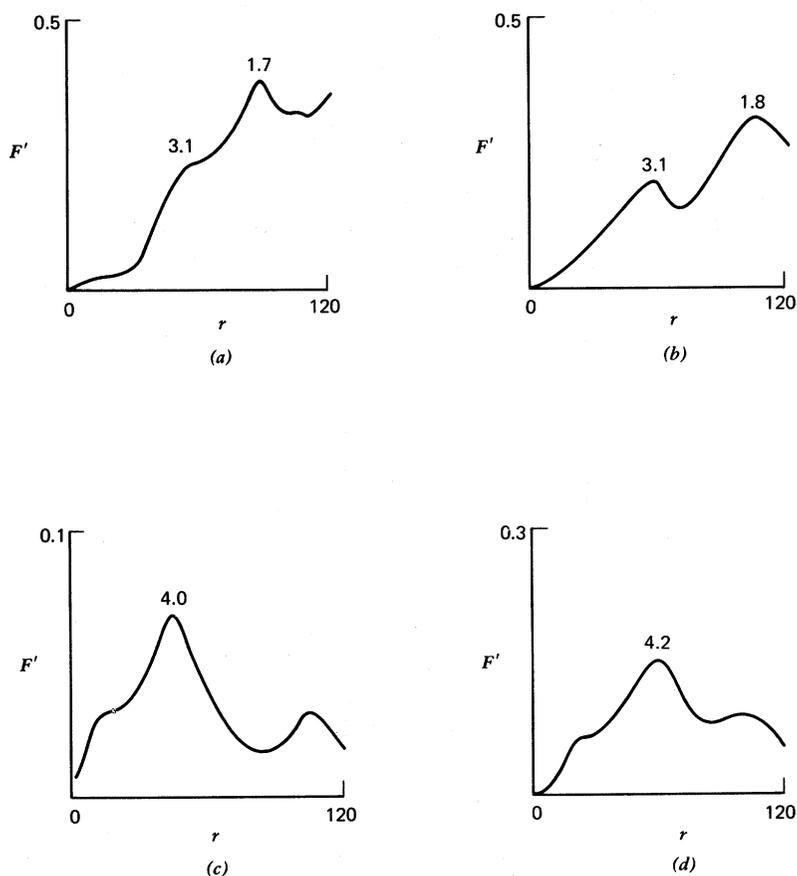


Fig. 12.36. Purification of alanine acceptor s-RNA ( $K = 4.2$ ) using transfer programming;  $N = 120$ . (a) Profile after 230  $n$  as in CCD; (b) profile after application of 380 upper-phase transfers and 50 lower-phase transfers; (c) 761 upper- and 139 lower-phase transfers; (d) 837 upper- and 139 lower-phase transfers (70). The numbers above profiles are  $K$  values.

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unwanted materials with  $K$  values lower and higher than the desired were removed. Program modifications were made to compensate for variable  $K$  of the product. A second and possibly a third alanine acceptor were shown to be present.

## D. MULTIPLE INPUTS

Transfer programming is also useful for isolating a minor component of a mixture, because the ratio of upper-to-lower phase transfers may be chosen so that the desired solute stays at the feed tube. If a new increment of solute is fed at every cycle, buildup of this component will occur as shown in Fig. 12.37. When sufficient solute level is attained, feed is stopped and the program could be modified to achieve further purification.

If a very large number of solute inputs are made, a steady-state condition is reached at which the weight of each component in the effluents equals the weight in the feed. This mode of operation, which permits large amounts of a solute to be isolated in a continuous fashion, is probably the most widely used CDCD method. Purification by steady state CDCD is limited to either the highest or lowest  $K$  solute in a multicomponent mixture. In a typical application, 2.25 grams of methyl linolenate of 99.9% purity was produced per hour from linseed oil esters (17) using a system in which the solutes were automatically recovered and the solvents continuously recycled.

The closer  $K/s$  is to 1, the greater the number of solute inputs required to reach steady state. If  $K/s < 1$ , the steady-state concentration will be greatest

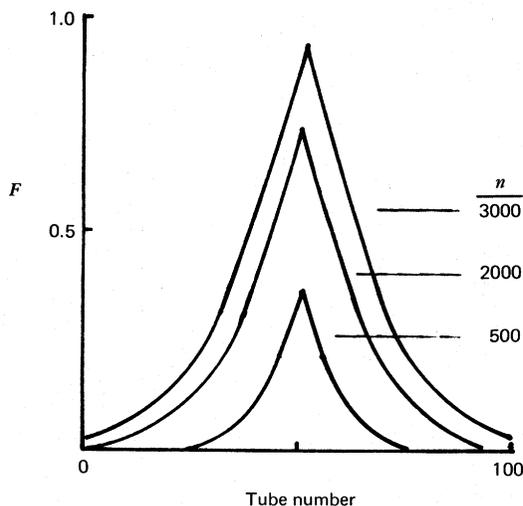


Fig. 12.37 Concentration of a minor component using transfer programming to keep it near center of distributor while the other components were eluted. The ratio of lower- to upper  $n$ -phase transfers is 2;  $K = 0.5$ . One unit was fed per transfer ( $n$ ) (71).

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in the upper phase; if  $K/s > 1$ , the concentration will be greatest in the lower phase. Increasing the train length also increases the number of transfers to reach steady state. Number of transfers to achieve steady state can be reduced by adding much greater amounts of solute for the first several inputs than will be fed subsequently. Caution must be exercised, however, so that effects such as phase inversion or phase coalescence do not occur.

It was shown (91) that when  $K$  is constant and no solvent is added with the solutes, one solvent ratio enables a separation to be achieved with the minimum number of tubes. In Fig. 12.38, solvent ratio is plotted against number of tubes for a system in which it is desired to obtain a solute at a specified purity and percent recovery. The number of tubes to the right and left of the feed tube required to fulfill this requirement with different solvent ratios was calculated and graphed. The summation plot shows the optimum solvent ratio. When the fraction of solute 1 in the upper-phase output equals the fraction of solute 2 in the lower phase and a center feed is used,  $s = (K_1 K_2)^{0.5}$ . The use of a computer facilitates such calculations (18,29) and allows greater flexibility in calculating separation parameters, as was the case with other types of liquid-liquid extraction that have been discussed.

Another variable is the position of the feed tube. Figure 12.39 shows the condition of a distributor at steady state. Shifting the feed tube to lower tube numbers would decrease purity of the desired solute, while moving it toward higher tubes would decrease yield (17). Transfer ratio variation has also been evaluated in steady-state extraction (28).

As in CCD, nonideal effects influence CDCD separations. Volume reorganizations, variable  $K$ , and so on, alter the purity of products and the number of transfers needed to reach steady state, but their impact has not been studied in great detail as in CCD. However, just as CCD is a model for chromatography, CDCD is a stagewise model for continuous liquid-liquid extraction (64). For further reading in this area, the reader is referred to an excellent critical review by Pollock (65).

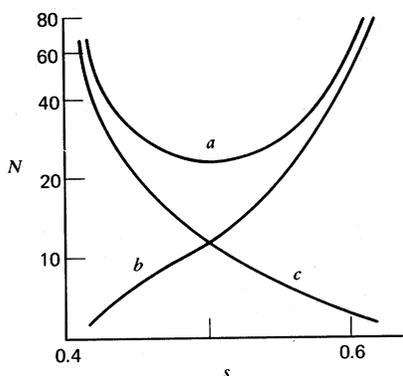


Fig. 12.38. Effect of solvent ratios ( $S$ ) on number of tubes ( $N$ ) required for a given separation by CDCD (91). (a) Total number of tubes; (b) number to right of feed tube; (c) number to left.

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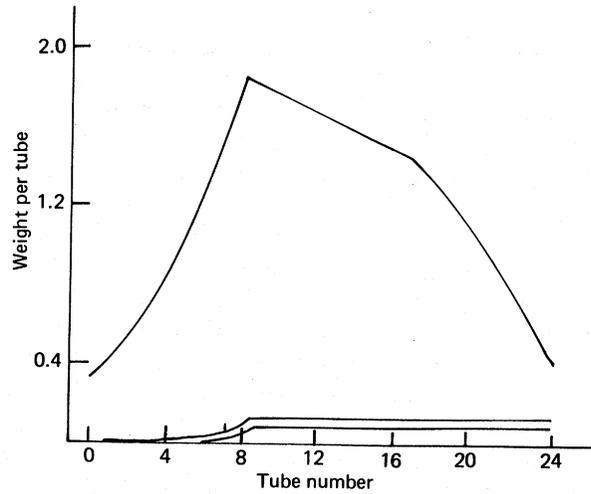


Fig. 12.39. CDCD at steady-state condition. The weights of three components in each tube are shown. The pure component is withdrawn at tube 0.

## IV. SPECIALIZED APPROACHES

### A. LIQUID INTERFACE DISTRIBUTION

When large particles such as blood cells, viruses, and large proteins are dispersed in a two-phase system, their distribution is related to the surface properties of the system. Consider the particle adsorbed at the interface as demonstrated in Fig. 12.40. If the particle is considered to be spherical, its potential energy caused by interfacial forces is (2)

$$G^s = [(4\pi R^2 - 2\pi R h)\gamma_L] + 2\pi R h \gamma_U - \pi r^2 \gamma_{UL} \quad (61)$$

where the surface free energy ( $G^s$ ) is given by the product of the contact areas and the various interfacial tensions ( $\gamma_U$ ,  $\gamma_L$ ,  $\gamma_{UL}$ ) between the particle and each of the phases or the liquid-liquid interface.

Then, after substituting for  $r^2$ , which from trigonometry equals  $h(2R - h)$ , and evaluating the derivative, it is demonstrated that  $G^s$  is a minimum when

$$h = R \left( 1 - \frac{\gamma_U - \gamma_L}{\gamma_{UL}} \right)$$

if  $|(\gamma_U - \gamma_L)/\gamma_{UL}| < 1$ . When this quantity is equal to or greater than 1, the particle has its lowest energy in one of the phases. For a number of similar particles, according to the theory of Brownian motion,

$$K = \exp \left[ \frac{4\pi R^2 (\gamma_U - \gamma_L)}{kT} \right] = \frac{C_L}{C_U} \quad (62)$$

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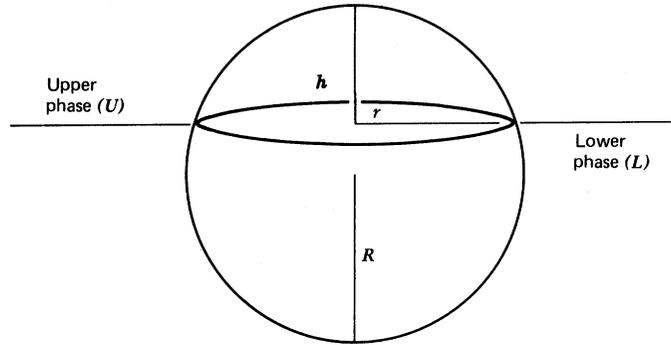


Fig. 12.40. Particle suspended at interface between two immiscible phases.  $R$  is radius of spherical particle;  $r$  is radius of particle cross section at interface;  $h$  is distance the particle penetrates into the upper phase.

or when interfacial adsorption occurs,

$$K_{\gamma} = \frac{\text{number of particles per cm}^3 \text{ at interface}}{\text{concentration in upper phase}} = \left[ \frac{-\pi R^2(\gamma_U - \gamma_L - \gamma_{UL})^2}{\gamma_{UL}kT} \right] \quad (63)$$

In real distributions, it is impossible to determine particle interfacial area because it would be dependent on their dispersion. In such distributions, the solvent ratio is adjusted so that the phase interface is below the cutoff point of the cell. The two-phase flow expressions derived earlier for CCD can then be employed to describe the process. In this case,  $p''$  would be the particle fraction transferred and  $q''$  the fraction retained. If the particles are charged, the Donnan effect must also be considered (2).

Albertsson has studied a number of phase systems for particle separation. The separation of a mixture of microorganisms is shown in Fig. 12.41. Because of the nature of the systems, the phases are formed into thin layers to facilitate settling. Suitable automated devices are commercially available.

### B. COUNTERCURRENT CHROMATOGRAPHY

Countercurrent chromatography is an ingenious semicontinuous form of CCD in which two liquids contact one another in a long tube of small ( $< 1$  mm) diameter (Fig. 12.42). Droplets or regions of the stationary phase are retained in the coiled tubing, and mobile phase is pumped through the tubing and out through a detector and to fraction collectors if desired. In order to maintain gravitational stability of the fluids, the tube is rotated along its long axis as well as in another plane. The small droplets provide the equivalent of many equilibrium stages in a relatively small space. A separation and the conditions are given in Fig. 12.43.

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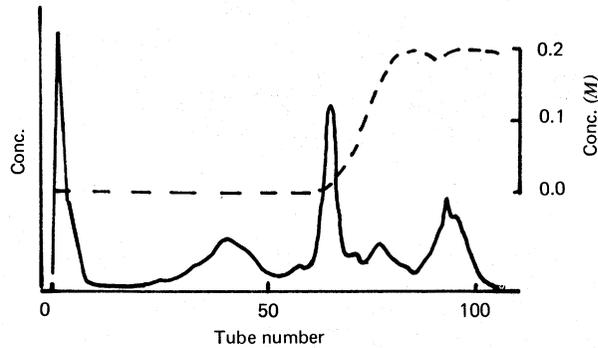


Fig. 12.41. Separation of mixture of microorganisms by liquid-interface countercurrent distribution (solid curve). Peaks represent from left: yeast cells; *Escherichia coli* K12, W1177; *E. coli* K12, 58; *Chlorella pyrenoidosa* (two small peaks); and *E. coli* ML2081 (2). Dashed curve indicates NaCl gradient with concentrations listed at the right.

Two devices of somewhat different configurations have been developed: the flow-through coil planet centrifuge and the elution centrifuge. Some results are compared in Table 12.VI. In both devices, there are two critical criteria: (a) an adequate amount of stationary phase must be maintained in the tube (column), and (b) segmentation of the stationary phase must occur to provide a large number of partition units with high interfacial area. Some of the variables that may be manipulated to produce the criteria are: (1) column configuration, (2) inside tube diameter, (3) revolutionary speeds, and (4) flow rate of mobile phase. Solvent properties, such as density, viscosity,

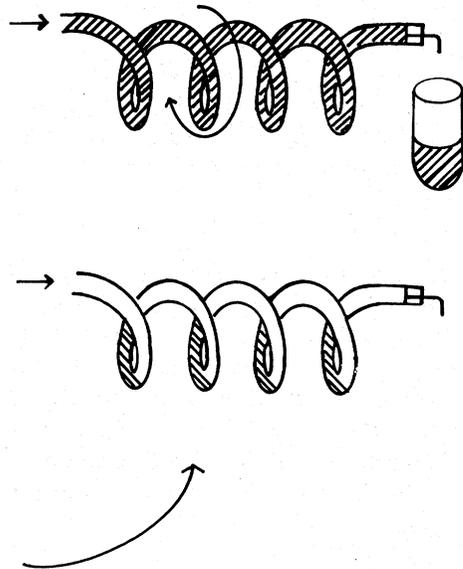


Fig. 12.42. Schematic diagram of elution centrifuge. In top portion of the figure, the coiled tube is filled (horizontal arrow) with stationary phase (lined region) and rotated about its long axis. The lower part of the figure depicts continued rotation and then the tube is spun through the plane of the paper. Mobile phase (clear) region is introduced (horizontal arrow), and eventually stationary phase is held in part of the loop. Mobile phase flows around the drops of stationary phase and out the end at right. Partition between the many increments of mobile and stationary phase provides the separation efficiency (48).

TABLE 12. VI  
 Comparison of Results for Two Centrifuges Used for Countercurrent Chromatography<sup>a</sup>

Type	Length (m)	Tube diameter (mm)	Time (hr)	Plate number range	Time per transfer equivalent (sec)
Coil planet centrifuge					
Analytical	100	0.30	10	3000-10000	~ 5
Preparative	100	1.4	7	500-2000	~ 25
Elution centrifuge	60	0.2	26	2500-6000	~ 25

<sup>a</sup> Time for elution of the DNP amino acids as in Fig. 12.43.

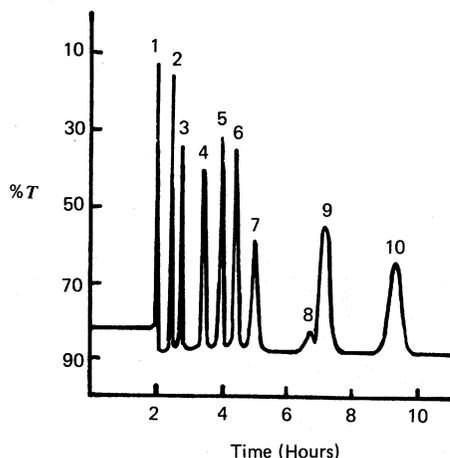


Fig. 12.43. Separation of N-DNP-amino acids by analytical coiled planet centrifuge (100 by 0.30 mm i.d. Teflon tubing). Chloroform/acetic acid/0.1 N HCl (2/2/1, v/v). Compounds: (1) N-DNP-L-ornithine; (2) N-DNP-L-aspartic acid; (3) N-DNP-L-glutamic acid; (4) *N,N'*-di-DNP-L-cystine; (5) N-DNP- $\beta$ -alanine; (6) N-DNP-L-alanine; (7) N-DNP-L-proline; (8) unknown; (9) N-DNP-L-valine; (10) N-DNP-L-leucine (48).

and interfacial tensions, will influence the requirements for the variables. It appears that on a per-transfer basis, the system is more rapid than ordinary CCD, no solid support phase is required as in liquid chromatography, and small sample sizes may be used (48). The technique is apparently applicable to the separation of particulates as in liquid-interfacial distribution. Emulsification, however, must be avoided, since it results in transport of both phases out of the instrument. This new development appears to be a useful extension of CCD.

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