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THE USE OF THE COMPUTER IN TEACHING THE THEORY OF CHROMATOGRAPHY

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SUMMARY

The problem of teaching the principles of chromatography to undergraduate students is greatly simplified by the use of the computer. The programs discussed take real or imaginary data and calculate how the system should develop. The effect of variation of chromatographic parameters is readily tested. Copies of the programs written in Fortran IV are available from the author.

When training students, how far should one go beyond teaching the mechanics of some actual separations? Without some understanding of chromatographic principles, each separation becomes a magical rite, impossible to perform unless one has been given the exact recipe. Yet, to most students in undergraduate laboratory classes, a thorough presentation of the theory of chromatography would prove formidable and rather useless. It is desirable for these students to have answers to three basic questions about chromatography:

(A) Why do solutes move at different rates in a chromatographic system?

(B) How does a band of solute, initially very narrow, distribute during development?

(C) What factors govern degree of separation of two bands?

The first question can be answered easily and in a variety of ways. However, it is convenient to prepare for answering B and C by discussing A in terms of countercurrent distribution (CCD). The validity of this time-honored approach to the theory of chromatography is well established[1]. Starting with two immiscible liquids in a separatory funnel, these represent the two phases necessary for any chromatographic system; one may add a solute, shake, and measure the concentration of solute in each phase at equilibrium. The ratio of these concentrations is the partition coefficient (K) and the ratio of the amounts of solute in each phase is the distribution ratio (R).

$$K = \frac{\text{concn. in phase } m}{\text{concn. in phase } s} \tag{1}$$

$$R = \frac{\text{amount in phase } m}{\text{amount in phase } s} = K\frac{V_m}{V_s} \tag{2}$$

Where V_m = volume of phase m; V_s = volume of phase s.

Students then recognize that for any solvent system different solutes will have different partition coefficients. Next the concept that one phase moves while the other remains stationary must be introduced. This can be illustrated by a row of separatory funnels each containing the same amount of phase *s*. Phase *m* is then transferred from the first funnel (tube 0) to the second (tube 1), and a new phase *m* is added to the first funnel. Such an operation, called a transfer, is analogous to one theoretical plate in more conventional chromatography. A continuous repetition of this process shifts phase *m* along the row of funnels. The solute is carried along at a lesser rate and at the same time spreads into an increasing number of funnels. The solute becomes distributed in decreasing concentrations before and behind the funnel of maximum concentration. This is typical of chromatographic separations.

The student must appreciate the following points: (1) Phase *m* is a moving phase, while phase *s* is a stationary phase. (2) At each transfer some portion of the solute is moved to higher numbered tubes. (3) The higher the partition coefficient, *i.e.* the greater the relative solubility in moving phase, the larger the portion of solute moving at each transfer will be. (4) A plot of concentration of solute *versus* tube number produces a peaked curve tapering out from a maximum point smoothly in both directions. Thus, in conventional chromatography solute moves as a band because, just as with CCD, equilibration between moving and stationary phase is rapid relative to the movement of the phases. Solute moves at a fraction of the rate of the moving phase, this fraction increasing with increasing K.

The advantage of using the CCD analogy is clear when attempting to answer questions B and C. CCD lends itself readily to mathematical analysis producing equations which are useful in teaching chromatographic principles. In the simplest sense it is possible to have even mathematically unsophisticated students take partition coefficients such as 1 or 9, assume unit volumes of both stationary and moving phase, and calculate the transfer of one gram through several transfers (Table I). However, to deal with realistic problems, especially to predict the behavior of a real solute whose partition coefficient has been measured, requires the use of more sophisticated calculations. Using the distribution ratio (Eqn. 2), the proportion of

TABLE I

HAND CALCULATION OF THE DISTRIBUTION OF A COMPOUND WITH $K = 9$ BY CCD

It is assumed that there is 1 g of solute and that equal volumes of each phase are present. The calculation is simply to transfer 90% of the solute of each tube, one tube ahead at each transfer, and to leave 10% behind. This is shown clearly in the first transfer. In tube 1 after the second transfer the 0.180 g arise from 0.090 g being transferred in from tube 0 and 0.090 g being left behind when 90% of tube 1 transfers to tube 2. Similary $K = 1$ provides an easy hand calculation since half the contents of each tube moves and half remains behind.

Transfer number	*Tube number*							
	0	1	2	3	4	5	6	7
0	1.0							
1	0.1	0.9						
2	0.01	0.180	0.810					
3	0.001	0.027	0.243	0.729				
4	0.0001	0.0036	0.0486	0.2916	0.6561			
5	—	0.0005	0.0081	0.0729	0.3281	0.5905		
6	—	0.0001	0.0012	0.0146	0.0984	0.3543	0.5314	
7	—	—	0.0002	0.0026	0.0230	0.1240	0.3720	0.4783

solute in moving phase a and in the stationary phase b can be calculated:

$$a = \frac{R}{R + 1} \tag{3}$$

$$b = \frac{1}{R + 1} = 1 - a \tag{4}$$

The amount of compound $C_{n,x}$ in a specific tube X (distance in theoretical plates along the column in conventional chromatography) after n transfers (total theoretical plates in conventional chromatography) is calculated by the Pascal distribution formula:

$$C_{n,x} = \frac{n! a^x b^{(n-x)}}{x!(n - x)!} \tag{5}$$

This calculation has been applied not only to CCD[2] but also to partition column[3] and ion-exchange[4] chromatography. In order for students to gain a feeling for the effect of changing the partition coefficient and varying the relative volumes of the phases for various numbers of transfers a prohibitive number of calculations would be required. Since it is the result and not the calculation that is important, a computer program has been prepared allowing the student to select and test these variables rapidly and easily. The output of the program is shown in Table II.

Question C concerns the separation of two solutes in a chromatographic system. The variables are the partition coefficients of the two components (K_A, K_B), the volumes of stationary and moving phases (V_s, V_m), and the number of transfers n. If the distribution of solute concentration in a developing band is considered to be well represented by a Gaussian curve, standard statistical approaches may then be applied. In practice the actual distributions may not conform to this assumption, but the use of the assumption is instructionally useful.

It is possible, given distribution coefficients and volumes of the two phases, to calculate the number of transfers or theoretical plates required to resolve two components. One must include also a criterion of what is meant by the resolution *i.e.* how much band-overlap is acceptable. Fig. 1 illustrates the percentage of total area under various portions of the curve, the X-distance being expressed in terms of standard deviations σ. If it is sufficient to collect a rich cut which contains 84% of the desired component and can contain 16% of the other peak as an impurity, it is necessary only to have the intersection (point of maximum separation) of the two peaks occur at a distance of one σ from the maximum of each peak. However, if it is desired to have 99.98% of one component and only 0.02% of the other in the "rich cut" it is necessary to have the separation occur 3.5 σ from each maximum. When discussing the number of transfers or theoretical plates required to separate two peaks it is necessary to indicate the degree of resolution, here expressed as the number of standard deviations from peak to intersection, that is expected (see Table III).

The number of transfers (theoretical plates) required to achieve a desired degree of resolution can be calculated by eqn. 6.

$$n = \sigma_{ov} \left[\frac{R_2^{\frac{1}{2}}(R_1 + 1) + R_1^{\frac{1}{2}}(R_2 + 1)}{R_2 - R_1}\right]^2 \tag{6}$$

Where σ_{ov} = number of standard deviations from center of the peak to the center of band-overlap. R_1,R_2 = The distribution coefficients of components 1 and 2.

TABLE II

DISTRIBUTION THROUGH FOURTEEN TRANSFERS OF 1 g OF A COMPOUND BETWEEN 200.0 ml MOVING PHASE AND 100.0 ml STATIONARY PHASE WHEN THE PARTITION COEFFICIENT IS 7.35 (SOL. MOBILE PHASE/SOL. STATIONARY PHASE)

This is the output of a program predicting the distribution of a solute through several transfers in a CCD system when the distribution coefficient and volumes of both phases are known. Of interest here to the student is not only the distribution of solute after the last transfer, but also the shifting distribution in the intermediate stages. The effect of varying the paramaters on the final distribution can be easily observed by use of such a program.

Number of transfers	*Contents of each tube after each transfer*														
	0	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*
0	1.000														
1	0.063	0.936													
2	0.004	0.119	0.876												
3	0.000	0.011	0.167	0.820											
4	0.000	0.000	0.021	0.209	0.768										
5	0.000	0.000	0.002	0.033	0.244	0.719									
6	0.000	0.000	0.000	0.004	0.046	0.275	0.673								
7	0.000	0.000	0.000	0.000	0.006	0.061	0.300	0.630							
8	0.000	0.000	0.000	0.000	0.000	0.010	0.076	0.321	0.590						
9	0.000	0.000	0.000	0.000	0.000	0.001	0.014	0.092	0.338	0.553					
10	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.019	0.107	0.352	0.517				
11	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.025	0.123	0.362	0.484			
12	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.031	0.138	0.370	0.453		
13	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.038	0.153	0.375	0.425	
14	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.008	0.045	0.167	0.379	0.397

The distribution coefficient is 14.70000, the proportion of molecules in the moving phase is 0.93, and the proportion in the stationary phase is 0.06.

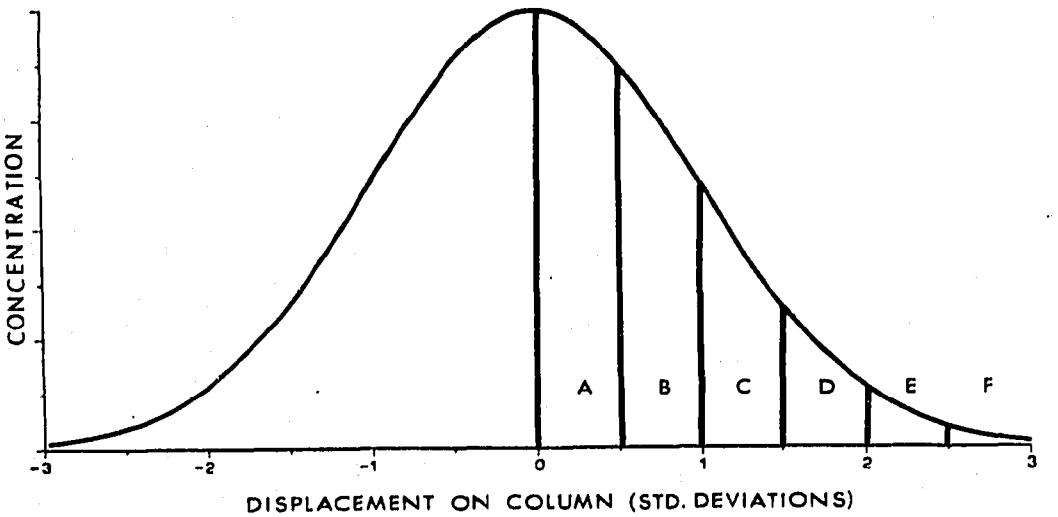

Fig. 1. The Gaussian curve.

TABLE III

THE PERCENTAGE OF THE TOTAL AREA UNDER EACH PORTION OF THE CURVE

Deviation from peak	*Percentage of area*	*Designation on figure*
0.0–0.5	19.15	A
0.5–1.0	14.98	B
1.0–1.5	9.19	C
1.5–2.0	4.41	D
2.0–2.5	1.65	E
2.5–3.0	0.49	F
3.0–3.5	0.11	—
3.5–4.0	0.02	—

TABLE IV

PREDICTION OF THE NUMBER OF TRANSFERS (THEORETICAL PLATES) REQUIRED TO ACHIEVE THE DEGREE OF SEPARATION INFERRED IN THE SECOND COLUMN "AREA IN RICH CUT" GIVEN THE PARTITION COEFFICIENTS FOR BOTH COMPONENTS IN THE SOLVENT SYSTEM AND THE VOLUMES OF BOTH PHASES

The distribution coefficients and β value are also presented. Programs are available from the author.

σ	*Area in rich cut*	*No. transfers*
0.5	69.15	6.7
1.0	84.13	27.1
1.5	93.32	61.0
2.0	97.73	108.4
2.5	99.38	169.4
3.0	99.87	244.0
3.5	99.98	332.1
4.0	99.99	433.8

The partition coefficient A = 2.20, the partition coefficient B = 7.35, the volume mobile phase = 200.00, the volume stationary phase = 100.00, the distribution coefficient A = 4.40, the distribution coefficient B = 14.70, β = 3.34.

Once again the calculation is awkward, particularly if realistic values are used. A computer program has been prepared for this calculation which allows students to supply partition coefficients and phase volumes and learn the number of transfers required to achieve various degrees of separation. The output is shown in Table IV.

It is worthwhile to establish the validity of calculations by a simple experiment. The distribution of bromcresol purple between a lower phase of 2% KCl in aqueous HCl (pH 1.9) and an upper phase of *n*-butanol–ligroin (35:65) is convenient because the distribution of the dye can be followed visually and can be conveniently assayed in a colorimeter. Prepare the solvents beforehand and store them together to allow each layer to saturate the other. Prepare a 0.005% solution of bromcresol purple in lower phase. Have students establish the absorptivity and the validity of Beer's law for dilutions of the dye in the lower phase. Read in a spectrophotometer at 430 mμ or in a filter photometer with an appropriate filter. Then in a separatory funnel place measured volumes of the upper and lower phase, add a measured amount of dye solution, and shake thoroughly. Withdraw some lower phase, read its absorbance in the spectrophotometer, and from the Beer's law curve calculate the concentration in the lower phase. Calculate the total amount of dye in the lower phase and substract this from the total amount added to give the amount in the upper phase. Then from these values and the known volumes of upper and lower phases added, calculate the partition coefficient (Eqn. 7). If the distribution is to be run in a Craig machine assume upper phase to be moving phase, while if separatory funnels are to be used assume lower phase to be the moving phase.

$$K = \frac{\text{(amount in moving phase) (volume of stationary phase)}}{\text{(amount in stationary phase) (volume of moving phase)}} \qquad (7)$$

The value obtained can vary with the exact composition of the solvents, but should be reasonably close to 1.

Using the computer program calculate the distribution of dye through fourteen transfers given the K determined and the volumes of stationary and moving phase to be used.

Finally, perform the distribution. If a Craig machine is not available, use either fifteen separatory funnels or fifteen glass or teflon stoppered burets. Without a Craig machine it is necessary to shake each vessel individually, and to transfer lower phase beginning with the highest numbered tube and proceeding back to tube zero. After the last transfer, shake all the vessels and remove the lower phase for assay in the spectrophotometer. Knowing the concentration in the lower phase and the distribution ratio (program output) the concentration in the upper phase can be calculated. From this the total amount of dye in each vessel is determined and this is plotted against the predicted values of the computer print out.

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