

LETTERS TO THE EDITORS

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Analysis of Counter-Current Distribution Curves

THE increasing use of counter-current distribution between solvents, in machines of the kind developed by Craig¹, for the purification of natural products prompts us to direct attention to simple relationships which exist between successive fractions withdrawn from such machines but which do not appear to be generally appreciated.

After the 'fundamental procedure'² under ideal conditions, the fractions of a single substance in successive tubes of the machine are given by the terms of a binomial expansion³⁻⁶. If K is the partition coefficient $\left[= \frac{C, \text{ upper phase}}{C, \text{ lower phase}} \right]$ of a substance distributed in a machine having a series of tubes numbered from 0 to R , and if the upper phase is mobile, the fraction of the total material $T_{n,r}$ in tube r after n transfers is given by:

$$T_{n,r} = \frac{n!}{r!(n-r)!} \left[\frac{1}{K+1} \right]^n K^r, \quad (1)$$

whence $T_r/T_{r-1} = FK$, (2)

where $F = (n+1-r)/r$. When the mobile solvent has reached the last tube of the machine, $n = R$.

After the procedure called 'single withdrawal'², the distribution of a single substance in the withdrawn series has been described by the following continuous function²:

$$y = \frac{1}{\sqrt{2\pi n/k}} \exp \frac{x^2}{2n/k}, \quad (3)$$

where y is the fraction in a sample that is a distance x from the maximum and n the number of transfers at the maximum. This equation is convenient to use, but it is an approximation which becomes nearly exact only when n is large. Moreover, a value for K must be deduced, by a formula which is itself only approximate, from the position of the maximum, and the latter does not necessarily correspond to the purest material when resolution is incomplete. An exact relationship between successive fractions, however, may easily be derived⁷. If withdrawal of the upper phase takes place from tube R , and if T'_n is the fraction of the total material in the sample withdrawn during the n^{th} transfer, then T'_n is equal to fraction in tube R after $(n-1)$ transfers multiplied by $K/(K+1)$.

Whence from equation (1)

$$T'_n/T'_{n-1} = F_w/(K+1), \quad (4)$$

where $F_w = n-1/(n-R-1)$.

Similarly, if withdrawal of the lower phase begins from tube 0, the fraction withdrawn during the n^{th} transfer is T''_n , which is equal to [fraction in tube $(n-R-1)$ after n transfers] - [fraction in tube $(n-R-2)$ after $n-1$ transfers] $\times K/(K+1)$;

whence $T''_n/T''_{n-1} = F_w K/(K+1)$, (5)

where $F_w = n-1/(n-R-1)$.

Equation (5) also follows directly from (4) if the lower phase is imagined as mobile, so that K is replaced by $1/K$.

Equations (4) and (5) enable values of K to be calculated over any portion of the curve derived from the fractions withdrawn, and the constancy of these values provides a criterion of the purity of the material in the samples concerned. For example, application of the equations to a curve given for fractions of crystalline neamine withdrawn from a 41-tube machine⁸ indicates that, if the distributions were carried out under ideal conditions, the material concerned was not homogeneous. The experimental curve can be fitted closely to a theoretical curve calculated for a mixture containing 62 per cent of a substance with $K = 1.79$, 35 per cent of a substance with $K = 1.18$ and possibly 3-4 per cent of a substance with $K > 2$.

In the course of an extensive investigation of the theory of systematic extraction, Stene⁴ showed that the fractions obtained in a process equivalent to 'single withdrawal' formed a Pascal distribution. Equations (4) and (5) are also readily derived from successive terms of this distribution. More recently, Karlson and Hecker⁹ have treated the problem in a similar fashion and obtained essentially the same solution. They have also solved the more difficult problem of the relation between successive fractions in the procedure known as 'alternate withdrawal'².

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⁴ Stene, S., *Arkiv Kemi. Min. Geol.*, **A**, **18**, No. 18 (1944).

⁵ Williamson, B., and Craig, L. C., *J. Biol. Chem.*, **168**, 687 (1947).

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Relationship of Electron Distribution in Amines to Ammine Stability

H. C. Longuet-Higgins and C. A. Coulson¹ calculated the π -electron distribution in certain heterocyclic molecules by the method of molecular orbitals. It was thought that it would be of considerable interest to measure the formation constants of the silver complexes formed with some of these amines. The relative formation constants of the complex silver ions formed by acridine, quinoline, isoquinoline and pyridine were measured at 25°C. in 59 weight per cent ethanol-water solution. The formation constants express the equilibrium:



$$K_f = \frac{(\text{Ag Amine}_2)^+}{(\text{Ag}^+) (\text{Amine})^2},$$

where (Ag^+) , etc., represent the activities of the species.

In nearly all such measurements, either the metal ion concentration is determined by the use of a