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Paper Chromatography of Alkaloids Using Liquid Ion Exchangers as Developing Solvents

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Summary

The R_F vs. pH relationships of a number of more important alkaloids were determined for several solvent systems of the type oleic acid + diluting solvent/aqueous buffer solutions. The chromatographic data seem to indicate a high solvent power of the organic phase, belonging to the class of liquid ion exchangers. The regular R_F vs. pH relationships obtained can be quantitatively interpreted both by physical and by ion-exchange partition; the latter mechanism seems to be indicated by the exceptionally high extraction affinity of the organic phase (much higher, for instance, than for chloroform), difficult to explain by ordinary physical interactions. A marked effect of the diluting solvent on the solvent power and selectivity was found. In view of a certain parallelity of static (batch) extraction coefficients and chromatographic parameters, the latter can be employed for the estimation of suitable solvent systems for countercurrent separation of organic electrolytes on a preparative scale.

In the last decade, the increasing application of the so-called liquid ion exchangers has been observed; these solvents are lipophilic organic acids or bases, usually diluted with neutral solvents to decrease the viscosity and mutual solubility with aqueous solutions and to control the extraction coefficients. So far, liquid ion exchangers have been used almost exclusively for the extraction and chromatography of inorganic ions; reviews on these applica-

tions have been published by Moore (*1*), Coleman et al. (*2*), and Cerrai (*3,4*). A few publications in the extraction of organic electrolytes have been reviewed by Wolf and Käupke (*5*), who investigated the partition of phenols between water and some liquid ion exchangers. Several solvent systems of possible ion-exchange mechanism have been reported by Craig and Craig (*6*).

In the preceding paper (*7*) we have shown that paper impregnated with di(2-ethylhexyl)orthophosphoric acid (HDEHP, a liquid cationite) behaves like an ion-exchange paper, controlling the partition and chromatographic behavior of organic electrolytes by the acidity of the mobile aqueous phase; it has also been suggested that paper-chromatographic data can be employed for the approximate estimation of extraction coefficients and optimal solvent systems for the cascade separation of electrolytes on a preparative scale [cf. also (*3,4,8–11*)].

In the present work we have employed in analogous investigations another organic solvent possessing potential ion-exchange properties, namely, oleic acid diluted with some organic liquids. Oleic acid has been chosen in view of its numerous advantages, e.g., relatively low price, high chemical stability, low mutual solubility with water, and low vapor pressure. Applications of higher fatty acids in the extraction of metals have been reviewed elsewhere (*12*).

The partition system investigated (diluting solvent + oleic acid/aqueous buffer solution) shows certain analogies to the Partridge system (butanol + acetic acid + water, 4 : 1 : 5) employed extensively in the chromatography of alkaloids, amino acids, and other organic electrolytes. The distinct advantage of the former system is, however, that it is by its nature a two-phase system of low mutual solubility of the two phases; it is, therefore, suitable for application in countercurrent cascade processes on a preparative or even industrial scale, the optimal conditions being theoretically predictable from paper-chromatographic data.

The experiments had the following purposes.

1. Investigation of the effect of pH on the R_F and R_M values.
2. Investigation of the effect of the diluting solvent on the solvent power and selectivity of the organic liquid and comparison of liquid ion exchangers with neutral solvents.
3. Determination of batch-extraction coefficients in analogous systems and comparison of calculated per cent extraction with chromatographic data.

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EXPERIMENTAL

To obtain appropriate amounts of aqueous phase in the paper and comparable values of the volume ratio ($r = v_{org}/v_w$ in the chromatographic system), the "moist buffered paper" technique (*18*) was employed, the degree of impregnation (0.5 ml of buffer solution per 1 g of dry paper) being controlled by weighing. (A similar degree of impregnation could probably be obtained by prolonged conditioning of previously impregnated and dried strips in a water-saturated atmosphere.)

Whatman No. 4 paper was cut at right angles to the machine direction into strips 6 × 23.5 cm; the distance of development was 16 cm (in tanks 6 × 14 × 21 cm, descending flow). The strips were weighed, immersed in a suitable buffer solution, excess liquid removed by pressing between two sheets of filter paper, and dried in a horizontal position. When the moisture content dropped to ca. 0.6 ml/g, the solutions of the alkaloids were spotted (1 to 3 ml of chloroform or aqueous 0.1 to 0.5 w/v % solution). The strips were dried further until the impregnation degree dropped to 0.5 ml/g and then transferred immediately to the tank and developed without conditioning. The solvent systems were quite fast ones, the time of development ranging from 1.5 to 6 hours.

The R_F vs. pH relationships were determined in the pH range 1.9 to 7.2. Oleic acid was diluted with organic solvents in the volume ratio 1:1, which corresponds to 1.5 *M* solutions of oleic acid as the mobile phase.

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DISCUSSION OF RESULTS

Effect of pH

The experimental results are presented as $R_F = f(\text{pH})$ curves for 15 of the more important alkaloids, grouped after their molecular structure, in Figs. 1 to 4. It can be seen that in most cases regular S-shaped curves have been obtained. This regular R_F vs. pH relationship can be explained as follows.

For monovalent cations distributing between the cationite phase and buffer solution we have [after Lederer and Kertes (*13*), assuming certain simplifications]

$$R_M = -\log Dr = \log [(1 - R_F)/R_F] = C - \text{pH} \qquad (1)$$

where D is the equilibrium extraction coefficient ($D = C_{org}/C_w$) and C is a constant.

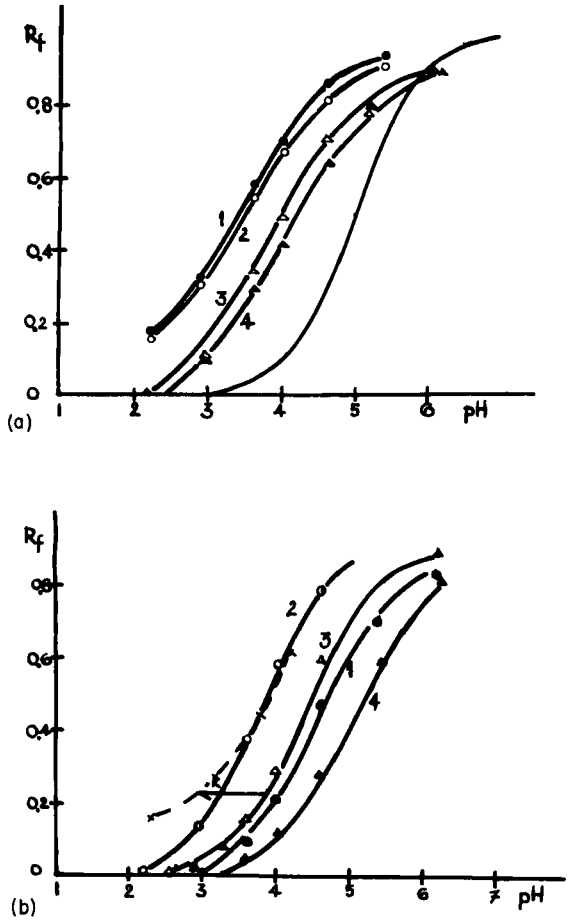

FIG. 1

FIGS. 1 to 4. R_f vs. pH curves of some of the more important alkaloids: 1, iohimbine; 2, strychnine; 3, brucine; 4, physostygmine; 5, cocaine; 6, atropine; 7, scopolamine; 8, cinchonine; 9, cinchonidine; 10, emetine; 11, sparteine; 12, lobeline; 13, veratrine; 14, codeine; 15, pilocarpine.

Mobile phase: (a) isoamyl alcohol + oleic acid; (b) decalin + oleic acid. Dashed lines denote batch extraction fractions of brucine (Fig. 1b) and cinchonine (Fig. 3b). The last curve in Fig. 1a has been calculated from Eq. (2) assuming that const = 10^5 [or Eq. (3), $krK_A = 10^{-5}$].

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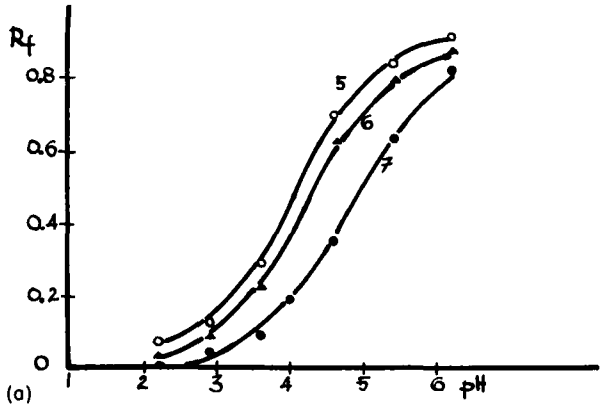

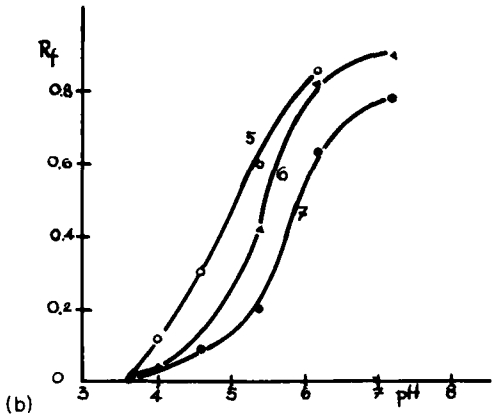

FIG. 2

Therefore,

$$R_F = \frac{Dr}{Dr+1} = \frac{1}{1+10^C \times 10^{-pH}} = \frac{1}{1+\text{const} \times 10^{-pH}} \qquad (2)$$

A theoretical curve calculated from the last equation is shown for comparison in Fig. 1a; it is seen that its shape is very similar to experimental curves. Correspondingly, experimental R_M vs. pH relationships are, as expected from Eq. (1), almost linear, with slope equal approximately to -1, as shown in Fig. 5 (the straight lines cross the pH axis at pH values corresponding to $R_F = 0.5$).

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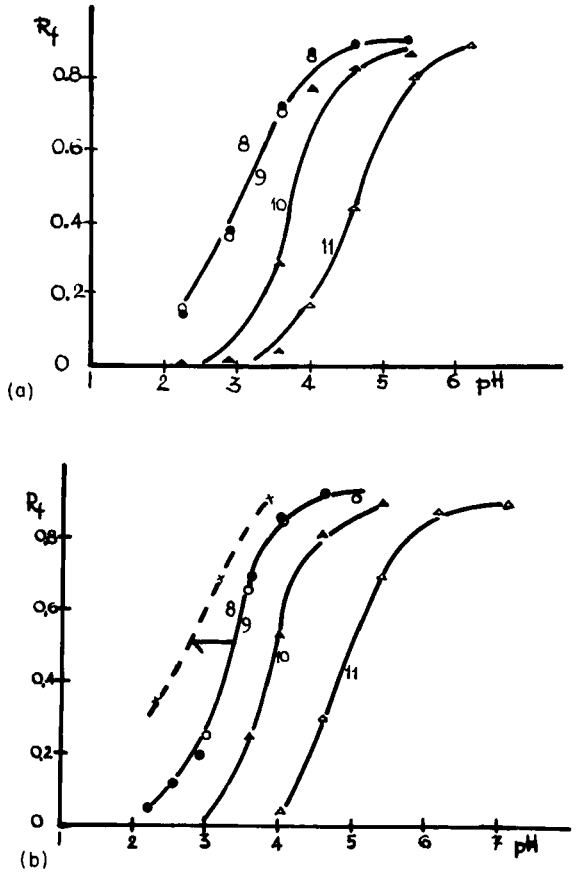

FIG. 3

This accordance of experimental data with the theory of ion-exchange partition cannot be considered, however, as an unambiguous proof of ion-exchange mechanism, since in buffered paper chromatography with neutral (i.e., nonionizing) developing solvents analogous equations are obtained (*14,15*):

$$R_F = \frac{kr}{kr + 10^{pK_A - pH}} = \frac{1}{1 + \frac{1}{krK_A} \times 10^{-pH}}$$

$$R_M = pK_A - \log kr - pH \qquad \text{(bases)} \tag{3}$$

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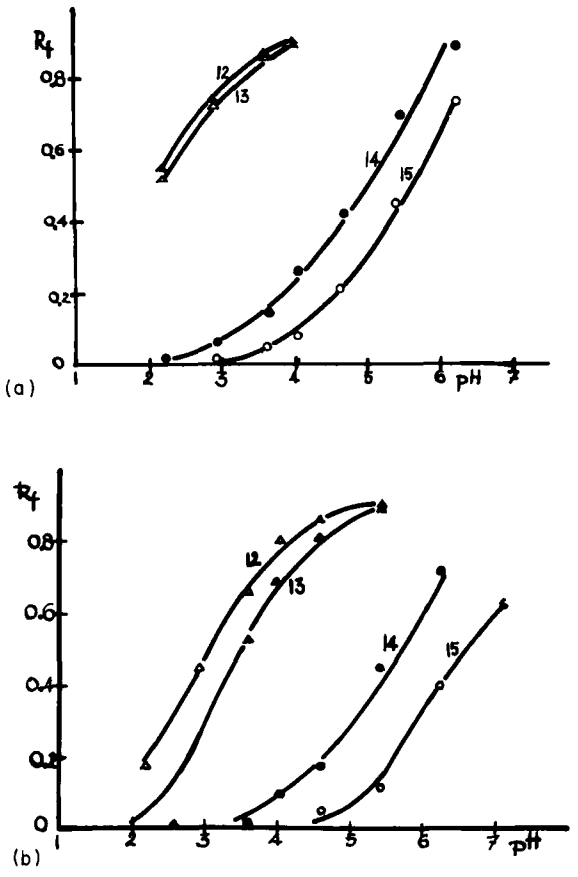

FIG. 4

where k is the partition coefficient of free base ($kr > 100$) and K_A is its acidic ionization constant.

The very high solvent power of the organic phases investigated (see below) seems to confirm the ion-exchange partition mechanism. However, as it is known that carboxylic groups interact very strongly with electron donor centers (as witnessed, for instance, by the high positive heat of mixing of acetic acid with acetone or dimethylsulfoxide, higher than that of chloroform), then a mixed partition mechanism is possible and it is likely that Lewis-type acid-base interactions also play a significant role (also with electron

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donor centers other than ionizable nitrogen atoms, for instance, ether oxygens, common in alkaloids).

Effect of Diluting Solvent

In most cases the substitution of decalin (Figs. 1 to 4b) by isoamyl alcohol as diluting solvent (Figs. 1 to 4a) caused a marked shift of the R_F vs. pH curves toward lower pH values, which indicates higher solvent power of oleic acid + isoamyl alcohol mixtures. The shift, however, was different for various alkaloids, which in a few cases even caused a changed sequence of spots. Thus the diluting

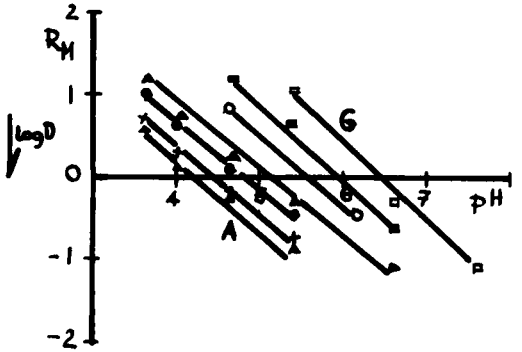

FIG. 5. R_M vs. pH relationships for novocaine and various diluting solvents (same data and notation as in Fig. 6).

solvent may markedly influence the selectivity of the liquid ion exchanger, and use of a suitable diluting solvent may permit the separation of pairs of alkaloids not separable in other systems.

Therefore, in the second part of the work, the effect of a number of typical diluting solvents was investigated. The solvents represented various donor-acceptor properties (*16,17*): decalin (N), benzene (N,B), chloroform (A), diethyl ether (B), butanol, isopentanol (AB). Four alkaloids were investigated, possessing partly hydrophilic properties and thus less strongly extracted by nonionizing organic solvents (even by chloroform). The choice was suggested by the fact that the liquid ion exchangers investigated had been found to possess much greater solvent power than chloroform, one of the best solvent for alkaloids (compare Figs. 1 to 4 and the R_F vs. pH curves for the system chloroform/buffer solution published in an earlier paper (*18*); in the latter system the corresponding R_F vs.

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pH curves are in higher ranges of pH, which indicates lower extraction coefficients).

The experimental data obtained in the second series of experiments are presented in Figs. 5 to 9. It follows from comparison of the diagrams that the improvement of solvent power of the liquid ion exchanger, reflected by shifts of the R_F vs. pH curves to the left, decreases generally in the following order of the diluting solvents:

chloroform (A) > butanol (AB) > isoamyl alcohol (AB) > benzene (N,B) > decalin (N) > ethyl ether (B)

This sequence is not consistent with the order of polarities or the eluotropic order and is probably determined by the force and type of interaction between the diluting solvent, the solute, and the oleic acid. In addition to the acid-base interaction between the alkaloid and oleic acid, which depends on the nature of the organic medium, the following main effects may play a significant part:

1. Solvation of alkaloid molecules (i.e., their electron donor groups) by diluting solvents of classes A and AB and single (nondimerized) molecules of oleic acid.
2. Breaking of dimers of oleic acid by diluting solvents (especially of classes AB, B, and A).
3. Solubility of water in the organic phase, enhancing ionic interaction between the alkaloid and ion exchanger and influencing the degree of hydration of organic molecules.

It seems that consideration of these effects explains the observed sequence of activity of the diluting solvents. The place of ethyl ether in this sequence (last) is probably due to the fact that ether molecules cannot form solvates with B groups, which predominate in alkaloid molecules; and although the solvent causes the dissociation of oleic acid dimers, its molecules strongly compete with alkaloid molecules for oleic acid in view of strong interactions of the —COOH · · · O= type.

To illustrate more clearly the effect of the diluting solvent on the solvent power, in Fig. 10 the experimental data from Figs. 5 to 9 are presented in the form of four chromatographic "spectra"; pH_i values corresponding to $R_F = 0.5$ (i.e., $R_M = 0$) are plotted against the six diluting solvents used. Since the R_F vs. pH curves in Figs. 6 to 9 are high in all cases ($R_{F\max} = 1$), the log D axis in Fig. 10 is directed downward, its zero point being shifted in comparison to the pH_i axis by a value depending on the volume ratio r [cf. Eq.

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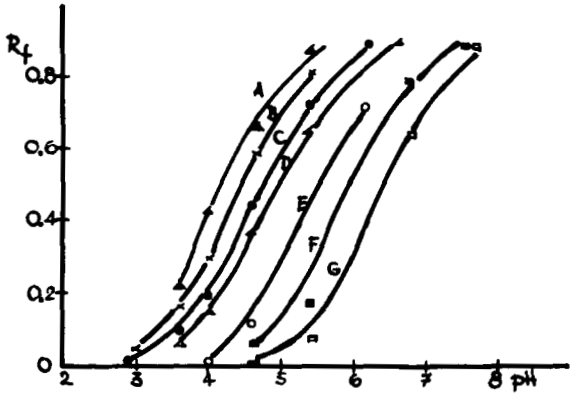

FIG. 6

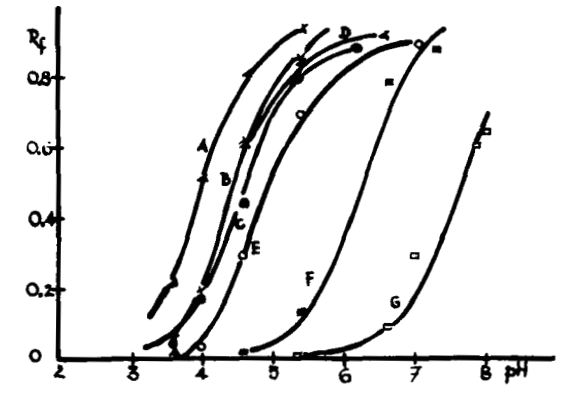

FIG. 7

FIGS. 6 to 9. Effect of diluting solvents on the position of R_F vs. pH curves of four alkaloids: A, chloroform; B, butanol; C, isopentanol; D, benzene; E, decalin; F, diethyl ether. For comparison, R_F vs. pH curves are also given for the pure chloroform/buffer solution system (G). The alkaloids are: Fig. 6, novocaine; Fig. 7, sparteine; Fig. 8, atropine; Fig. 9, pilocarpine.

(1)]. Thus, for instance, the shift of the pH_i value of a given alkaloid downward by one unit denotes a tenfold increase of its extraction coefficient (since log 10 = 1); on the other hand, the distance of points of two different alkaloids for a given solvent (i.e., in mixture with oleic acid) is equal to the logarithm of the separation factor

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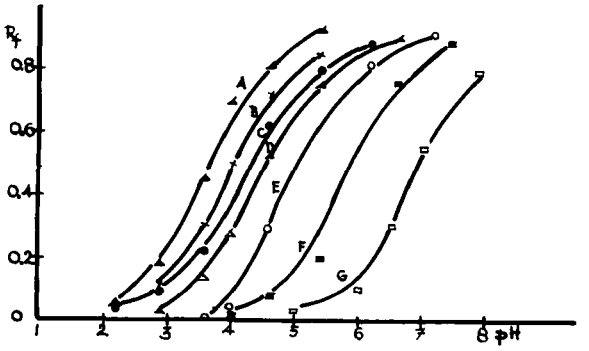

FIG. 8

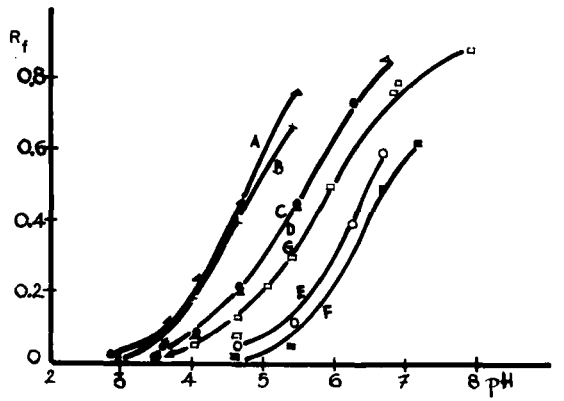

FIG. 9

($\beta = D''/D'$) and is thus a measure of the selectivity of the system relative to the given pair of solutes [cf. (*19*)]. It is seen that the "spectra" are not parallel and even cross occasionally; this means that the selectivity depends strongly on the diluting solvent used.

It can also be seen that in most cases oleic acid diluted with the solvents in volume ratio 1:1 is a stronger extractant than chloroform; only for pilocarpine are higher R_F values observed for pure chloroform than for oleic acid diluted with decalin or ethyl ether. Thus the chromatographic data seem to indicate a very high solvent power of oleic acid, especially when it is diluted with chloroform or butanol.

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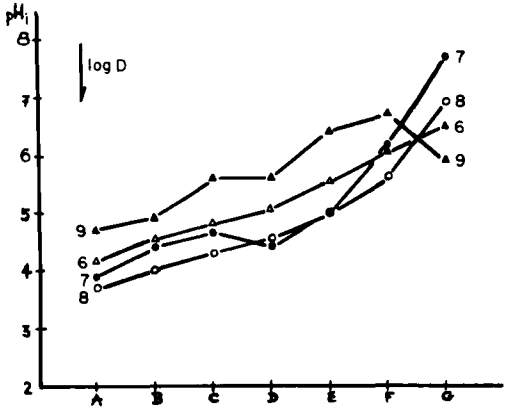

FIG. 10. R_M vs. solvent spectra plotted from data of Figs. 6 to 9. Ordinate: pH_i/values of pH for which $R_F = 0.5$, i.e., $R_M = 0$/; abscissa: six solvents of the type oleic acid + diluting solvent (A to F; for notation cf. preceding figures) and pure chloroform (G).

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R_F Values and Batch Partition

In the idealized model of the chromatographic process, it is assumed that the migration rate is determined by Eq. (2). In order to investigate the usefulness of paper-chromatographic data for the estimation of extraction coefficients which is necessary in the choice of optimal systems for preparative separations, in the third series of experiments a number of static extraction coefficients were determined for several solvent systems.

The batch-extraction coefficients were determined by shaking solutions of the alkaloids in the liquid ion exchanger (oleic acid + decalin 1:1) with buffer solutions. The equilibrium concentration of the alkaloid in the aqueous phase was determined by the precipitation of reineckates. Extraction coefficients of cynchonine and brucine were obtained for pH 2.3, 3.2, and 3.8; then static extraction fractions were calculated $\left[E = \frac{D}{(D+1)}, \text{ analogous to } R_F \text{ for } r = 1\right]$ and plotted in Figs. 1b and 3b (dashed lines). Although chromatographic and static experiments are not directly comparable, a certain parallelism is evident, the two E vs. pH curves being shifted by ca. 1 pH unit to the left in comparison to the respective R_F vs. pH curves. This parallelism permits approximate estimation of the variation of static extraction coefficients from easily obtain-

able chromatographic data, and thus the choice of suitable solvent systems for extraction and re-extraction, or countercurrent separation of alkaloids (and other electrolytes). It is also possible to estimate the selectivity of the system, since, for cases in which Eq. (2) or (3) is valid, the horizontal distance of two R_F vs. pH curves is equal to the log separation factor (log β) of the pair of solutes for the given organic phase.

The high solvent power, in addition to good solubility of alkaloids in the acidic aqueous solutions employed, leads probably to very high capacities of the solvent systems investigated, so that high concentrations of alkaloids can be employed. The partition of alkaloids can be varied within wide limits by the pH of the aqueous phase. Therefore, systems of this type can find increasing application not only in chromatographic microanalysis, but also in the isolation, purification, and separation of organic electrolytes.

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REFERENCES

1. F. L. Moore, U.S. At. Energy Comm., *Nuclear Sci. Ser. NAS-NS3101*, 1962.
2. C. F. Coleman, C. A. Blake, Jr., and K. B. Brown, *Talanta*, **9**, 297 (1962).
3. E. Cerrai, *Chromatog. Rev.*, **6**, 129 (1964).
4. E. Cerrai, in *Stationary Phase in Paper and Thin-Layer Chromatography* (K. Macek and I. M. Hais, eds.), *Proc. 2nd Liblice Symp.*, Elsevier, New York, 1965, p. 180.
5. K. Käupke and F. Wolf, *Chem. Techn. (Berlin)*, **18**, 405 (1966).
6. L. C. Craig and D. Craig, in *Technique of Organic Chemistry*, Vol. III (A. Weissberger, ed.), Wiley (Interscience), New York, 1950, Chap. IV.
7. E. Soczewiński and M. Rojowska, *J. Chromatog.*, in press.
8. V. Betina, *Nature*, **182**, 796 (1958).
9. E. Soczewiński, A. Waksmundzki, and W. Maciejewicz, *Anal. Chem.*, **36**, 1903 (1964).
10. Ž. Prochazka, in *Some General Problems of Paper Chromatography* (I. M. Hais and K. Macek, eds.), *1st Liblice Symp.*, CŠAV, Prague, 1962, p. 113.
11. P. Kabasakalian, *Anal. Chem.*, **36**, 2202 (1964).
12. E. Soczewiński, St. Przeszlakowski, and A. Flieger, *Ann. Univ. M. Curie-Skłodowska, Lublin-Polonia, Sec. AA* in press.
13. M. Lederer and S. Kertes, *Anal. Chim. Acta*, **15**, 226 (1956).
14. E. Soczewiński, *Roczniki Chem.*, **37**, 467 (1963); *Nature*, **188**, 391 (1960).
15. D. Rybar, B. Tousek, and I. M. Hais, *Chem. Listy*, **48**, 1532 (1954).
16. R. H. Ewell, J. M. Harrison, and L. Berg, *Ind. Eng. Chem.*, **36**, 871 (1944).
17. G. C. Pimentel and A. L. McClellan, *The Hydrogen Bond*, Freeman, San Francisco, 1960.
18. E. Soczewiński and B. Szabelska, *Dissertation Pharm.*, **17**, 53 (1965).
19. E. Soczewiński, *Bull. Acad. Polon. Sci. Ser. Sci. Chim.*, **13**, 209, 213 (1965).

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