

THE RELATION BETWEEN THE COMPOSITION OF CERTAIN TERNARY TWO-PHASE SOLVENT SYSTEMS AND R_M VALUES

EDWARD SOCZEWIŃSKI* AND CARL AXEL WACHTMEISTER

*Institute of Organic Chemistry and Biochemistry,
University of Stockholm
(Sweden)*

(Received June 12th, 1961)

In paper chromatography differences in the relative migration rates (R_F) of the components of a mixture have fundamental significance for the efficiency of separation. In general, best resolution is obtained when the components—which are frequently closely related compounds—possess intermediate R_F values; on the other hand, when they remain near the starting line or migrate close to the front of the developer, the resolution is usually poor.

The R_F value of a substance in a given partition system is given by the formula:

$$R_F = \frac{K\gamma}{K\gamma + 1} \quad (1)$$

where K = partition ratio of substance = ratio of overall concentrations of substance in non-polar and polar phase respectively, at equilibrium; γ = ratio of cross-sectional areas of non-polar and polar phases, or ratio of volumes of the two phases in a sufficiently narrow section of the system.

Thus, the rates of migration in paper partition chromatography can be controlled mainly by varying the partition ratios of the substances to be separated (the volume coefficient γ may be varied only over a fairly narrow range). Two methods have been extensively used:

(1) In the case of weak electrolytes good results may often be obtained by using the buffered paper method^{1,2} to control the rate of migration.

(2) The most common technique is the use of mixed solvents of a suitable optimum composition.

Although the latter technique has been used from the very beginning of paper chromatography, little attempt has been made to derive quantitative relationships in this field; the phenomena involved are usually of a complex nature and result from superimposition of several effects. However, a number of qualitative relations has been

* Permanent address: Department of Inorganic Chemistry, Academy of Medicine, Lublin, Poland.

found; the most general may be formulated as follows: the R_F of a solute increases with its solubility in the mobile phase. There are many examples to illustrate this rule, for instance, increase of water content in the organic solvent entails increase of R_F values of such hydrophilic substances as amino acids^{3,4} or sugars⁵. Increased water content may also be brought about indirectly, *e.g.* by a change of temperature^{3,6} or by the addition of a third component, which increases the mutual solubility of water and organic solvent⁷. For sugars the water content is of such importance that the nature of the organic component plays only a minor part, as exemplified by the linear relationship between R_M and solubility of water in different organic solvents (strictly speaking, the logarithm of the molar fraction of water in the organic solvent saturated with water), found empirically by ISHERWOOD AND JERMYN⁸ and confirmed by ALCOCK AND CANNELL⁹. On the other hand, in reversed phase chromatography of higher fatty acids (paper impregnated with liquid hydrocarbons and developed with water-alcohol or water-acetic acid mixtures), the water decreases their solubility in the mobile phase and then the R_F values decrease with increasing water content^{10,11}.

In paper chromatography of organic electrolytes the addition of acids and bases (*e.g.* acetic acid, ammonia) has a particularly marked effect upon the R_F values. These effects have been investigated in paper chromatography of amino acids (*e.g.*^{3,12,13}), alkaloids (*e.g.*^{14,15}), nucleic acids¹⁶ etc.

The influence of solvent composition has been extensively studied in inorganic paper chromatography. The presence of complexing agents is of primary importance here, such as hydrochloric, hydrobromic and nitric acid, or chelating agents¹⁷. Numerous examples are to be found in monographs on chromatography¹⁸, only a few references will be mentioned here. LEDERER¹⁹ has derived an equation anticipating a linear relationship between the R_M and the logarithm of the concentration of complexing chloride ions; this equation has been confirmed experimentally for a number of cations. HARTKAMP AND SPECKER²⁰ have found that, within certain groups, cations behave similarly as the composition of the developer changes. The influence of various components of the developer has also been investigated by LACOURT *et al.*²¹, LEDERER²², DE CARVALHO AND LEDERER²³, DE CARVALHO²⁴, REEVES AND CRUMPLER²³, BERGAMINI AND ROVAI²⁶, KERTES²⁷ and others.

In most of the works cited one-phase developers were used or solvents in which the composition of the phases changes readily (except refs.^{10,11}). The immobile phase was thus formed mainly by conditioning and adsorption of more polar solvents from the developer. Thus demixing leading to composition gradients along the paper strip was to be expected in these cases.

The aim of the present work was to give a theoretical relationship for certain simple cases of paper chromatography, using ternary liquid systems. In the systems considered both phases are practically immiscible at any overall composition of the system (*cf.* Fig. 8). In these systems demixing is practically eliminated and conditions are much more similar to those of continuous counter-current extraction, so that the process could be considered as "real partition chromatography" (TSCHESCHE *et al.*²⁸). The effect of solvent composition could be investigated over a very wide range of compositions.

When one of the liquid phases forming the chromatographic system consists of a two-component solvent and the other phase is a pure liquid, practically immiscible both with the solvent mixture and its components (*cf.* Fig. 8), then the partition ratio of a substance between the two phases—if certain additional requirements are fulfilled—will be given by the KEMULA-BUCHOWSKI equation²⁹:

$$\log K = u_1 \log K_1 + u_2 \log K_2 \quad (2)$$

where u_1, u_2 are volume fractions of components 1 and 2, forming the mixed solvent phase; and K_1 (K_2) is the partition ratio of substance between pure solvent 1 (2) and the other phase.

It can be seen from eqn. (2) that, using a mixed solvent of an appropriate composition, any value of partition ratio of the solute can be obtained in the range limited by the extreme values K_2 and K_1 . When the logarithm of partition ratio ($\log K$) is plotted against the volume composition of the mixed solvent phase, a straight line should be obtained, connecting the values of $\log K_1$ and $\log K_2$ ^{29,30}. As, however, in partition chromatography (considered as a counter-current extraction process) it is more convenient to use not the partition ratio K but the partition number Kr (ratio of amounts of solute in non-polar and polar phase, at equilibrium), eqn. (2) can be written as follows:

$$\log Kr = u_1 \log K_1 r + u_2 \log K_2 r \quad (\text{as } u_1 + u_2 = 1) \quad (3)$$

or

$$R_M = u_1 R_{M1} + u_2 R_{M2} \quad (3a)$$

Assuming that the volume coefficient r is constant and independent of the composition of the mixed solvent phase, the logarithm of the partition number, or the R_M value, should also be a linear function of the volume composition of the mixed solvent phase. This is illustrated in Fig. 1, where the relation between R_M and the composition of the mixed solvent phase (1 + 2) is represented diagrammatically for a number of imagi-

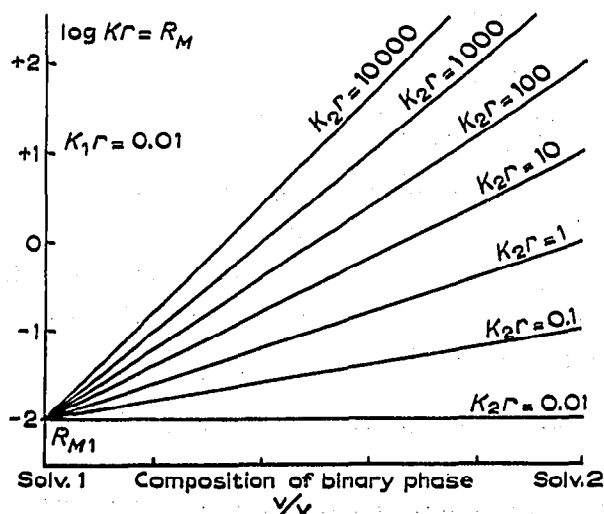


Fig. 1. Theoretical R_M vs. volume composition curves according to eqn. (3).

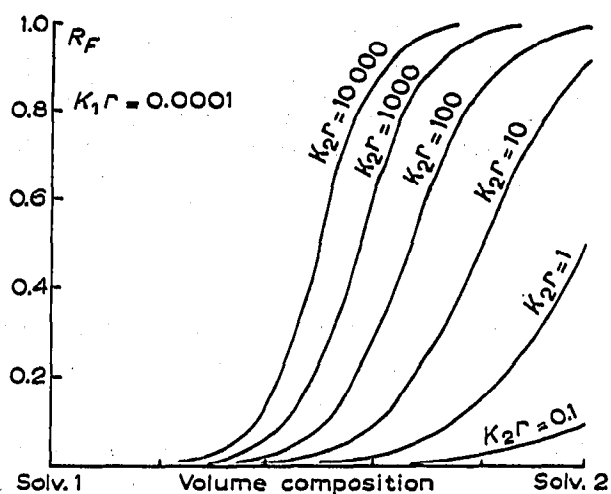


Fig. 2.

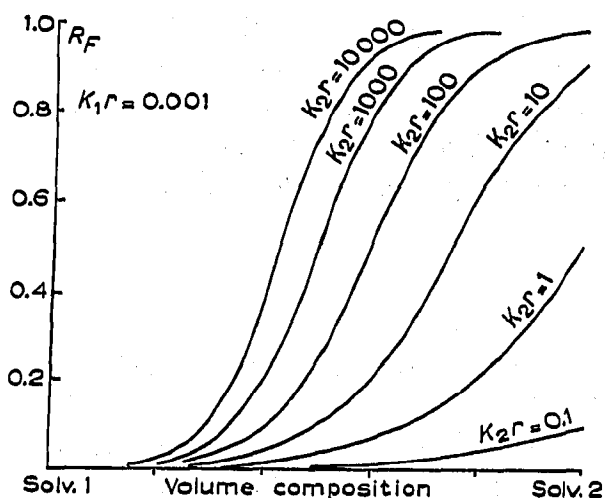


Fig. 3.

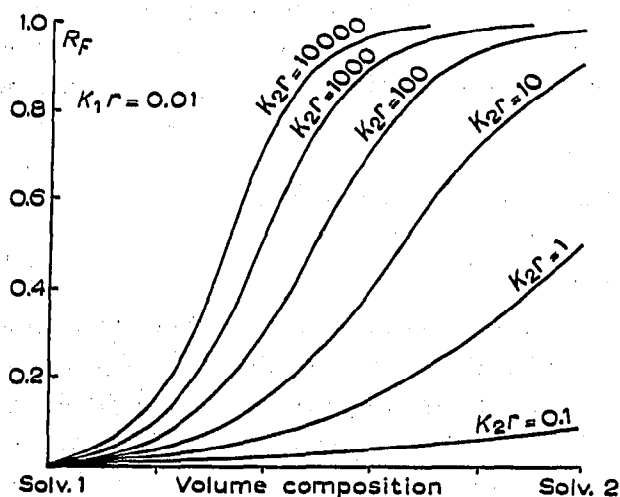


Fig. 4.

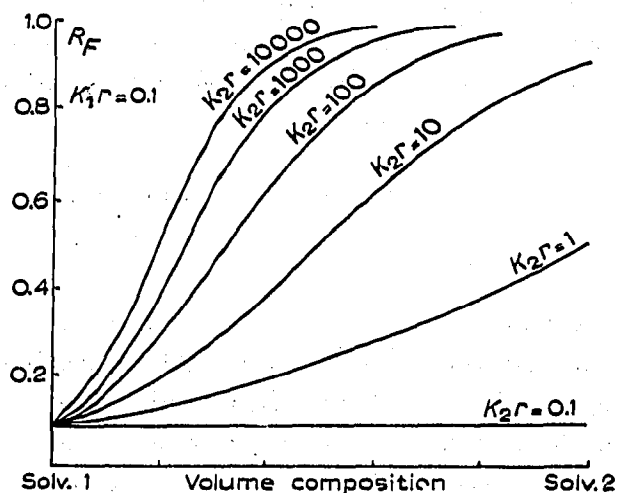


Fig. 5.

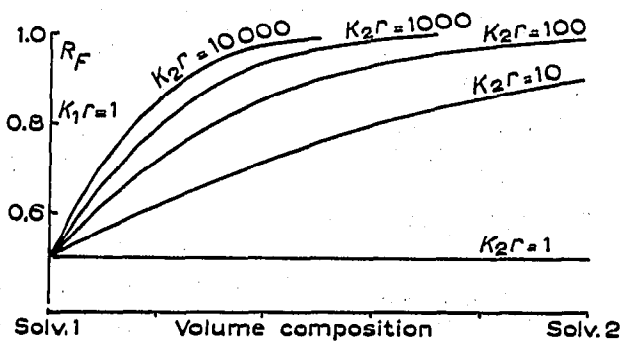


Fig. 6.

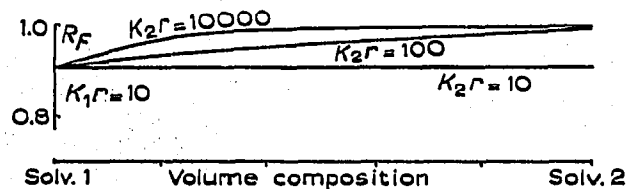


Fig. 7.

Figs. 2-7. Theoretical R_F vs. volume composition curves for different pairs of values of $K_1 r$ and $K_2 r$

nary substances whose partition numbers between the pure solvent 1 and the other phase are $K_{1r} = 0.01$. In the system solvent 2/other phase, K_{2r} values are 0.1; 1; 10; 100; 1000; 10000, respectively.

Variations of composition of the mixed solvent phase will also change the R_F values in accordance with eqn. (1). In Figs. 2-7 some typical theoretical R_F vs. volume composition relationships are illustrated. The solvent giving lower values of Kr has been arbitrarily chosen as solvent 1. (Fig. 4 corresponds to the conditions of Fig. 1.) The importance of the composition of the mixed solvent phase for the efficiency of separation is evident.

In order to illustrate these relationships experimentally, distribution systems had to be chosen which would at least approximately fulfil the simplifying assumptions made by KEMULA AND BUCHOWSKI²⁰. The mixed solvent may form either the non-polar or the polar phase, so that two possibilities could be taken into account, as illustrated schematically in Fig. 8. The latter alternative (II) was chosen in the ex-

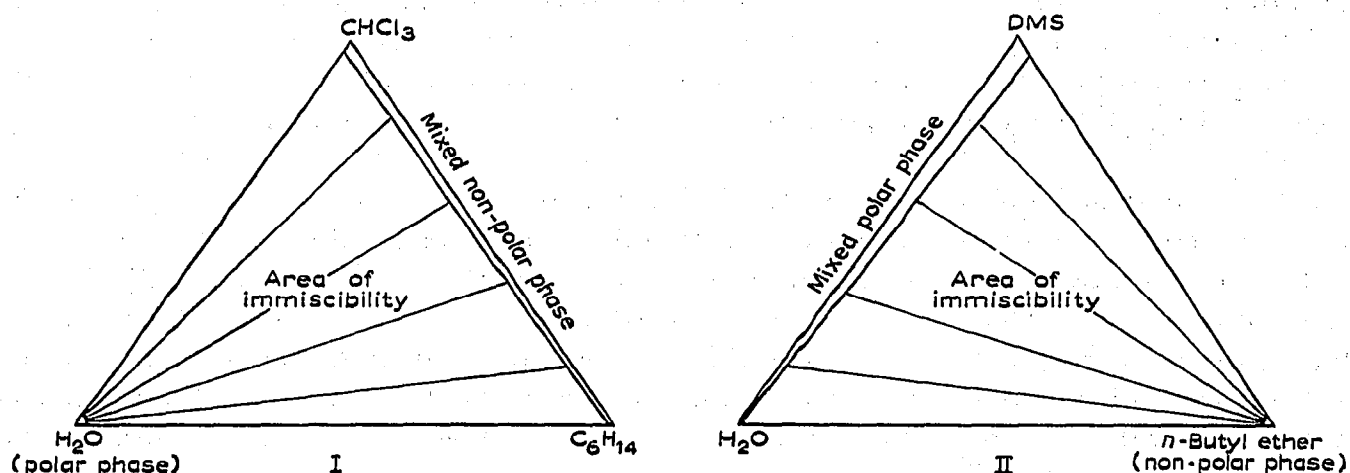


Fig. 8. Gibbs' diagrams illustrating two cases of mixed solvents used for extraction processes: I. The polar phase is practically pure water, the non-polar phase composed of chloroform and hexane (practically dry). II. The polar phase is composed of water and dimethylsulphoxide (DMS), whereas the non-polar phase is almost pure *n*-butyl ether.

perimental part, although this particular system, dimethylsulphoxide + water/*n*-butyl ether, does not fulfil strictly all requirements of KEMULA-BUCHOWSKI's theory. Systems of this type have been less commonly used in partition chromatography (except for reversed phase techniques). It is well known, however, that the use of non-aqueous polar phases, *e.g.*, dimethylsulphoxide (DMS) or formamide makes it possible to perform paper chromatography even of fairly lipophilic compounds, giving too high R_F values in conventional systems³¹. Moreover, the use of highly polar organic liquids—which are strongly adsorbed on cellulose—is often accompanied by a pronounced reduction in tailing tendency of the spots.

EXPERIMENTAL

The phenols, derived from dibenzofuran (see Fig. 9a), were available through earlier work on the chemistry of lichens (for references, *cf.*³²). They were chosen merely as

examples of compounds of moderate molecular weight, with a pronounced tailing tendency in common solvent systems. *n*-Butyl ether and isopropyl ether were of reagent grade. DMS was of a technical quality, containing at the most 0.5 % water (Nitroglycerin AB, Gyttop, Sweden).

Whatman No. 4 paper was used, cut along the machine direction into strips 10×28.5 cm.

The "moist paper method"^{33,34} was used, in order to secure an appropriate and comparable amount of polar phase in the paper (0.5 ml/1 g air-dry paper). This in turn was expected to result in a fairly constant value of the mean volume coefficient r in all experiments, an assumption based on an earlier investigation³⁵. The paper strips were passed through solutions of known concentrations of DMS in a dilute acidic solution (0.3 ml conc. hydrochloric acid + 1 g NaCl diluted to 200 ml with water) and the excess of liquid removed by blotting between sheets of filter paper. The phenols, as 1 % solutions in acetone, were then applied to the starting line (0.5–1 μ l, corresponding to 5–10 μ g) and the strips air-dried to the humidity coefficient (weight of moist paper: weight of dry paper) corresponding to polar phase content 0.5 ml/1 g dry paper, as controlled by weighing. It was assumed that only water evaporates, which is justified by the very low vapour pressure of DMS³⁶ at the temperature used ($21.5 \pm 0.5^\circ$).

In calculations, for the sake of simplicity, linear density–composition relationship was assumed between 100 % DMS ($d = 1.1$) and 0 % DMS ($d = 1.0$); thus the humidity coefficients employed were in the range 1.55 (100 % DMS) to 1.50 (0 % DMS). It was taken into account that the concentration of DMS approximately doubled during drying of the strips; thus with a starting concentration of 30 % DMS, final concentrations were obtained mostly in the range 65–70 % DMS. With high final concentrations of DMS ($> 80\%$) loss of weight was very slow and it was accelerated by drying, for short intervals of time at 60° . Due to absorption of water from the atmosphere, strips containing 100 % DMS are difficult to obtain without special precautions, but *ca.* 96–98 % DMS was reached by impregnation with a 30 % solution of DMS in toluene, as described by HAMMARBERG AND WICKBERG³¹.

After reaching the appropriate humidity coefficient, the strips were immediately transferred to the chromatographic chamber ($20 \times 10 \times 28$ cm) containing an excess of the non-polar solvent. The chromatograms were developed by the descending technique (distance of development 16 cm; time *ca.* 25 min; temperature $21.5 \pm 0.5^\circ$). The spots were detected by spraying the strips with a saturated solution of NaHCO_3 and then passing through a solution of bis-diazotised benzidine, obtained by mixing equal volumes of solution A (5 g benzidine + 15 ml conc. HCl diluted to 1000 ml) and B (10 % NaNO_2) (for reference, see³²). The strips were finally washed in running water and dried. Distances travelled by the spots were measured from their centres.

The experimental R_F vs. volume composition diagrams obtained in this way are given in Fig. 9 (mobile phase: (a) isopropyl ether; (b) *n*-butyl ether).

The accumulation of points in Fig. 9b in the range 30–40 % H_2O (70–60 % DMS) corresponds to a series of experiments with a constant starting concentration of DMS

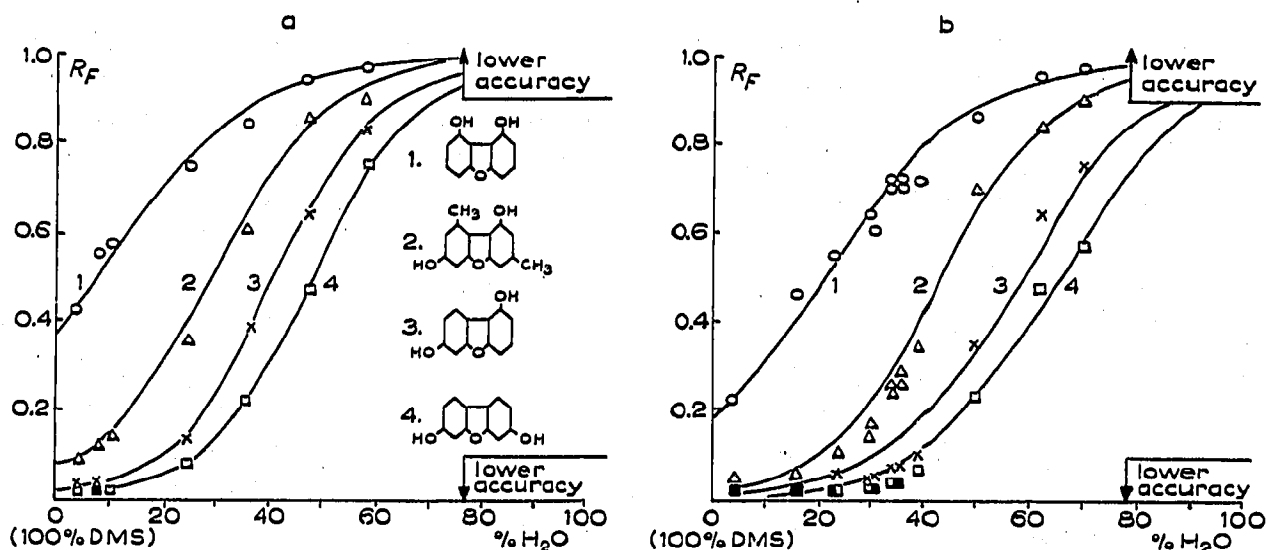


Fig. 9. R_F vs. composition plots. The points are experimental, the curves theoretical. Mobile phase: (a) isopropyl ether; (b) *n*-butyl ether.

(30%). Continuous lines are theoretical curves obtained by the method described below.

In order to test the validity of eqn. (3), the experimental data of Fig. 9 should be given as $\log Kr$ vs. composition plots. The logarithms of partition numbers R_M could be calculated from R_F values; however, a simpler graphical method was used, illustrated in Fig. 10, which is self-explanatory.

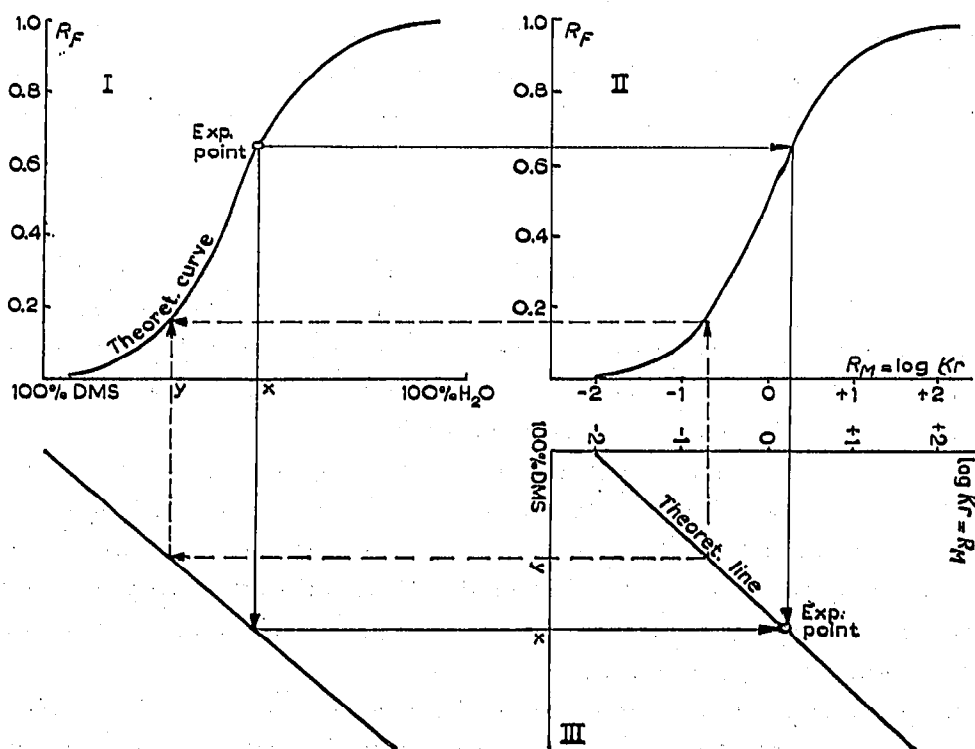


Fig. 10. A graphical method of transformation of R_F vs. composition plots (I) into R_M vs. composition plots (III) (continuous arrows) and *vice versa* (broken arrows) using an auxiliary plot (II) of the function $R_F = F(R_M)$.

The R_M vs. volume composition diagrams are represented in Fig. 11. Points are experimental R_M values found graphically; straight lines were drawn in the most appropriate position. Points within the range of R_M from -1 to $+1$ (which corresponds to R_F 0.1–0.9) were considered rather more reliable than the points outside this range (Fig. 11, broken lines): at low and high R_F values deviations from uniform

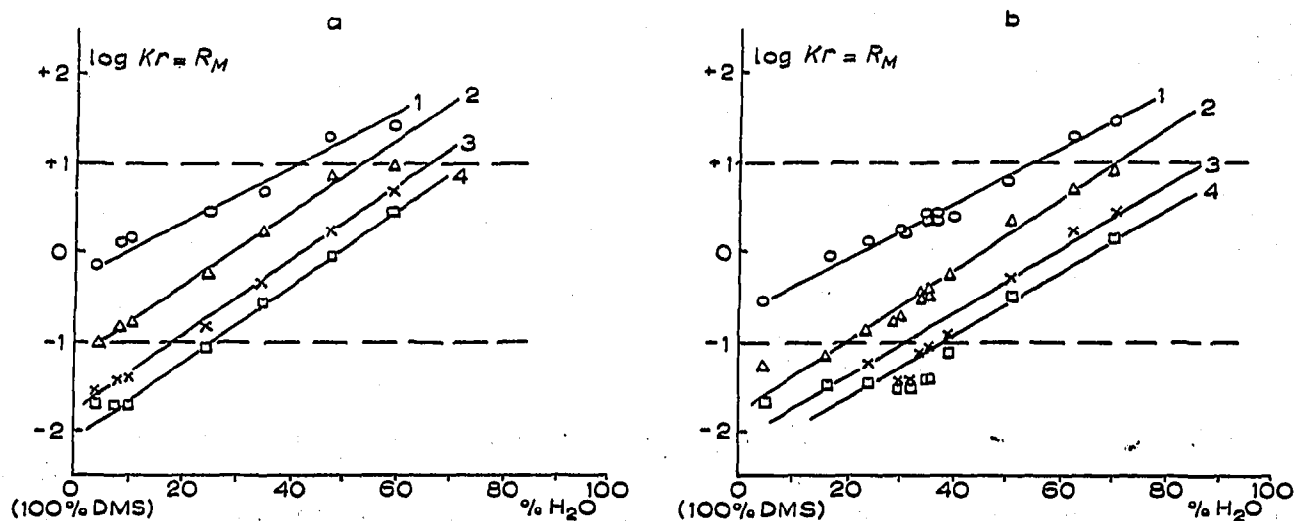


Fig. 11. R_M vs. composition plots. The points are experimental, the straight lines are drawn in the most appropriate position. Mobile phase: (a) isopropyl ether; (b) *n*-butyl ether. Stationary phases as in Fig. 9.

conditions are to be expected due to formation of gradients of the volume coefficient v , etc. From these straight lines (considered theoretical) R_F vs. composition curves (theoretical) were determined graphically, working backwards as indicated by broken arrows in Fig. 10.

DISCUSSION OF RESULTS

The experimental results seem to indicate that the rate of migration of the phenols studied is fairly well described by the simple eqn. (3), in spite of the fact that the solvent systems used are certainly not strictly ideal. Thus, heat is evolved and a volume contraction of up to 2% is observed when DMS and water are mixed, indicating marked interaction between the components.

Considering the pronounced tailing tendency of the dibenzofurans employed, the appearance of the spots was generally satisfactory for concentrations of DMS in the range down to 30–40%; at low R_F values almost circular spots were obtained, whereas at higher values the spots were more elongated. Pronounced tailing was only observed at lower DMS concentrations; with pure water as the stationary phase, some phenols trailed from the point of application.

Relatively good appearance of spots and agreements of results with theory seem to justify the statement that certain chromatographic systems can be approximately described by simple equations derived from the theory of extraction. Buffered paper chromatography (moist paper technique^{2,34}) may be referred to for further examples.

Too many assumptions, however, have to be fulfilled even for the simple equations (2) and (3) to be valid for all ternary solvent systems of the types discussed (Fig. 8). In actual practice, the picture might become more diffuse, distorted by deviations from these assumptions owing to adsorption of substance on paper, formation of composition gradients due to frontal analysis of the developer, etc.

In the simple cases mentioned above, valuable information can often be obtained from the experimental data; for instance, diagrams like Fig. 11 can be used to estimate optimal solvent systems for countercurrent distribution (Craig's method)³⁷. It is also possible to obtain a general idea of the possibilities of such systems from a few experimental data.

ACKNOWLEDGEMENTS

Thanks are due to Professor Dr. A. WAKSMUNDZKI (Dept. of Physical Chemistry, Academy of Medicine, Lublin, Poland) for suggesting the subject of investigation. One of the authors (E.S.) is indebted to the Ministry of Health and Social Welfare of the Polish People's Republic for a scholarship.

SUMMARY

A modified KEMULA-BUCHOWSKI equation for partition ratio in certain simple ternary solvent systems can be used for deriving a simple relationship between R_M and R_F values and volume composition of the mixed solvent phase. Experiments using mixtures of dimethylsulphoxide (DMS) and water as the polar phase have given results in fair accordance with theoretical anticipations. A method of interpretation of experimental data is described.

REFERENCES

- ¹ R. R. GOODALL AND A. A. LEVI, *Analyst*, 72 (1946) 277.
- ² E. SOCZEWIŃSKI, *Nature*, 188 (1960) 391.
- ³ R. CONSDEN, A. H. GORDON AND A. J. P. MARTIN, *Biochem. J.*, 38 (1944) 224.
- ⁴ H. R. BENTLEY AND J. K. WHITEHEAD, *Biochem. J.*, 46 (1952) 341.
- ⁵ L. HOUGH, J. K. N. JONES AND W. H. WADMAN, *J. Chem. Soc.*, (1950) 1702.
- ⁶ J. N. COUNSELL, L. HOUGH AND W. H. WADMAN, *Research*, 4 (1951) 143.
- ⁷ D. P. BURMA, *J. Indian Chem. Soc.*, 28 (1951) 555.
- ⁸ F. A. ISHERWOOD AND M. A. JERMYN, *Biochem. J.*, 48 (1951) 515.
- ⁹ M. ALCOCK AND J. S. CANNELL, *Nature*, 177 (1956) 327.
- ¹⁰ H. P. KAUFMANN AND W. H. NITSCH, *Fette, Seifen, Anstrichmittel*, 56 (1954) 154.
- ¹¹ H. P. KAUFMANN AND W. H. NITSCH, *Fette, Seifen, Anstrichmittel*, 58 (1956) 234.
- ¹² E. F. MCFARREN, *Anal. Chem.*, 23 (1951) 168.
- ¹³ A. J. LANDUA, R. FÜRST AND J. AWAPARA, *Anal. Chem.*, 23 (1951) 163.
- ¹⁴ R. MUNIER, *Bull. soc. chim. France*, (1952) 852.
- ¹⁵ H. THIES AND F. W. REUTHER, *Arzneimittel-Forsch.*, 7 (1957) 63.
- ¹⁶ B. MAGASANIK, E. VISCHER, R. DONIGER, D. ELSON AND E. CHARGAFF, *J. Biol. Chem.*, 186 (1950) 37.
- ¹⁷ F. H. POLLARD, J. F. W. MCOMIE AND I. I. M. ELBEIH, *J. Chem. Soc.*, (1951) 466.
- ¹⁸ E. LEDERER AND M. LEDERER, *Chromatography*, 2nd Ed., Elsevier, Amsterdam, 1957.
- ¹⁹ M. LEDERER, *J. Chromatog.*, 1 (1958) 176.
- ²⁰ H. HARTKAMP AND H. SPECKER, *Z. anal. Chem.*, 158 (1957) 92, 161.
- ²¹ A. LACOURT, G. SOMMEREYNS, E. DE GEYENDT, J. BARUH AND J. GILLARD, *Nature*, 163 (1949) 999; *Metallurgia*, 40 (1949) 181.
- ²² M. LEDERER, *Anal. Chim. Acta*, 5 (1951) 185.

- ²³ W. A. REEVES AND T. B. CRUMPLER, *Anal. Chem.*, 23 (1951) 1576.
²⁴ R. G. DE CARVALHO, *Intern. Congress of Anal. Chem.*, Lisbon, 1956.
²⁵ R. G. DE CARVALHO AND M. LEDERER, *Anal. Chim. Acta*, 13 (1955) 437.
²⁶ C. BERGAMINI AND A. ROVAI, *Anal. Chim. Acta.*, 15 (1956) 43.
²⁷ S. KERTES, *Research Correspondence, Suppl. to Research (London)*, No. 2 (1956) S5.
²⁸ R. TSCHESCHE, G. K. GRIMMER AND F. SEEHOFER, *Chem. Ber.*, 86 (1953) 1235.
²⁹ W. KEMULA AND H. BUCHOWSKI, *Roczniki Chem.*, 29 (1955) 718.
³⁰ G. V. SCHULTZ, *Z. physik. Chem.*, A 179 (1937) 321.
³¹ G. HAMMARBERG AND B. WICKBERG, *Acta Chem. Scand.*, 14 (1960) 862.
³² B. ÅKERMARK, H. ERDTMAN AND C. A. WACHTMEISTER, *Acta Chem. Scand.*, 13 (1959) 1855.
³³ A. WAKSMUNDZKI, J. OŚCIK AND Z. FRELEK, *Ann. Univ. Mariae Curie-Skłodowska, Lublin-Polonia, Sect. AA*, 9 (1954) 83.
³⁴ A. WAKSMUNDZKI, E. SOCZEWIŃSKI AND R. AKSANOWSKI, *Chem. Anal. (Warsaw)*, 2 (1957) 459.
³⁵ A. WAKSMUNDZKI AND E. SOCZEWIŃSKI, *Roczniki Chem.*, 35 (1961) 1363.
³⁶ H. L. SCHLAFFER AND W. SCHAFFERNICHT, *Angew. Chem.*, 72 (1960) 618.
³⁷ A. WAKSMUNDZKI AND E. SOCZEWIŃSKI, *Bull. acad. sci. Polon., Sér. sci. chim.*, 9 (1961) 155.

J. Chromatog., 7 (1962) 311-320