

SOME PROBLEMS OF REPRODUCIBILITY OF R_F VALUES IN LIQUID-LIQUID PARTITION CHROMATOGRAPHY

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SUMMARY

The effect of the variation of the distribution coefficient and the cross-sectional area ratio on the reproducibility of R_F values in ideal liquid-liquid partition chromatography is discussed. In the case of formamide-impregnated papers developed with nonpolar solvents, the degree of loading (defined as ml of formamide per one gram of dry paper) has been found to influence the R_F values through the volume ratio of the two liquid phases.

Certain types of partition chromatography system fit quite well the idealized partition model¹ when a suitable support and experimental technique are employed (e.g., Zaffaroni systems, in which the support is impregnated with a non-volatile liquid and developed with a solvent immiscible with the stationary phase).

In ideal partition chromatography, the R_F value is determined by the ratio of cross-sectional areas (or volumes) of the two phases ($r = V_{mob}/V_{st}$) and by the distribution coefficient of the solute ($D = C_{mob}/C_{st}$, total concentrations, mole/l):

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

Therefore, any variations in the experimental conditions and experimental technique will then influence the reproducibility of R_F values through their effect on the distribution coefficient D and on the volume ratio r .

Factors influencing the reproducibility through the distribution coefficient D

The variations of the distribution coefficient can in certain cases be expressed by quantitative relationships. The main methods of controlling the distribution behaviour (and thus R_F values) are the use of mixed solvents or the utilization of formation equilibria of extractable complexes; the problems have been recently reviewed by one of us² (cf. also ref. 3). It seems interesting to consider the problems from the viewpoint of reproducibility of paper chromatographic data.

It should be pointed out in the first place that simpler relationships are usually obtained for R_M values (see e.g. refs. 1, 2 and 4) and not R_F coefficients which are extensively employed for practical reasons. For instance, the R_M value is often linearly dependent on the composition of the mixed phase or on the log concentration

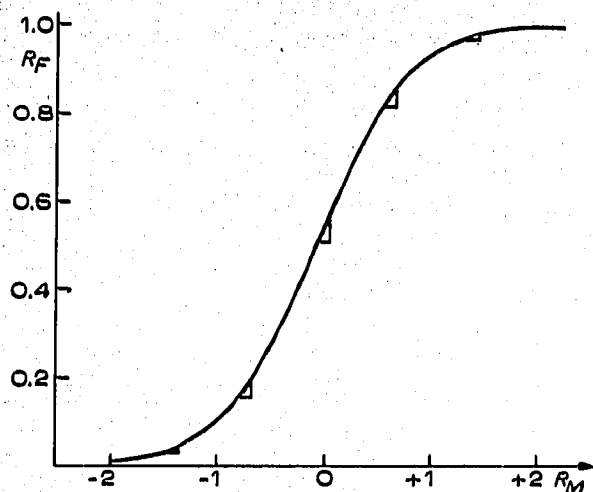


Fig. 1. R_M vs. R_F plot illustrating changes in R_F values (ΔR_F , vertical lines) due to constant changes in R_M values ($\Delta R_M = 0.1$, horizontal lines).

of the ligand necessary to form the extractable species (e.g., hydrogen ions in the aqueous phase or the complexing solvent in the organic phase²). In view of the peculiar sigmoid shape of the R_F vs. R_M curve (Fig. 1), identical changes of R_M (caused, for instance, by a change of pH or composition of the mixed solvent) will be reflected by various changes of the R_F coefficient. It can be seen from Fig. 1 that identical changes of R_M ($\Delta R_M = 0.1$ units) correspond to various ΔR_F values; the ΔR_F values which are a direct measure of irreproducibility of paper chromatographic results, are highest in the proximity of $R_F = 0.5$ (ca. $0.06 R_F$ units per $0.1 R_M$ unit), lower in the range $0.1 < R_F < 0.3$, and quite small for R_F values below 0.1 and above 0.9 . It follows, therefore, that for linear R_M relationships the lowest reproducibility is obtained in the proximity of $R_F = 0.5$.

Among the parameters determining the distribution coefficient D , the temperature should be mentioned⁴ and for experiments carried out at a constant temperature, the pH of the aqueous phase in buffered paper chromatography and composition of the mixed solvent. Irreproducibility of $\log D$ values ($\log D = R_M - \log r$) is then caused by variations of pH (or composition), and, in addition, by variations

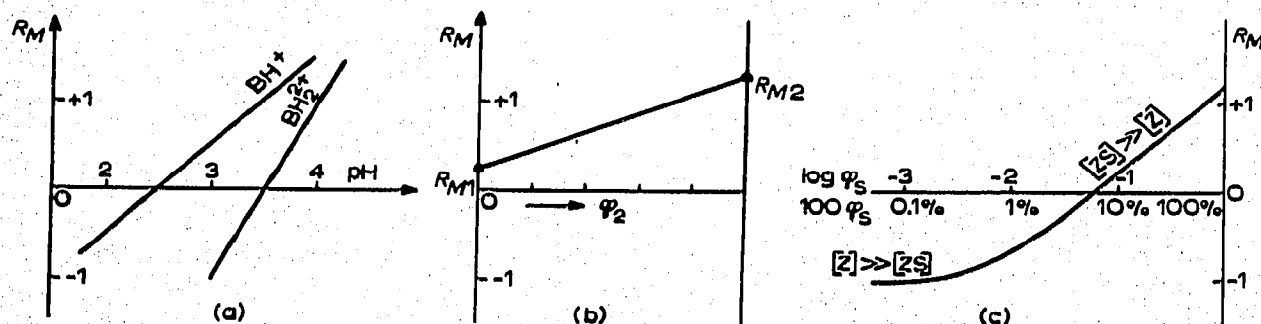


Fig. 2. (a) R_M vs. pH lines (fragments corresponding to double and single ionization; $kr > 100$, where k = partition coefficient of unionized base). (b) Semiempirical linear R_M vs. φ relationship. (c) Semiempirical linear R_M vs. $\log \varphi_S$ relationship; solute Z forms a 1:1 solvation complex with the active component solvent S.

in the slope of the R_M vs. pH (or R_M vs. composition) line (dR_M/dpH , $dR_M/d\varphi$ or, in certain cases, $dR_M/d \log \varphi$, where φ = volume fraction).

In the case of monoprotic electrolytes, under conditions of strong ionization, the slope of the R_M vs. pH line is $+1$ (bases) or -1 (acids), *i.e.*, $\Delta pH = 0.1$ corresponds also to $\Delta R_M = 0.1$. For decreasing ionization, the slope approaches zero; however, for lipophilic electrolytes this occurs beyond the range of accurate R_F values. On the other hand, in the case of biprotic electrolytes the slope may increase to $+2$ (bases) or decrease to -2 (acids) in the range of pH values corresponding to the second degree of ionization² (Fig. 2a).

In the case of solvent systems of the type: binary phase/pure solvent (*e.g.*, cyclohexane + benzene/water), if the component solvents of the mixed phase do not interact strongly with the solute and between themselves, R_M is often linear with respect to the volume composition of the mixed phase^{2,3} (Fig. 2b), the slope of the R_M vs. φ line usually being small and determined by the difference of R_M values obtained for the two simple systems (in the system given above, for the systems cyclohexane/water and benzene/water). The difference is usually greater for solutes of polyfunctional molecules, *e.g.*, alkaloids.

When one of the component solvents of the mixed binary phase can form stable solvation complexes with the solute (*e.g.*, through H-bonding), linear relationships are often obtained when R_M is plotted against \log volume fraction of the active solvent in the mixed phase (strictly speaking, the R_M vs. $\log \varphi$ line deviates to a horizontal asymptote at decreasing concentration of the active solvent, like R_M vs. pH lines). Irreproducibility of R_M values due to a casual change of composition of the mixed solvent in this case also depends on the slope of the R_M vs. $\log \varphi$ line; the slope is in turn determined by the composition of the extractable complex (solute-solvent), association of the solvent etc. It should be pointed out that, in view of the logarithmic horizontal scale, in the linear R_M vs. $\log \varphi$ range a change of concentration of the active solvent from 1 to 2 v/v % has the same effect on R_M as a change from 10 to 20 v/v %. In the linear range, the slope of the line should be *ca.* 1 if the solute Z is largely present as complex ZS with the active solvent S (*cf.* Fig. 2c) and increases to 2 when increasing proportion of solute is present as 2:1 complex, ZS₂ (compare the case of biprotic electrolytes). In the case of ZS₂ complexes the R_M values are, of course, much more sensitive to variations in the composition of the mixed phase. Association of the active solvent (dimerization—carboxylic acids; polymerization—alcohols) tends to decrease the slope of the R_M vs. $\log \varphi$ line⁵.

From the variation of R_M values the corresponding changes in R_F coefficients can easily be estimated on the basis of Fig. 1.

Factors influencing the reproducibility through the volume ratio r

The problem constitutes the main topic of the present communication.

As demonstrated at the preceding Liblice Symposium⁶, the volume ratio of the two liquid phases is mainly determined by the type of paper and the degree of impregnation (or loading); the latter is best defined in terms of milliliters of the fixed phase per one gram of dry paper. The latter parameter depends on the method of impregnation (concentration of nonvolatile liquid in the diluting volatile solvent, repeated impregnation, blotting etc.).

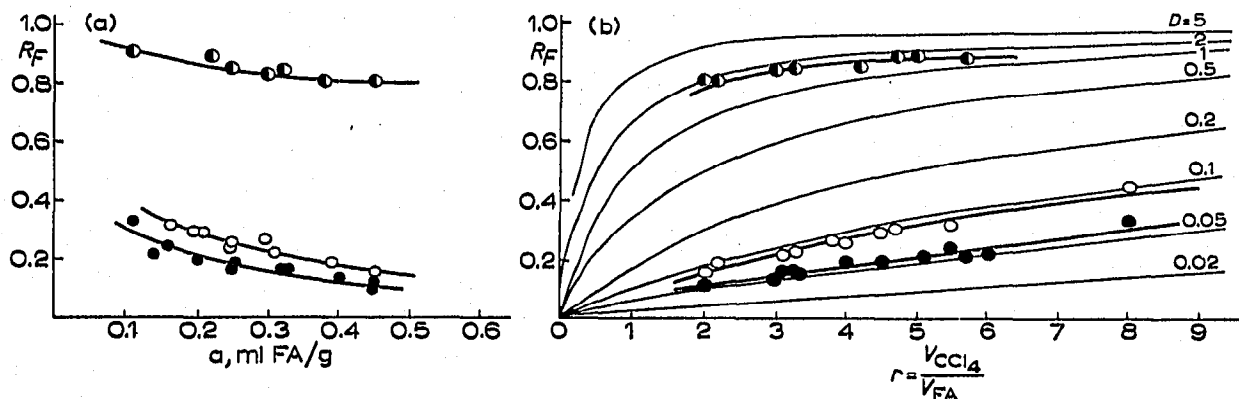


Fig. 3. (a) R_F values of 1-naphthol (O); 2-naphthol (●) and 8-hydroxyquinoline (◐) plotted against degree of loading. (b) Same data, R_F values plotted against volume ratio r . Thin lines: calculated R_F vs. r curves for various values of distribution coefficient. Mobile phase: carbon tetrachloride.

We have carried out a series of investigations using formamide-impregnated Whatman No. 4 paper strips (5×23.5 cm, distance of development by the descending technique 16 cm + 6 cm of solvent level-start line distance).

In Fig. 3a the R_F values of α -naphthol, β -naphthol and 8-hydroxyquinoline are plotted against the degree of impregnation expressed in ml formamide (FA) per one gram of dry paper. As expected, the R_F values decrease with loading in view of the increase of the volume of fixed phase in the paper what entails decrease of the volume ratio r .

In order to investigate if these changes of R_F (irreproducibility due to variation in degree of loading) are in quantitative agreement with decreased values of the volume ratio r , the data are replotted in Fig. 3b against numerical values of the volume ratio reported at the preceding symposium⁰. The experimental data are presented against a background of a family of theoretical R_F vs. r curves calculated from eqn. (1) for a number of values of the distribution coefficient D . It can be seen that the experimental points follow the theoretical lines, which indicates that for the system studied the variations in the degree of loading affect the reproducibility of the R_F values through the volume ratio r .

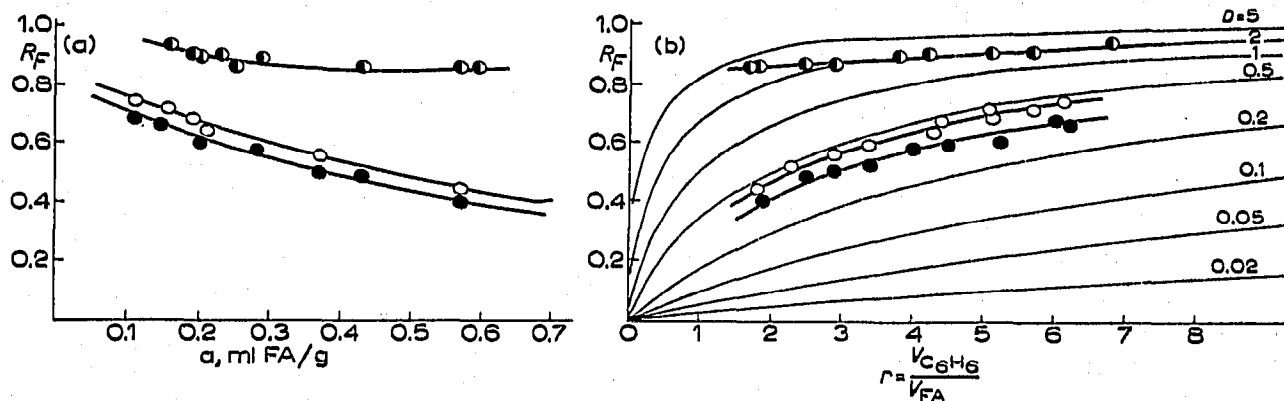


Fig. 4. As in Fig. 3. Mobile phase: benzene.

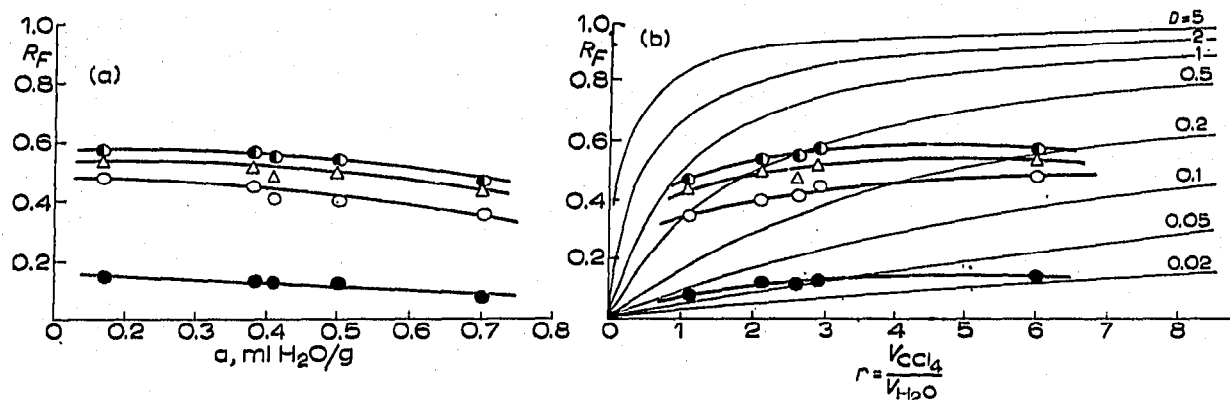


Fig. 5. R_F values of isoquinoline (○); 2-methyl quinoline (●); 3-methylisoquinoline (▲); 2,6-dimethylquinoline (●) plotted against degree of loading (a) and volume ratio r (b). Mobile phase: carbon tetrachloride. Stationary phase: McIlvaine's buffer solution (0.1 M citric acid + 0.2 M Na_2HPO_4).

Further examples are given in Figs. 4a and b (the same solutes in the system benzene/formamide).

Less consistent results have been obtained for some quinoline bases chromatographed in the system carbon tetrachloride/McIlvaine's buffer solution, pH = 3.0 (Fig. 5a, b). Although the R_F values decrease with the degree of loading (ml buffer solution per one gram of dry paper, controlled by weighing), the experimental points deviate from the theoretical R_F vs. r lines. The deviations are probably due to increased concentrations of the buffer solution at lower loadings (lower a and higher r values) causing stronger interactions of the quinoline bases with citric acid ($\geq N \dots HOOC-$).

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