INVESTIGATIONS ON THE MECHANISM AND SELECTIVITY OF CHROMATOGRAPHY ON THIN LAYERS OF POLYAMIDE

I. SYSTEMS OF THE TYPE CYCLOHEXANE + POLAR SOLVENT–POLYAMIDE

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SUMMARY

  For numerous phenols and their derivatives chromatographed in different binary solvent mixtures composed of cyclohexane plus a polar solvent (methylene chloride, *n*-butyl acetate, C_2–C_5 alcohols) the R_M values were found to be linearly dependent on the logarithm of the mole fraction (log X_S) of the polar solvent. The slopes of the graphs of R_M *versus* log X_S could be related to the presumable molecular solvation mechanism only for the non-associated solvents, whereas for solvent systems that contained alcohols only a qualitative interpretation of the semi-empirical relationships could be attempted.

INTRODUCTION

  In a earlier paper[1], we derived an equation that related the R_M values of proton-donor solutes to the concentration of the electron-donor component in the developing binary solvent; the slopes of the graphs of R_M *versus* log X_S, where X_S is the mole fraction of the solvent, provide simple information about the molecular mechanism of sorption and the role of the solvation of the chromatographed solutes in the mobile phase, which depends on the presence of certain functional groups in the solute molecule. On the other hand, the variation of R_F values with the composition of the solvent is of fundamental importance in the optimization of solvent systems, including gradient elution[2,3], and the relationship of the chromatographic behaviour to the molecular structure of the separated solutes may permit a more detailed interpretation of chromatographic data for unknown solutes and a more rational choice of solvent systems for use with solutes of known molecular structure.

  In the earlier work[1], we restricted ourselves to non-associated solvents of moderate polarity (electron-donor solvents in class B of the system of Pimentel and McClellan[4], *e.g.*, ketones, ethers and esters). The experimental data could be interpreted in terms of both adsorption and partition mechanisms (see also ref. 5), the main cause of the variation of R_F values with composition being the gradual shift of

solvation equilibria in the mobile phase. In the present investigation, the range of solvents has been extended to include proton-donor solvents (class A) and strongly polar donor–acceptor solvents (class AB), which are frequently used in thin-layer chromatography (TLC) on polyamides. A wide range of solvents was investigated because the solvent systems reported so far, often multi-component systems[6,7], have not provided clear information about the effect of the mobile phase on the selectivity. The use of different binary solvent mixtures enables the optimal composition to be chosen and facilitates the interpretation of experimental data, especially when one of the components is an inert, non-polar solvent.

EXPERIMENTAL

Thin layers of polyamide (Woelm, G.F.R.) were spread on glass plates; a suspension of 4 g of polyamide in 36 ml of absolute ethanol was used to prepare five 14 × 22 cm plates, the layer thickness being *ca.* 0.1 mm. The plates were dried in air for 24 h and activated before development for 15 min at 80° (ref. 8). Ascending development was carried out in 14 × 7 × 24 cm glass tanks saturated with the vapours of the binary developing solvent, which consisted of cyclohexane plus a polar solvent; the plates were then dried and the solutes detected by spraying the plates with freshly prepared bis-diazotized benzidine[9].

RESULTS AND DISCUSSION

The experimental results (mean values from three runs) are presented as graphs of R_M *versus* log X_S, where X_S is the mole fraction of polar solvent; the R_M axis is directed downwards, as in preceding papers, so that the R_F values increase upwards (see Fig. 1, with the parallel R_F axis). The solutes are denoted by abbreviations derived from the names of the compounds: thus, phenol is denoted by P, naphthalene by N, hydroxyl group by H, methyl group by M, etc.; *e.g.*, 2MP denotes 2-methylphenol, 16HN, 1,6-dihydroxynaphthalene, etc. (for the full list of abbreviations, see Table I).

The difficulties of interpreting the results obtained in open-bed chromatography should be stressed. The X_S values are those of the solvent introduced into the tank; the actual solvent composition in the chromatographic system is changed owing to evaporation of the solvent, especially when the volatilities of the component solvents are different[3]. The formation of composition gradients along the chromatogram (demixting) in both phases and gradual changes in the properties of the polyamide gel with the composition of the binary solvent are further complications that are neglected in the simplified theoretical approach.

In spite of the complexity of the system, in most instances linear R_M *versus* log X_S relationships are observed. Deviations from linearity depend on the individual properties of the solute and on the type of polar solvent involved. For example, with 2,6-xylenol in solvent systems that contain methylene chloride (Fig. 1), at $X_S = 0.6$ the R_M *versus* log X_S line becomes much steeper, the slope increasing almost three times, which indicates a significant increase in affinity for the developing solvent. Linear relationships can be expected when the diluting solvent, cyclohexane, has a much weaker elution strength than the polar solvent. This is not so for 2,6-dimethyl-
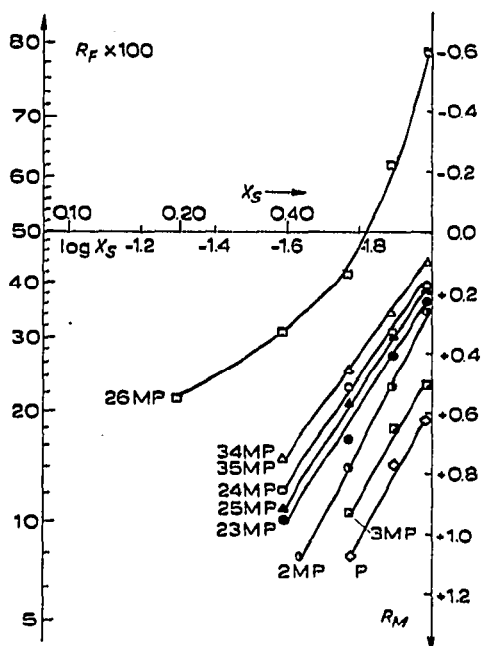
Fig. 1. R_M versus log X_S relationships for phenols. Polar solvent (S): methylene chloride.

dhenol, whose two methyl groups in the *ortho* position strongly decrease the inter-action of the hydroxyl group with polyamide[10]. The increase of the (absolute) slope at higher concentrations of methylene chloride may be due to demixting effects (the spot of 2,6-dimethylphenol is overtaken by the zone containing the increased concen-tration of methylene chloride).

Xylenols and cresols migrate more rapidly than phenol, owing to weaker sorption by polyamide. For non-aqueous developing solvents, the polyamide gel is the more polar, more strongly associated phase[11], and enlargement of the non-polar part of the solute molecule shifts its partition in favour of the mobile phase (*cf.*, Graham and Daly for alkylphenols in polyamide and formamide systems[10] and Przyborowska[12,13] for alkylanilines in formamide systems). The slopes of the graphs of R_M versus log X_S for phenol, cresols and xylenols are approximately 2.0, which could indicate the formation of solvation complexes with a ratio of 2:1; however, a more probable explanation is that the increased concentration of methylene chloride shifts the partition of phenols in favour of the mobile phase by two parallel mecha-nisms: by solvation of phenol molecules in the developing solvent (1:1 ratio for sol-vents of class A) and by competitive solvation of carbonyl groups in the polyamide gel (compare an analogous situation in adsorption chromatography[14,15]). Dihydroxy and trihydroxy derivatives of benzene, as well as nitrophenols, were very strongly sorbed by polyamide, even from pure methylene chloride, so that their R_M versus log X_S relationships could not be determined.

For alkylphenols and polyhydroxyphenols, good selectivity was obtained for the system cyclohexane + *n*-butyl acetate (Figs. 2 and 3), complete separation of phenol, three cresols and four xylenols being achieved, as well as good group sepa-
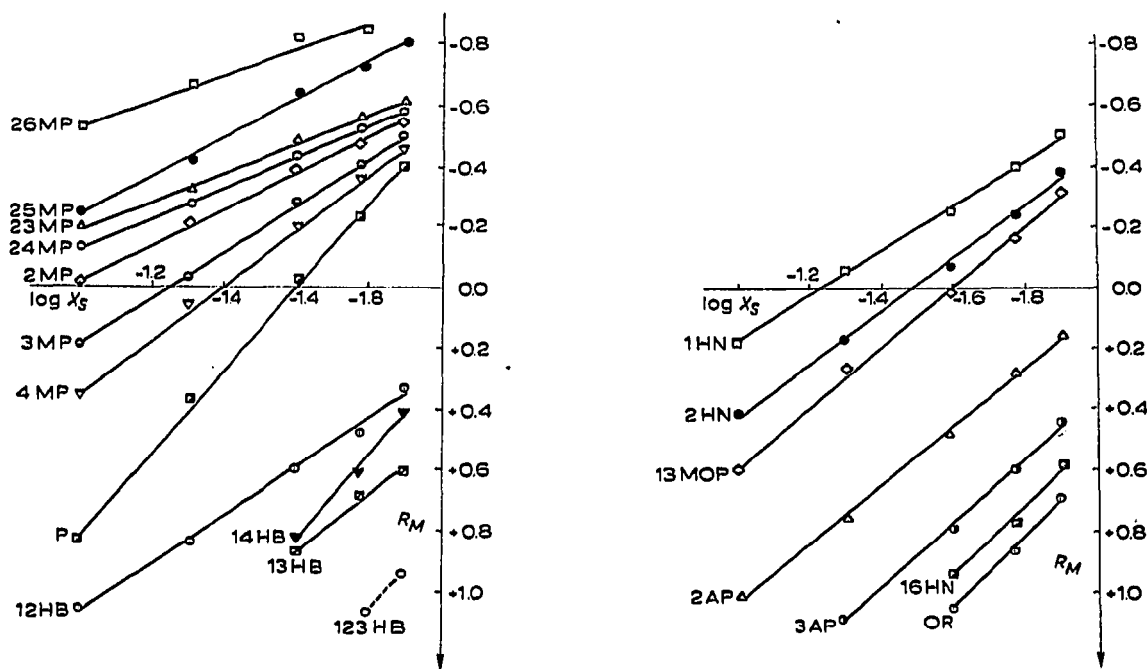
Fig. 2. R_M versus log X_S relationships for phenols. Polar solvent (S): n-butyl acetate.

Fig. 3. R_M versus log X_S relationships for phenols. Polar solvent (S): n-butyl acetate.

ration of polyhydroxy compounds. The cresols were found to migrate in the following order (at $X_S = 0.2$): o-cresol (0.61) > m-cresol (0.52) > p-cresol (0.45), the migration rate decreasing with the distance between the methyl and the hydroxyl groups.

The constitutional effects are also apparent with xylenols, the R_F values decreasing in the following order (for $X_S = 0.2$): 2,6-xylenol (0.82) > 2,5-xylenol (0.73) > 2,3-xylenol (0.68) > 2,4-xylenol (0.63).

The slopes of the graphs of R_M versus log X_S are lower than those theoretically expected for both alkylphenols and dihydroxy compounds (cf. Table I).

The use of solvents of class A and B for the chromatography of phenols on polyamide gave unsatisfactory results in many instances: some solutes were strongly sorbed by polyamide and the R_F values were too low, especially for di- and tri-hydroxybenzenes, dihydroxynaphthalenes and nitrophenols. It might be expected that the replacement of solvents of class B by more polar solvents of class AB would permit higher R_F values to be obtained. Four n-alcohols were chosen as typical donor–acceptor solvents: ethanol, propanol, butanol and pentanol. Using solutions of these alcohols in cyclohexane, R_M versus log X_S relationships were determined for a number of phenols (Figs. 4–10). The relationships were found to be linear over the whole range of compositions ($0.1 < X_S < 0.8$) only for ethanol and propanol; for the two higher alcohols, linear plots were obtained only for lower R_F values of the solutes. The graphs for phenol and its methyl derivatives, the R_F values of which vary in the range 0.20–0.85, were found to deviate downwards at X_S values greater than 0.4 for butanol and 0.6 for pentanol.

TABLE I

ABSOLUTE SLOPES OF THE GRAPHS OF R_M versus LOG X_S FOR PHENOL AND NAPH-THOL DERIVATIVES

Polar solvents S of class A, B and AB; diluting solvent, cyclohexane.

Solute	Abbreviation	Methyl-ene chloride	n-Butyl acetate	Ethanol	Propa-nol	Butanol	Pentanol
Phenol	P	2.3	1.3	1.4	1.3	1.6*	0.9*
o-Cresol	2MP	2.2	0.6	1.6	1.4	1.5*	0.9*
m-Cresol	3MP	2.0	0.7	1.6	1.4	1.5*	0.9*
p-Cresol	4MP	—	0.9	—	—	—	—
2,3-Xylenol	23MP	2.0	0.5	1.6	1.2	1.3*	1.2*
2,4-Xylenol	24MP	1.9	0.5	1.6	1.1	1.3*	1.2*
2,5-Xylenol	25MP	1.9	0.6	1.6	1.1	1.2*	1.2*
2,6-Xylenol	26MP	0.9/2.7	0.45	1.6	1.1	1.2*	0.9*
3,4-Xylenol	34MP	1.6	0.9	1.6	1.1	1.2*	1.2*
3,5-Xylenol	35MP	1.6	0.9	1.6	1.1	1.2*	1.2*
Pyrocatechol	12HB	—	0.85	1.4	1.5	1.2	1.3
Resorcinol	13HB	—	0.9	1.65	1.9	1.5	1.3
Hydroquinone	14HB	—	1.4	1.65	2.4	1.8	1.3
Phloroglucinol	135HB	—	—	1.65	2.0	1.5	1.6
Pyrogallol	123HB	—	—	1.7	2.4	1.2	1.2
p-Methoxyphenol	14MOP	—	1.1	1.8	—	2.1	0.9
Orcinol (3,5-di-hydroxytoluene)	OR	—	1.2	1.3	1.5	1.4	1.2
m-Nitrophenol	3NP	—	—	1.7	1.6	—	—
p-Nitrophenol	4NP	—	—	2.2	1.6	1.3	1.3
o-Aminophenol	2AP	—	0.9	1.9	1.6	1.3	1.3*
m-Aminophenol	3AP	—	1.1	1.9	1.6	1.3	1.3*
p-Aminophenol	4AP	—	—	2.9	2.0	1.4	1.4
o-Chlorophenol	2CP	—	—	1.6	1.4	0.9	1.0
m-Chlorophenol	3CP	—	—	1.6	—	0.9	—
2,6-Dichlorophenol	26CP	—	—	1.5	1.0	—	—
o-Bromophenol	2BP	—	—	1.4	1.4	1.2	—
o-Iodophenol	2IP	1.4	0.6	1.3	1.0	0.9	—
1-Naphthol	1HN	—	0.75	1.6	1.5	1.3	1.5*
2-Naphthol	2HN	—	1.3	1.6	1.5	1.3	1.5*
1,6-Dihydroxy-naphthalene	16HN	—	—	2.2	1.8	1.7	1.7
1,5-Dihydroxy-naphthalene	15HN	—	—	2.5	2.0	1.9	1.6
2,3-Dihydroxy-naphthalene	23HN	—	—	2.0	1.7	1.3	1.5
2,7-Dihydroxy-naphthalene	27HN	—	—	2.2	1.8	1.3	1.7

* The slope increases at $X_S = 0.4$ for butanol and $X_S = 0.6$ for pentanol.

The spots were found to be more diffuse for the higher alcohols, possibly owing to the decreased ability of the solvents of larger molecular size to penetrate the polymer, with a resulting lower linear capacity of the stationary phase and greater viscosity. For the homologous series of alcohols, only a slight variation in the R_F values of individual solutes was observed, which probably was also caused by a parallel decrease in solubility in both phases of the higher alcohols.
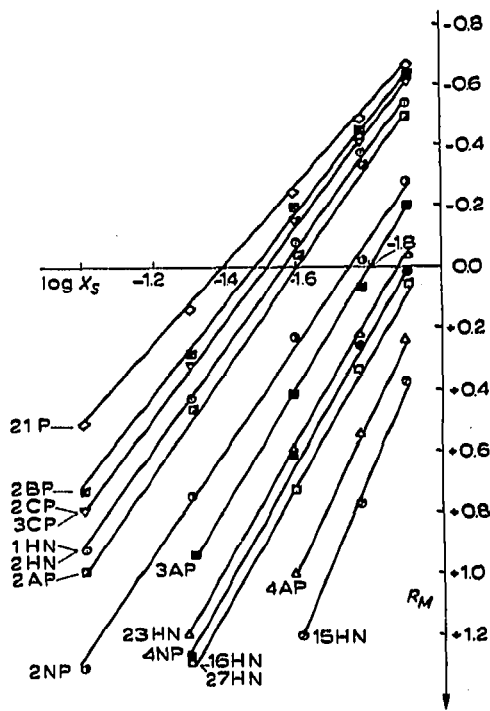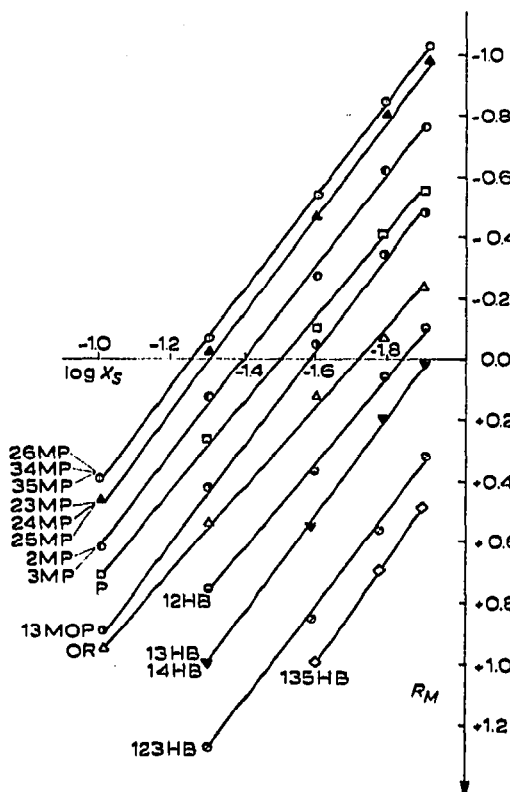
Fig. 4. R_M *versus* log X_S relationships for phenols. Polar solvent (S): ethanol.

Fig. 5. R_M *versus* log X_S relationships for phenols. Polar solvent (S): ethanol.

Owing to the greater solvent strength of solvents of class AB, the R_F values of phenols chromatographed in alcohol systems were markedly higher than those obtained in solvents of classes A and B. The slopes of the graphs of R_M *versus* log X_S (Table I) are less sensitive to differences in the molecular structures of phenols; thus, for ethanol the slopes are in the range 1.4–2.0 for most solutes containing one, two or even three polar groups, so that the graphs of R_M *versus* log X_S are usually parallel.

The fundamental causes of this difference in behaviour of N + AB solvent systems are presumably the strong tendency of alcohol molecules to associate and their strong interaction with the carbonyl groups of polyamide. In pure alcohols and their concentrated solutions, dimers are mostly formed, their association energies being *ca.* 13 kcal/mole[16]; in more dilute solutions in inert solvents, linear associates of lower association energy are formed, the degree of association decreasing with dilution. The R_M *versus* log X_S relationships were determined for a wide range of concentrations, so that varying association of the alcohol molecules was certainly superimposed on the over-all variation of R_M values. Furthermore, the use of solvents of class AB alters the pattern of molecular interactions between the polar groups of polyamide (A), solute (Z) and polar solvent molecules (S) (hydrogen bonding is
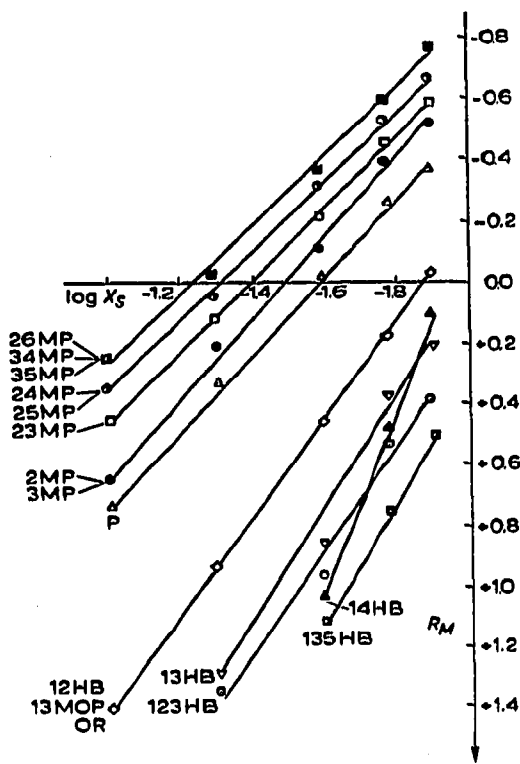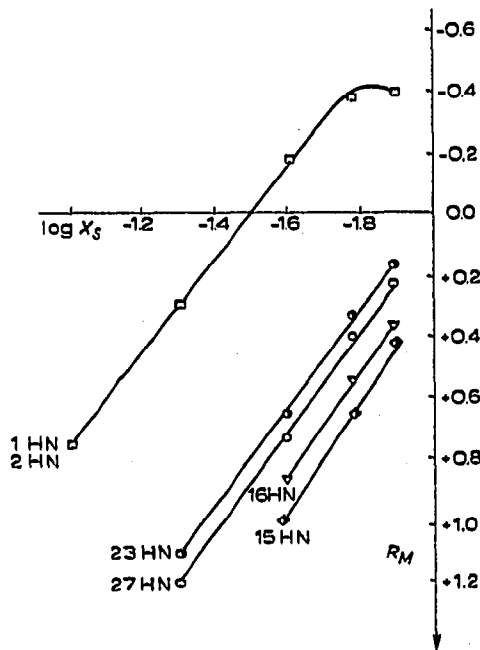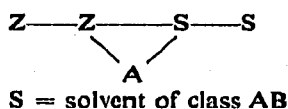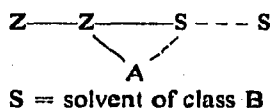
Fig. 6. R_M *versus* log X_S relationships for phenols. Polar solvent (S): *n*-propanol.

Fig. 7. R_M *versus* log X_S relationships for phenols. Polar solvent (S): *n*-propanol.

represented by solid lines and weaker interactions by dashed lines):

S = solvent of class B   S = solvent of class AB

     Therefore, the model discussed in the preceding paper[1], in which the S–S and A–S interactions were neglected as being weaker than the A–Z and Z–S interactions, cannot be applied to alcoholic systems without corrections being made. The association of alcohol molecules (S–S) probably plays a less significant role, as it was demonstrated[17,18] that solute hydroxyl groups can be included in the association chain at any position so that self-association is not competitive and does not decrease the solvent strength of the alcohol; thus, the slope of the graphs of R_M *versus* log X_S is presumably influenced by A–S interactions (solvation of carbonyl groups in the polyamide gel, competitive with phenol–polyamide interactions) and the variation of the degree of swelling of polyamide with the composition of the developing solvent[1]. The latter effect is probably responsible for the similar slopes obtained for mono- and dihydroxy compounds; increasing sorption strength of the polyamide gel should have a greater effect for polyfunctional solutes, and a partial counteracting of increased
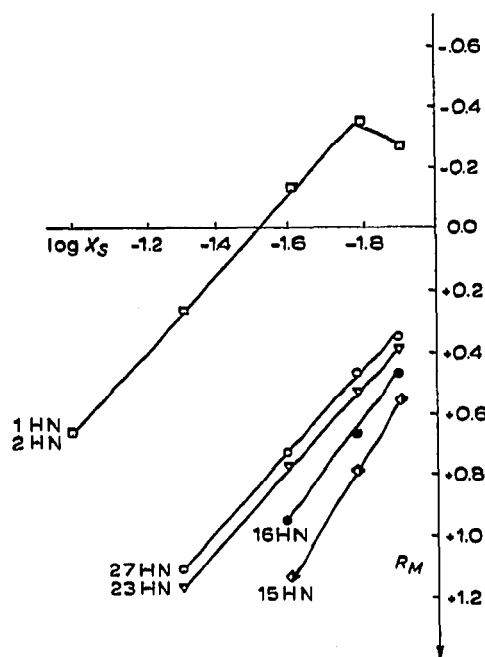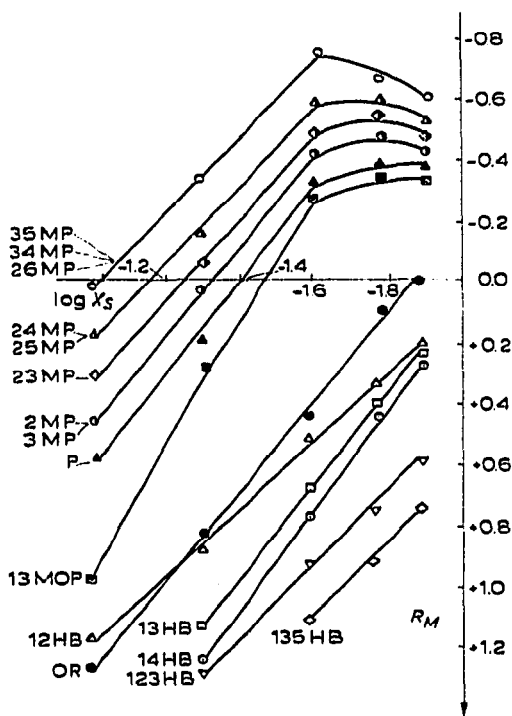
Fig. 8. R_M versus log X_S relationships for phenols. Polar solvent (S): n-butanol.

Fig. 9. R_M versus log X_S relationships for phenols. Polar solvent (S): n-butanol.

solubility in the developing solvent can then be the cause of decreased slopes of the graphs of R_M versus log X_S, this decrease being more pronounced for more polar solutes.

Although the interaction pattern complicates the interpretation of the experimental relationships, the application of solvent mixtures of the N + AB type in chromatography on thin layers of polyamide can in many instances be advantageous or even necessary, especially for compounds with several polar groups that are strongly sorbed by polyamide. For the solvent systems investigated, the chromatographic partition process is largely based on solvation equilibria in the mobile phase (formation of solvation complexes of definite composition) and sorption in the polyamide gel, the contributions of adsorption and absorption being dependent on the type of solvent and its composition (compare the $R_M = f(\log X_S)$ relationships in refs. 1, 5 and 15). It is remarkable that even for more complex interaction patterns, the chromatographic behaviour of many solutes in binary solvent mixtures composed of a polar solvent and an inert diluting solvent can be described by simple equations that have been found to be characteristic for many partition and adsorption systems:

$$R_M = a - b \log X_S$$

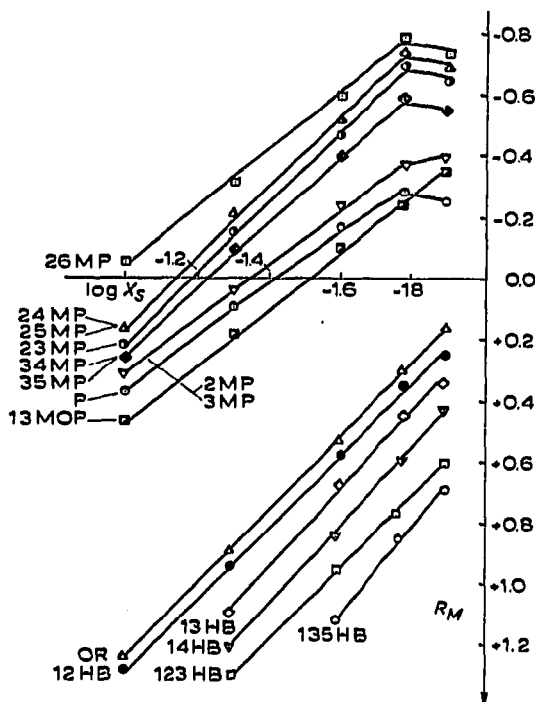where a and b are constants, the latter being simply related to the molecular sorption

Fig. 10. R_M *versus* log X_S relationships for phenols. Polar solvent (S): *n*-pentanol.
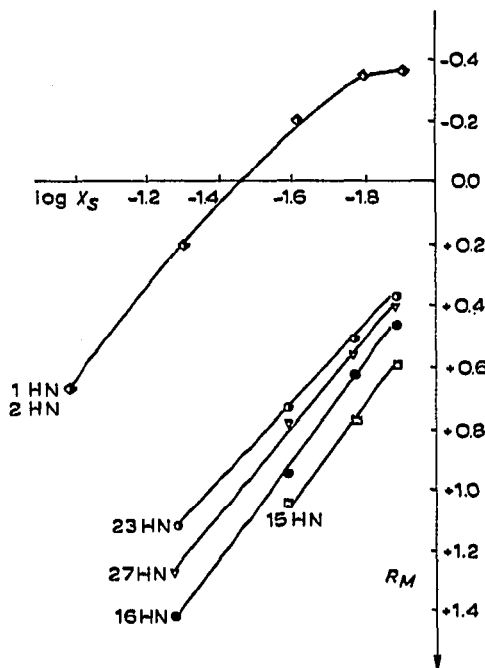
Fig. 11. R_M *versus* log X_S relationships for phenols. Polar solvent (S): *n*-pentanol.

mechanism for chromatographic systems that obey the simplification assumed in the theoretical interpretation.

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