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

III. MOBILE PHASES CONSISTING OF A COMBINATION OF POLAR SOLVENTS

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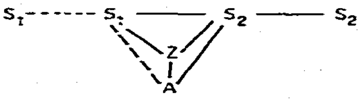
SUMMARY

A number of phenols was chromatographed using solvent systems composed of liquids of various hydrogen bonding abilities: A + B, A + AB, B + AB and AB + AB (where A = proton donor, B = electron donor and AB = proton and electron donor). The experimental results represented as R_M *versus* composition relationships are interpreted in terms of the molecular interactions involved.

INTRODUCTION

In previous investigations on polyamide TLC[1-3], thin layers of polyamide were developed with binary mixed solvents of the type N + A, N + B and N + AB[4], the non-polar diluent (N) being cyclohexane in all instances. These solvent systems are relatively simple, especially when the polar component is not associated; the effect of the composition on the chromatographic sorption coefficient can then be related to solvation equilibria in the mobile phase, and although the real situation is often different from the idealized model system and the chromatographic formation constants are based on the simplified version of the law of mass action, the R_M *versus* composition relationships can at least be regarded as semi-empirical (see Eon and Karger[5] for a discussion of the application of the law of mass action to partition equilibria; *cf.* also refs. 6 and 7). Further, the distortions due to the variation of the formation constants, use of other concentration scales, etc., are often similar for related substances so that analogies in behaviour are then apparent from chromatographic relationships.

It is frequently necessary to use mixed polar solvents; however, these systems have not been considered in detail theoretically owing to very complex intermolecular interactions involved. For adsorption chromatography, general equations relating the adsorption coefficient and the composition of the mixed solvent have been derived by Snyder[8] and Ościk and Różyło[9], and for liquid–liquid partition chromatography systematic studies on composition effects have been carried out by Gross[10]. However, no systematic investigations of this type have been carried out for polyamide systems. In the present study, composition effects were investigated for thin layers of polyamide developed with mixtures of polar solvents of the types A + B, A + AB, B + AB and AB + AB. The molecular interactions are even more complex than those for N + AB mixtures discussed in a previous paper[2]: for instance, the pattern of interactions of a solute Z with polar solvents S_1 (type B) and S_2 (type AB) and a sorption site A can be represented in a simplified manner as follows (neglecting the association of solute Z at low concentrations):



All components of the system, in addition to non-specific interactions, can form hydrogen bonds, including self-association of the solvent of the AB class (S_2–S_2 interactions). Contrary to the situation in previously studied systems, the solute can interact specifically with both solvents and association of the two component solvents also occurs. The formation constants of the various complexes influence to

TABLE I

$R_F \cdot 100$ VALUES OF PHENOLS FOR BINARY SOLVENTS CONTAINING VARIOUS MOLE FRACTIONS OF THE MORE POLAR SOLVENT (S)

Solute	*Chloroform - acetone (S)*					*Chloroform - diethyl ether (S)*					*Chloroform - ethanol (S)*				
	0.2	*0.4*	*0.6*	*0.8*	*0.9*	*0.2*	*0.4*	*0.6*	*0.8*	*0.9*	*0.2*	*0.4*	*0.6*	*0.8*	*0.9*
Phenol	47	58	65	71	80	48	55	62	68	77	72	82	85	86	86
o-Cresol	65	67	71	78	82	58	62	69	75	81	75	83	86	87	87
m-Cresol	62	64	69	72	75	56	59	64	72	79	—	—	—	—	—
p-Cresol	62	64	69	72	75	56	59	64	72	79	—	—	—	—	—
2,3-Xylenol	—	—	—	—	—	—	—	—	—	—	80	85	87	88	88
2,5-Xylenol	67	72	77	80	84	—	—	—	—	—	—	—	—	—	—
2,6-Xylenol	82	87	90	91	92	—	—	—	—	—	—	—	—	—	—
3,4-Xylenol	—	—	—	—	—	64	68	75	80	87	—	—	—	—	—
3,5-Xylenol	—	—	—	—	—	64	68	75	80	87	—	—	—	—	—
Pyrocatechol	20	32	45	53	56	13	19	27	32	35	41	64	76	67	64
Resorcinol	7	16	27	37	41	—	—	9	11	13	17	47	66	62	60
Hydroquinone	8	20	30	42	46	—	8	11	13	15	21	51	72	70	68
Phloroglucinol	—	—	—	9	13	—	—	—	—	—	—	17	30	40	45
Pyrogallol	—	11	21	31	35	—	—	—	—	—	15	29	45	51	54
p-Methoxyphenol	54	60	65	73	90	—	—	—	—	—	—	—	—	—	
Orcinol	—	—	—	—	—	—	10	13	15	17	—	—	—	—	—
o-Aminophenol	33	45	60	68	83	16	21	24	26	27	—	—	—	—	—
m-Aminophenol	24	39	52	65	80	—	—	—	—	—	46	68	75	76	72
p-Aminophenol	—	—	—	—	—	—	—	—	—	—	8	41	62	55	46
1-Naphthol	50	55	63	72	88	42	47	54	60	66	67	83	86	84	76
2-Naphthol	50	55	63	72	88	40	45	49	55	60	67	83	86	84	76
1,6-Hydroxynaphthalene	—	13	21	28	33	—	—	—	—	—	16	35	52	52	49
1,5-Hydroxynaphthalene	—	9	17	21	25	—	—	—	—	—	14	30	35	39	40
2,3-Hydroxynaphthalene	—	19	30	35	39	9	12	16	19	20	14	30	35	39	40
2,7-Hydroxynaphthalene	—	15	24	33	36	—	—	—	—	—	—	—	—	—	—

different extents the R_M *versus* composition relationships, depending on the molecular interaction forces, so that the R_M *versus* log X_S lines (X_S is the mole fraction of the more polar solvent, S) can have various slopes and deviate from linearity, which may even result in changes in the sequences of the spots. The system, although more complex, is therefore also more flexible and more promising as far as selectivity is concerned.

The chromatographic investigations have shown that for these complex solvent systems, a quantitative theoretical treatment is very difficult and attempts to introduce the various complexation constants fail because the systems are too complicated. At present, only attempts at a qualitative interpretation of the chromatographic relationships are possible.

EXPERIMENTAL

Thin layers of polyamide (Woelm, Eschwege, G.F.R.) were spread on mirror glass plates and activated as described previously[2]. The chromatograms were developed in glass tanks by the ascending technique, using mixtures of polar solvents belonging to classes A, B and AB in the classification of Pimentel and McClellan[1].

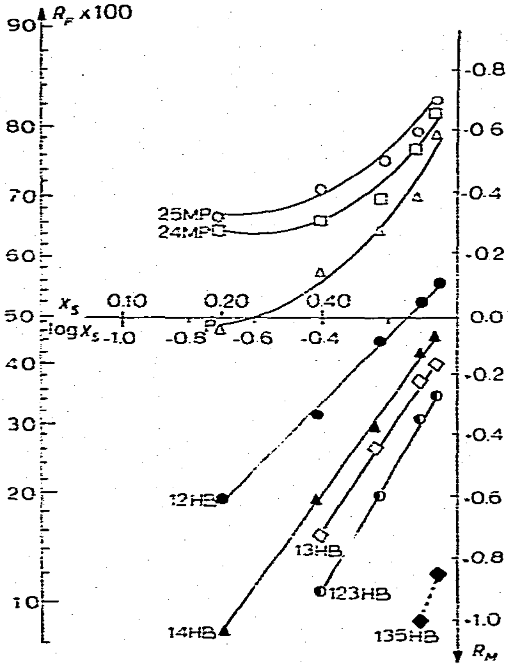
Chloroform - propanol (S)					*Chloroform - n-pentanol (S)*					*Acetone - ethanol (S)*					*Acetone - acetic acid (S)*					*Ethanol - acetic acid (S)*				
0.2	*0.4*	*0.6*	*0.8*	*0.9*	*0.2*	*0.4*	*0.6*	*0.8*	*0.9*	*0.2*	*0.4*	*0.6*	*0.8*	*0.9*	*0.2*	*0.4*	*0.6*	*0.8*	*0.9*	*0.2*	*0.4*	*0.6*	*0.8*	*0.9*
58	72	78	74	71	—	—	—	—	—	76	78	80	80	80	76	83	86	87	88	67	72	75	77	78
—	—	—	—	—	—	—	—	—	—	76	78	80	80	80	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	77	80	84	85	86
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
36	46	55	50	46	36	47	49	47	45	63	70	76	77	77	67	74	78	80	82	57	62	67	70	73
17	35	47	46	44	24	34	37	37	36	63	70	76	77	77	64	70	75	78	80	46	52	58	62	65
20	40	52	57	60	34	39	42	42	41	63	70	76	77	77	56	65	71	75	77	53	58	64	67	70
—	10	16	21	24		8	14	13	12	44	51	57	61	55	28	39	47	52	50	24	30	35	39	42
9	15	19	24	26	9	18	20	20	19	47	56	62	64	58	47	55	65	70	69	39	45	52	55	57
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
55	66	70	63	58	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
45	60	66	58	55	39	46	54	47	46	71	76	78	79	78	—	—	—	—	—	—	—	—	—	—
20	40	45	34	27	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
66	73	75	65	61	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
70	75	77	68	63	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
9	27	39	36	35	13	19	23	24	22	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
9	27	39	36	35	8	16	21	19	17	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
34	44	49	45	41	25	37	40	39	38	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
12	32	42	40	38	20	25	27	28	27	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

RESULTS AND DISCUSSION

The experimental results given in Table I are presented as R_M *versus* log X_s relationships in Figs. 1–13: the solutes are denoted by abbreviations as in Part II[3].

As binary solvents of the A + B type, mixtures of chloroform with acetone or diethyl ether were used (Figs. 1–3). The R_M *versus* log X_s relationships are linear for these compounds, which are strongly sorbed from the less polar solvent, *i.e.*, for di- and trihydroxybenzenes (Figs. 1 and 3), dihydroxynaphthalenes (Fig. 2) and *p*-nitro- and *o*-aminophenols (Fig. 3). For the remaining phenols, the R_M *versus* log X_s lines are characteristic for instances when the diluting solvent contributes to desorption (or extraction) of the chromatographed solute (see Part II[3]). The R_F values are higher than for analogous systems of the N + B type in which chloroform is replaced by cyclohexane. For this reason also, the R_M *versus* log X_s lines are less steep than for the analogous N + B systems. Hence, although chloroform is a much weaker solvent than acetone or diethyl ether and can be considered as a diluent, its contribution to the solvent strength of the mixtures is reflected by the non-linear R_M *versus* log X_s lines for the less polar solutes and generally by less steep relationships.

Mixed solvents of the A + AB type were composed of chloroform (A) and alcohols (AB), *viz.* ethanol, *n*-propanol and *n*-pentanol (Figs. 4–9). In most instances, the R_M *versus* log X_s relationships are non-linear, a distinct maximum being observed at *ca.* 0.6 mole fraction of the more polar solvent. This maximum becomes less appar-



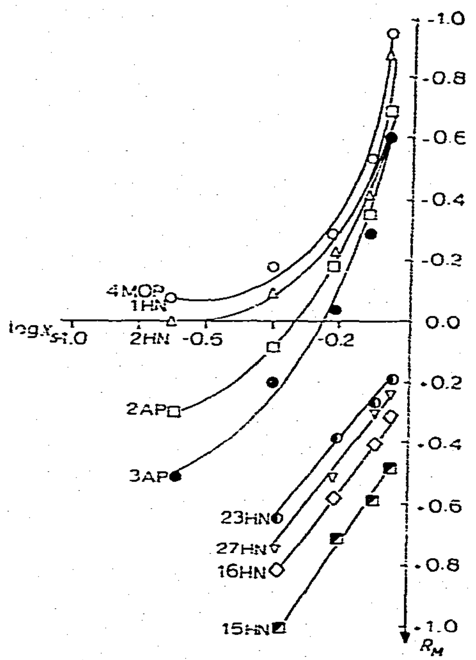
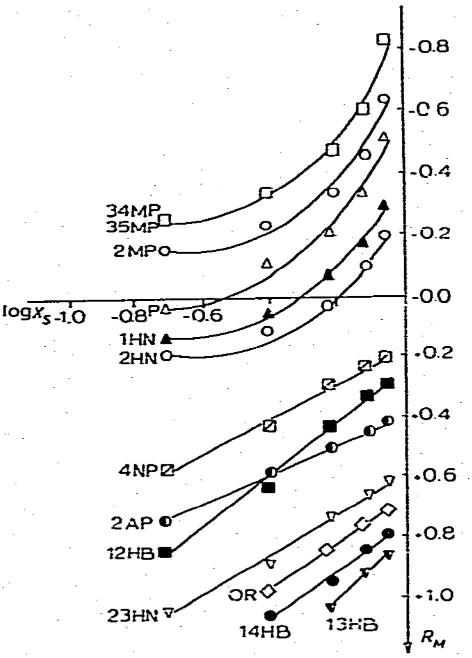


Fig. 1. R_M *versus* log X_s relationships for phenols. Polar solvents: chloroform + acetone (S). For full list of abbreviations, see refs. 2 and 3.

Fig. 2. R_M *versus* log X_s relationships for phenols. Polar solvents: chloroform + acetone (S).



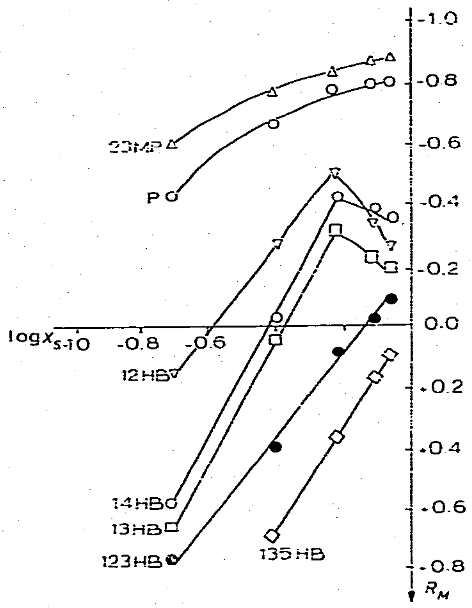
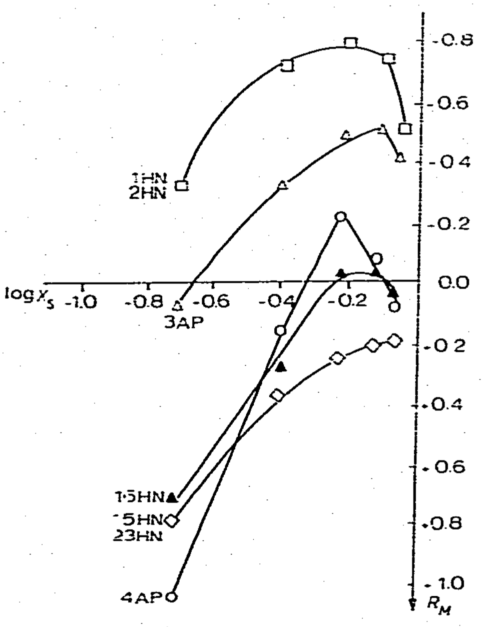


Fig. 3. R_M *versus* log X_S relationships for phenols. Polar solvents: chloroform + diethyl ether (S).

Fig. 4. R_M *versus* log X_S relationships for phenols. Polar solvents: chloroform + ethanol (S).



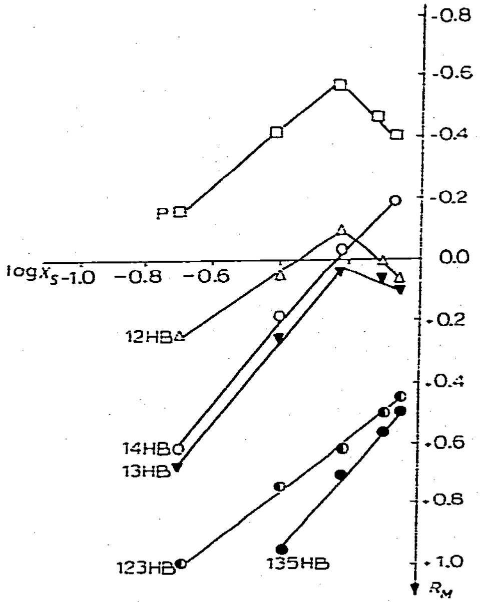
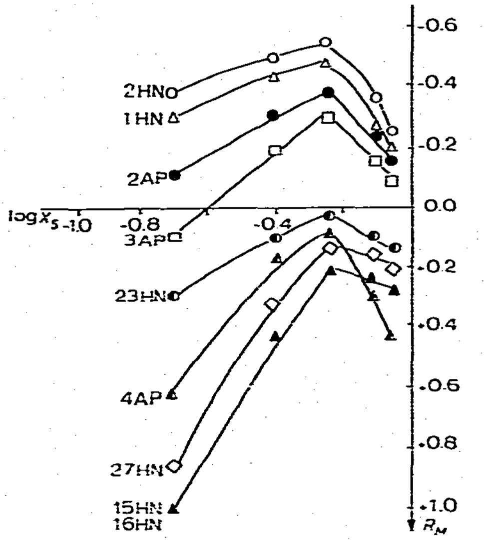


Fig. 5. R_M *versus* log X_S relationships for phenols. Polar solvents: chloroform + ethanol (S).

Fig. 6. R_M *versus* log X_S relationships for phenols. Polar solvents: chloroform + propanol (S).



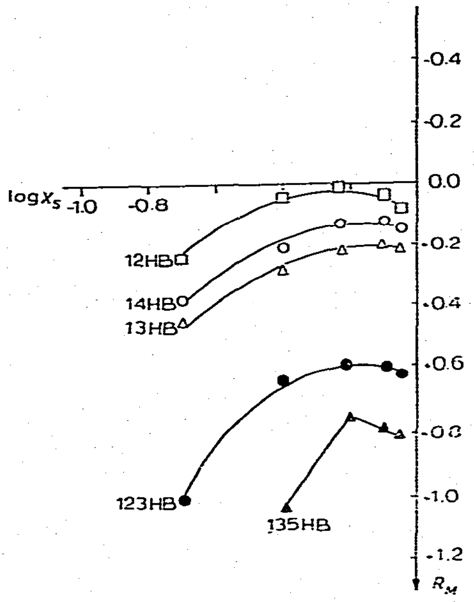
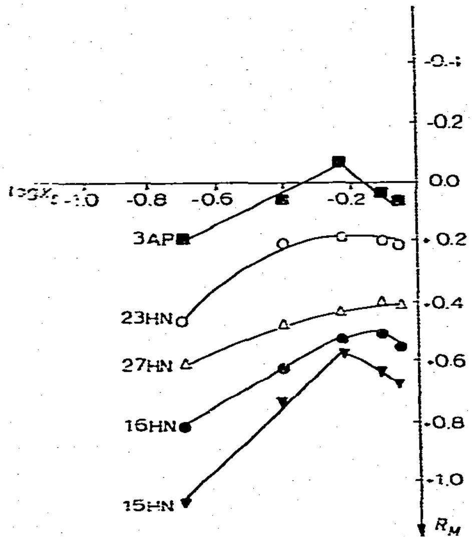


Fig. 7. R_M *versus* log X_S relationships for phenols. Polar solvents: chloroform + propanol (S).

Fig. 8. R_M *versus* log X_S relationships for phenols. Polar solvents: chloroform + *n*-pentanol (S).



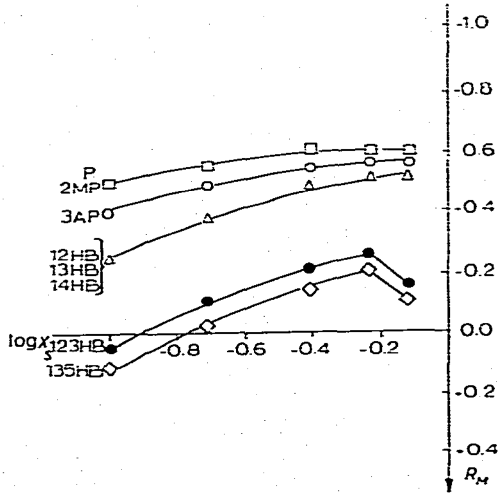
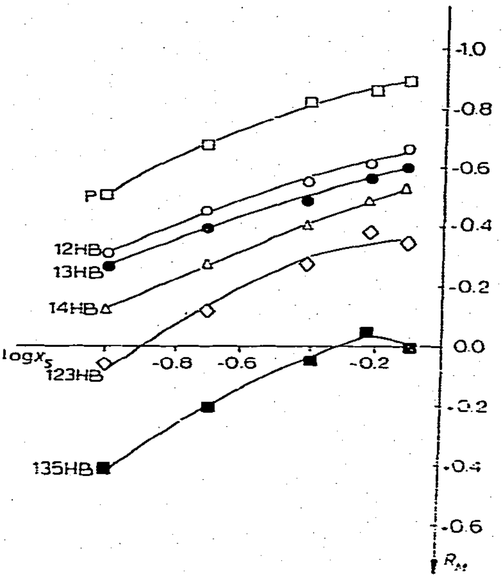


Fig. 9. R_M *versus* log X_S relationships for phenols. Polar solvents: chloroform + *n*-pentanol (S).

Fig. 10. R_M *versus* log X_S relationships for phenols. Polar solvents: acetone + ethanol (S).



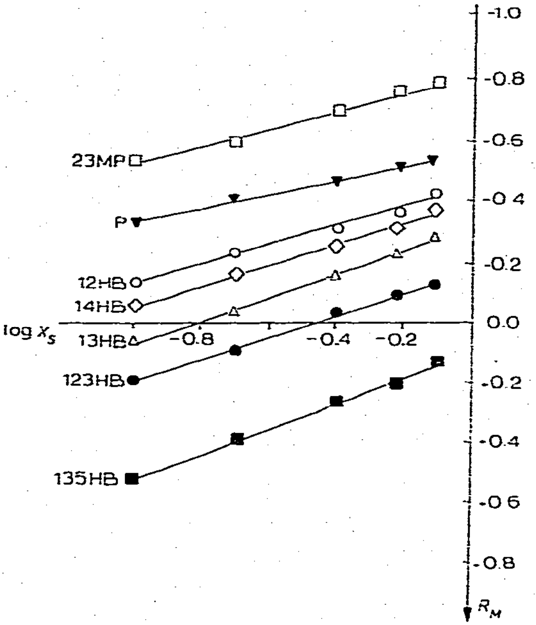


Fig. 11. R_M *versus* log X_S relationships for phenols. Polar solvents: acetone + acetic acid (S).

Fig. 12. R_M *versus* log X_S relationships for phenols. Polar solvents: ethanol + acetic acid (S).

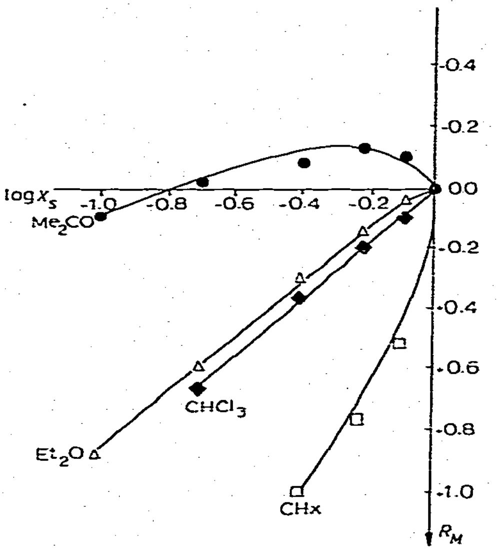


Fig. 13. Effect of less polar component on R_M *versus* log X_S relationships for phloroglucinol. Polar solvent (S): ethanol. Me$_2$CO = acetone; Et$_2$O = diethyl ether; CHCl$_3$ = chloroform; CHx = cyclohexane.

ent with increase in the molar volume of the alcohol (compare Figs. 4, 6 and 8). Only for trihydroxybenzenes, which are strongly sorbed from chloroform, are the R_M *versus* log X_s relationships linear, the lines being less steep than expected for trifunctional solutes. The contribution of chloroform to the solvent strength of the developing solvent is also reflected by increased R_F values for all compounds in A + B systems in comparison with N + B systems.

In further experiments, solvent mixtures of the B + AB and AB + AB types were investigated. For the former type, acetone (B) and ethanol or acetic acid (AB) were used; mixtures of the AB + AB type were composed of ethanol and acetic acid. The R_M *versus* composition relationships are presented in Figs. 10–12. In all instances high R_F values for most phenols were obtained, especially for the less polar cresols and xylenols. In most instances, the R_M *versus* log X_s relationships are linear and the slopes are low owing to the comparable solvent strengths of both components of the developing solvent. In Fig. 13, the effect of the diluting solvent is illustrated for ethanolic systems; it can be seen that the lowest and steepest relationships are obtained for cyclohexane; for the acetone–ethanol system the line is flat, with a weak maximum.

It is worth noting that substitution of cyclohexane by a more polar solvent, while reducing the range of variation of R_F values owing to the general increase in R_F values, increases the capacity of the system so that the spots are more compact and less diffuse. Therefore, solvent mixtures composed of two polar solvents are advantageous, especially for strongly sorbed polar compounds or those which tend to form tailing spots; on the other hand, differentiated R_M *versus* log X_s relationships for more complex solutes facilitate the choice of optimal conditions for the chromatographic analysis.

The quantitative interpretation of R_M *versus* composition relationships and molecular structure effects (for instance, based on the thermodynamic properties of mixed solvents) is additionally complicated by the variability of the properties of the polyamide gel; polyamide absorbs with some selectivity the more polar component of the mixture, so that the properties of the gel depend not only on the type of solvents in the mobile phase, but also on its composition. Further complications are introduced by demixing of the mobile phase, which leads to gradients of composition of both phases along the chromatogram. These phenomena will also depend on the molecular structure of the solute and its relative position on the chromatogram. Therefore, only empirical generalizations can be made that characterize the effects of the composition and molecular type of the solvents on the chromatographic parameters of various molecular structures; nevertheless, it seems that even a general description of R_M *versus* composition relationships can be helpful in establishing the optimal conditions in chromatographic systems.

The analysis of solvent composition effects is easier in the R_M *versus* log X_s coordinate system used in our papers than in R_F *versus* % S plots employed by other workers[11,12], because the former system is more simply related to equations derived from the law of mass action[1,5,7]. For 1:1 solvation complexes, simpler relationships should also be obtained by plotting the ratio of the amounts of the solute present in the mobile and stationary phases (inverse capacity factor, $R_F/(1-R_F)$) against % S (tables of $R_F/(1-R_F)$ values have been published[13,14]).

The effect of solvent composition on R_F values in polyamide systems could probably be considered as the sum of the contributions of adsorption and solubility in the polyamide gel. Assuming an idealized model of the chromatographic system[15]:

$$k' = k_p V_{st}/V_m + k_a S/V_m$$

where k' is the overall capacity factor, k_p is the (dynamic) partition coefficient (C_{st}/C_m, the amounts related to the volume of the liquid gel-like stationary phase, V_{st}, and the volume of the mobile phase, V_m) and k_a is the adsorption coefficient, the amounts of solute related to unit surface area (S) of the exposed crystalline regions and to the volume of the mobile phase, then

$$R_F = 1/(1 + k') \text{ and } R_M = \log k'.$$

Increased concentrations of the polar solvent in the mobile phase have complex effects on the parameters involved, depending also on the molecular structure of the solute. The following main contributions can be considered:

(*a*) Variation of interactions in the mobile phase ("solubility"). For less polar solutes, *e.g.*, anthracene, the "solubility" sometimes decreases with increase in the polarity of the mobile phase, especially when associating solvents of the AB class are used, and, when water is the more polar component, even the solubilities of moderately polar solutes such as phenol can decrease with increase in concentration of the more polar solvent.

(*b*) Variation of interactions in the polyamide gel, which can be considered as a viscous solution composed of solvents present in the mobile phase (changed in proportion more or less in favour of the component which has the greater tendency to be absorbed by the polyamide), -CO-NH- groups and hydrocarbon linkages. The gel is heterogeneous in nature and changes in time and along the system are possible as a result of demixing; the type and structure of the polyamide are important factors for these effects.

The effects discussed under (*a*) and (*b*) determine the liquid-liquid partition coefficient, k_p. However, the variation in composition and swelling of the polyamide gel should also influence the k' value indirectly through the diffusion coefficients, an effect which is recognized in gel chromatography (*cf.*, the "restricted diffusion model" [16,17]) and possibly through the V_{st}/V_m ratio.

(*c*) Variation in the contribution of adsorption effects: an increase in polarity should decrease the adsorption coefficient, k_a (through solvation of surface -CO-NH- groups) and decrease the crystalline surface area, S, due to swelling of the polyamide.

The adsorption mechanism predominates for non-polar developing solvents that cannot penetrate the polyamide structure to form a stationary gel phase. In such systems, polar solutes such as phenol are adsorbed by hydrogen bonding to the exposed carbonyl groups proportionally to the surface area of the polyamide particles: the adsorption of non-polar solutes such as anthracene is then very weak, even from non-polar solvents, and their sorption by polyamide is greater from polar solvents owing to the contribution of the partition mechanism (solubility in the gel phase).

The complex character of the chromatographic system explains the controversial interpretations of the chromatographic behaviour of polyamide systems, which have recently been reviewed by Tyukavkina *et al.*[12].

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