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INVESTIGATIONS ON THE RELATIONSHIP BETWEEN MOLECULAR STRUCTURE AND CHROMATOGRAPHIC PARAMETERS

VI. EFFECT OF ADSORPTION PHENOMENA IN LIQUID–LIQUID CHROMATOGRAPHY WITH BINARY MOBILE PHASES

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SUMMARY

Chromatographic and static distribution coefficients were compared for *m*- and *p*-nitroaniline and *p*-nitrophenol chromatographed in solvent systems of the type cyclohexane – electron donor solvent – water. Di-*n*-butyl ether, diisobutyl ketone and *n*-butyl acetate at concentrations up to 10% (v/v) were used as polar components of the mobile phase. The retention by the stationary aqueous phase for dilute solutions of polar solvents was found to be significantly stronger than expected from the static distribution coefficients, presumably owing to the contribution of adsorption on the liquid–liquid interface. Conclusions relating to investigations of solvation equilibria from R_M–composition relationships are given.

INTRODUCTION

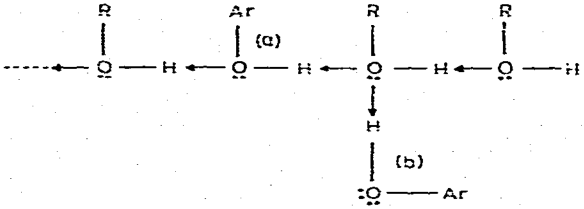
The investigation of reactions or interactions by varying the concentration of one of the interacting species is a well established experimental technique, especially for equilibria that can be described by the law of mass action, *e.g.*, complexation equilibria, acid–base interactions, solubility[1-5], ion exchange[6,7], adsorption from solutions[8] and gas–liquid and liquid–liquid partition[9-11]. Chromatography, especially gas chromatography, permits these equilibria to be studied in a simple and rapid manner; however, the complexity of the chromatographic process may introduce new effects which can distort the experimental results and lead to erroneous conclusions. For liquid–liquid partition, these effects have been discussed by Locke[11].

It is worthwhile to discuss in detail the phenomena that can be encountered in investigations of the molecular interaction mechanism by using series of liquid–liquid partition systems with a varying composition of one of the phases; the method, which can be considered a discontinuous analogue of the two-phase titration technique (see ref. 5, p. 111), has been especially useful in studies of the formation and extraction of metal complexes by both chromatographic and static techniques (see, for instance, Marcus and Kertes[6]).

(1) Mutual solubility of the two phases. For systems of the type binary solvent–pure solvent (*e.g.*, cyclohexane + di-*n*-butyl ether–water), the binary phase saturated with water is virtually a ternary system, and although the solubility of water in the non-polar diluent is negligible, it increases with increase in the concentration of the polar component; it should be pointed out that the mole fraction of water, owing to its low molecular volume, is usually several times greater than the volume fraction and its polarity and hydrogen bonding ability can strongly influence the properties of the organic phase. Some solvents are also markedly soluble in aqueous solutions, especially acidic solutions[6].

(2) Variation of the properties of the binary phase as an interaction medium (especially at higher concentrations of the additive)[13,15], leading to changes in the interaction constants (solvation, formation of donor–acceptor complexes).

(3) Self-association of the additive, leading to a decreased effective concentration[13,16–18]. For instance, self-association of alkanols, by bonding the active hydrogen atoms, leads to a significant decrease in their proton-donor ability so that they behave as solvents of class B (*cf.*, Part V[18], Figs. 6a, 6b). Their relatively high solvent strength for solutes of class AB (notation according to Pimentel and McClellan[19]) can be explained by two molecular mechanisms: enclosing the solute molecule (a) into the linear association chain in any position[16] or hydrogen bonding to any of the free electron pairs (b), as represented in the schematic diagram below (solute — phenol, ArOH; solvent —alkanol, ROH). Solutes of class B can only be hydrogen bonded to the terminal hydroxyl group[16] or interact by weak dipole–dipole orientation forces.



(4) Variation of the viscosity with the composition of the binary solvent can influence the kinetics of the chromatographic partition process. This effect, although more strongly pronounced with mixed stationary phases (availability of deeper pores), is usually neglected.

(5) Variation of other retention mechanisms with the composition of the binary solvent. In liquid–liquid partition chromatography, the additional mechanisms are mostly adsorption on the liquid–liquid interface and adsorption on the support from either the mobile phase (bare surface) or the stationary phase (covered surface)[14]. For simple aqueous systems, the last contribution can be considered to be small and in most instances can be neglected, as hydration of surface polar groups of the support virtually eliminates their interaction ability, even for such supports as cellulose (however, this is not true for certain specific groups, *e.g.*, –COOH). Expressing the contribution of various sorption mechanisms[13,14] in terms of the parameters used in paper and

thin-layer chromatography, we can write

$$R_F = \frac{1}{1 + K\,V_{st}/V_m + \sum_i K_{ai}A_i/V_i}$$

and

$$R_M = \log\,(KV_{st}/V_m + \sum_i K_{ai}\,A_i/V_i)$$

where K is the partition coefficient (C_{st}/C_m in terms of concentrations in moles per litre), V_{st} and V_m are the volumes of the two liquid phases and K_{ai} are the adsorption coefficients relative to the volume of the bulk phase (V_i) and surface area (A_i).

It is the last aspect of solvent composition effects that is the subject of the present study. In order to estimate the effects of adsorption phenomena, the chromatographic and static distributions of several solutes were investigated for solvent systems of the type cyclohexane + polar solvent–water. It can be expected that the contributions of adsorption phenomena in liquid chromatography would be much smaller than in gas–liquid partition chromatography; on the other hand, the use of a series of binary solvents with widely differing polarities can be expected to result in a variation in adsorption on the liquid–liquid interface of some solutes, especially those with marked surface activity. These effects should be considered if chromatography is to be used for the investigation of the molecular interactions involved.

In the chromatographic system investigated, the molecular complexes are formed by hydrogen bonding. Investigations of this type of molecular interaction by gas–liquid chromatography have been relatively scarce[13,15,16,20,21], while even less numerous have been static extraction studies (*e.g.*, refs. 6, 22 and 23) and liquid–liquid partition chromatographic investigations[17,18,21,25].

EXPERIMENTAL

As in Parts IV[24] and V[18], Whatman No. 4 paper strips were impregnated with water, blotted between two sheets of filter paper, dried to 50% (w/w) humidity content (controlled by weighing) and then transferred immediately into chromatographic tanks ($4 \times 7 \times 22$ cm) for descending development. The R_F values are given in Table I. For the experimental technique used, the volume ratio of the two phases, V_{org}/V_w is *ca.* 2.0 (ref. 26) so that

$$-R_M = \log R_F/(1-R_F) = \log D + 0.3$$

where $D = C_{org}/C_w$.

Static distribution coefficients were determined by equilibration of equal volumes of aqueous solutions of the solutes with the organic phase. The concentrations in the aqueous phase were determined by means of an LP 60 polarograph (Laboratorní Přístroje, Prague, Czechoslovakia), the solutions being diluted with equal volumes of 0.5 *M* potassium hydroxide solution. The distribution coefficients were calculated from the heights of the polarographic wave before and after extraction. The concentration range in the static extraction experiments was comparable with concentrations in the chromatographic systems (*ca.* $5 \cdot 10^{-3}$ *M*).

TABLE I

$R_F \times 100$ VALUES OF PHENOLS FOR VARIOUS CONCENTRATIONS OF THREE POLAR SOLVENTS IN CYCLOHEXANE

Polar component	*Solute*	*Solvent concentration (%, v/v)*							
		0.3	*0.5*	*0.7*	*1.0*	*3.0*	*5.0*	*7.0*	*10.0*
Di-*n*-butyl ether	4-Nitroaniline			–	8	18	25	–	40
Diisobutyl ketone	3-Nitroaniline	28	30	33	36	48	61	70	76
	4-Nitroaniline	6	7	8	9	16	28	36	48
	4-Nitrophenol	5	6	9	12	26	42	52	68
n-Butyl acetate	3-Nitroaniline	25	–	30	–	59	70	–	80

RESULTS AND DISCUSSION

The experimental results are presented as log *D versus* log % S plots (S = polar component), as in typical extraction diagrams for slope analysis[6]; the R_M axis is directed downwards so that the static (triangles) and chromatographic (circles) relationships are adjacent, the distance between them being 0.3 unit for $V_{org}/V_w = 2.0$ when a purely extractive chromatographic process is assumed. For practical reasons, the distribution coefficient is expressed in terms of concentrations in moles per litre and the composition scale of the binary solvent as log % (v/v) S, the latter being analogous to concentration scale in moles per litre of the polar solvent S (shifted horizontally by a constant value). The simplified concentration scales do not cause any significant distortions of the relationships owing to the narrow composition ranges investigated (up to 10% S) and the comparable molar volumes of cyclohexane and the polar solvents (for a discussion of the concentration scales in investigations of log *D versus* composition relationships, see refs. 6, 11 and 17).

The solubilities of cyclohexane and the polar components of the mobile phase in water are low, so that it can be assumed that the solution properties of the aqueous stationary phase are constant and comparable for all solvent systems, irrespective of the composition of the developing solvent; owing to the relatively high degree of impregnation (0.5 g of water per gram of dry paper), it can be expected that adsorption on the cellulose–mobile phase interface is negligible. Therefore, any adsorption effects that contribute to the liquid–liquid partition should be due mostly to the liquid–liquid interface, which is much larger in the chromatographic system.

Comparison of the $-R_M$ and log *D* values shows that the contribution of adsorption effects is small for moderate concentrations (above *ca.* 5%) of the polar solvent: both lines are approximately parallel and the distance between them is about 0.2–0.3 unit, in agreement with theoretical prediction. However, for more dilute solutions, the two lines converge and even cross, the R_M line being then lower than the log *D versus* log % S plot, indicating a contribution of an additional retention mechanism, probably adsorption of the solutes on the liquid–liquid interface (Figs. 1–4).

The contribution of adsorption effects should also depend on the structure of the polar solvent (S) and the molecular structure of the solute; for instance, for the diisobutyl ketone systems, the R_M line for 3-nitroaniline shows a less significant contribution of an additional retention mechanism (Fig. 3) than in the case of 4-nitro-

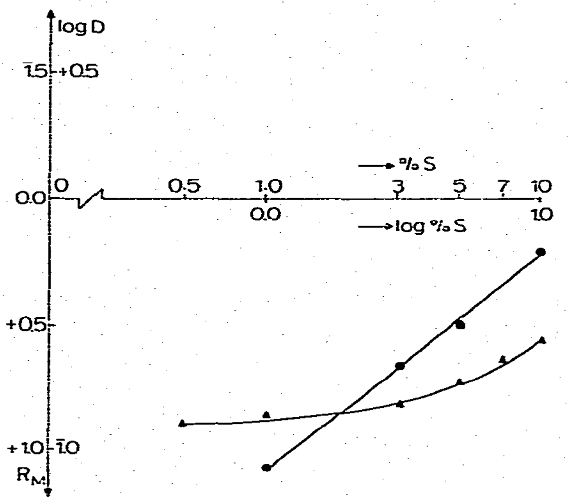


Fig. 1. R_M *versus* log % S (●) and log *D versus* log % S (▲) relationships for 4-nitroaniline for systems of the type cyclohexane – di-*n*-butyl ether (S)–water.

aniline and 4-nitrophenol (Figs. 3 and 4). The effect should obviously be much stronger for solutes that are surface-active compounds, which tend to accumulate at the liquid–liquid interface. This tendency varies in homologous series of polar compounds and the contribution of this effect could probably have an influence on the R_M *versus* chain length relationships.

In the systems investigated, only the amino and hydroxyl groups are able to form hydrogen bonds with the polar solvents of class B, so that the formation of

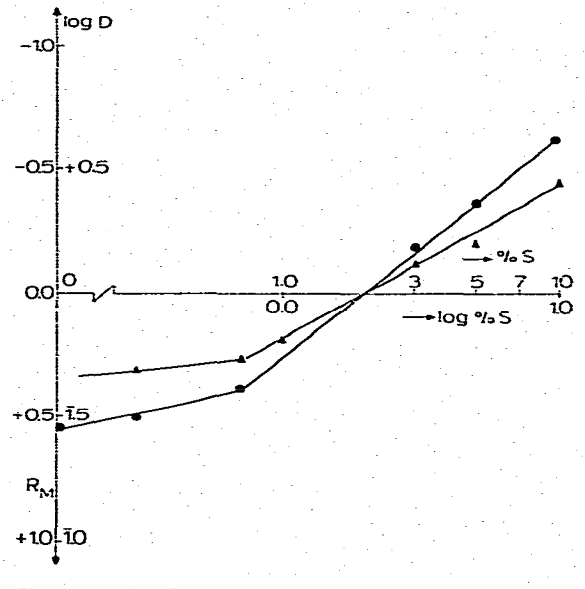


Fig. 2. As in Fig. 1, for 3-nitroaniline; S = *n*-butyl acetate.

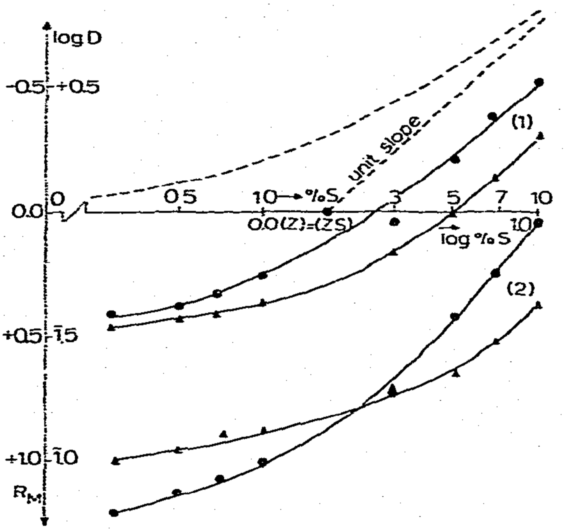


Fig. 3. As in Fig. 1, for 3-nitroaniline (1) and 4-nitroaniline (2); S = diisobutyl ketone. Dashed lines = theoretical log D-log [S] and $-R_M$-log [S] curves whose asymptotes cross at a composition corresponding to 50% solvation of solute.

simple 1:1 solvation complexes can be expected. The theoretical log D *versus* log % S relationship of the "hockey stick" type calculated by application of the law of mass action to the formation of the solvation complex[21] is shown in Fig. 3. The horizontal position of the theoretical curve is determined by the formation constant of the solvation complex and the vertical position by the log D value for the cyclohexane–water system. The experimental relationships are sometimes steeper for higher concentra-

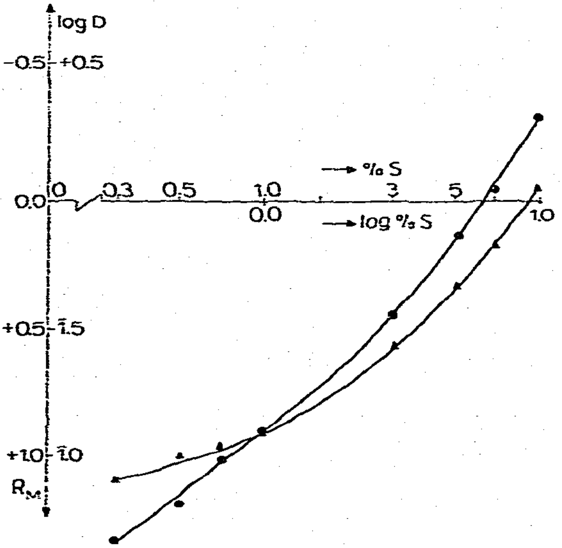


Fig. 4. As in Fig. 1, for 4-nitrophenol; S = diisobutyl ketone.

tions of the polar solvent, which may be due to the interaction of the nitro group with the polar solvent.

The experimental results indicate that the discrepancies between the chromatographic and static liquid–liquid partition parameters for the moderately polar solutes investigated are significant only for dilute solutions of electron donor solvents, for which additional static experiments should be carried out if solvation equilibria are investigated by chromatographic methods.

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