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SOLVENT COMPOSITION EFFECTS IN THE LIQUID-SOLID CHROMATOGRAPHY OF AZO DYES

EDWARD SOCZEWIŃSKI and JAN KUCZMIERCZYK

Department of Inorganic and Analytical Chemistry, Institute of Basic Chemical Sciences, Medical Academy, 20-081 Lublin (Poland)

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SUMMARY

Linear plots of R_M against the logarithm of the percentage concentration of polar solvents were obtained for chromatographic systems of the type silica-solution of polar solvent in *n*-heptane. The positions of the plots and their slopes were different depending on the chromatographic technique used, *i.e.*, thin-layer chromatography (TLC) in saturated tanks, TLC in sandwich tanks operated in a quasi-column manner (continuous development, samples spotted behind the solvent front) and column chromatography. It is demonstrated that the sandwich technique eliminates some effects that are specific to saturated tanks and also solvent demixing, so that the results are closer to those obtained in column chromatography. The chromatographic data are interpreted in terms of the presumed molecular mechanism of adsorption.

INTRODUCTION

Azo dyes are suitable for the investigation of molecular structure effects in adsorption from solutions; recorded chromatograms are easily obtained by densitometry of thin-layer chromatograms or by the application of detectors adapted from simple spectrophotometers. For these reasons, azo dyes are also convenient for the testing of adsorbents for quality control purposes.

Some information on the molecular mechanism of adsorption from solutions in polar solvents can be obtained from $\log k'$ versus \log composition relationships^{1,2} in a manner analogous to that employed in the investigation of complexation and partition equilibria³. Thin-layer chromatography (TLC) in saturated tanks is the simplest method that can be used in this approach; however, phenomena characteristic of TLC (gradient effects, solvent demixing, adsorption of solvent vapours) can cause deviations from ideal behaviour of the chromatographed solutes. For certain solvent systems and adsorbents, and especially for saturated tanks and pre-equilibration of the plates, some of these effects can be reduced or even eliminated; however, for adsorbents with a high specific surface area the pre-equilibration (necessary for reducing solvent demixing) may cause a significant decrease in the apparent R_F values, which requires the use of a correction factor, ξ (ref. 4).

For a series of binary solvents with various concentrations of the polar component, variation of ξ is likely to occur, which would change the slopes of the $\log k'$ versus log composition lines. Although for some types of solvent-adsorbent systems satisfactory correlations between TLC and column data were reported^{1,5}, the investigation of the mechanism in various chromatographic techniques is still a problem, especially when TLC is to be used as a reconnaissance technique prior to column separations^{6,7}.

EXPERIMENTAL

Azo dyes not commercially available were synthesised by coupling diazonium salts of aniline or 4-nitroaniline with phenols, aniline or N,N-dimethylaniline, followed by crystallization.

In TLC experiments, silica SI 60 for TLC (after Stahl, produced by E. Merck, Darmstadt, G.F.R.) was used. The adsorbent was mixed with 2.5 parts of water for 1 min; glass plates (20 × 12 cm for saturated tanks and 20 × 5 cm for sandwich-type tanks) were covered with a 0.5-mm layer of the suspension using a Quickfit spreader. The plates were dried at room temperature for 1 h and activated for 2 h at 130°. Glass tanks (25 × 14 × 7 cm) lined with filter-paper were used as saturated chambers. The solvent mixtures (*n*-heptane + polar solvent), dried over molecular sieve 5A, were poured into the tanks 1 h before the experiments. The dyes were spotted as 0.5% solutions with a capillary (2.5 mm³). The plates were pre-equilibrated in the tank atmosphere for 30 min before development.

In another series of TLC experiments, home-made horizontal sandwich tanks⁸ were used, which permitted continuous development (due to evaporation of the solvent at the other end of the TLC plate⁹) and application of the samples through a slit in the cover-plate after equilibration of the liquid-solid system. Any gradient and solvent demixing effects could thus be eliminated and the conditions were similar to those in column development. Azobenzene was used as a reference compound (indicator of the solvent flow-rate). The delivery of the developing solvent through a 0.5-mm I.D. stainless-steel U-tubing was accelerated by using as a distributor a glass bar (45 × 4 × 1 mm), which forms above the carrier plate a 0.4-mm slit filled automatically with the solvent due to capillary forces (Fig. 1).

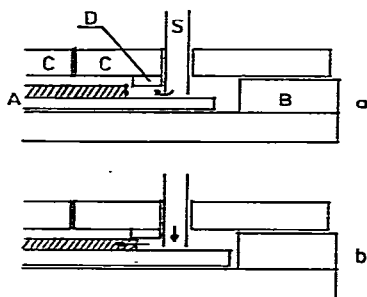


Fig. 1. Schematic representation of solvent delivery system for wide plates (longitudinal cross-section of the tank). A, Carrier plate with thin layer of adsorbent (dashed area); B, spacing plate; C, two adjacent cover-plates; D, distributors; S, capillary siphon. (a) The space between A and D is filled with the developing solvent. (b) Development.

In column experiments, a home-made liquid chromatograph was used with a pneumatic solvent delivery system¹⁰ (similar to the Varian Aerograph Model 4000 liquid chromatograph) and a Spekol detector (Zeiss, Jena, G.D.R.) with a 8 mm × 1 mm I.D. cuvette. A stainless-steel column (25 cm × 3.5 mm I.D.) was dry-packed with LiChrosorb SI 60, 30 μm (Merck). The solutions of the dyes were introduced through a septum injector with a 5-μl Hamilton syringe after equilibration of the column with the developing solvent. The flow-rate was *ca.* 0.5 cm³/min.

The experimental results are presented as R_M versus log %S plots, where S is the polar component of the developing solvent (Figs. 2-6).

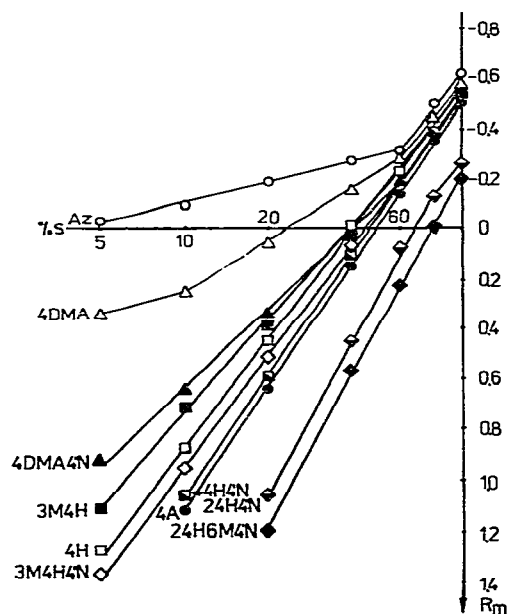


Fig. 2. R_M values of azo dyes plotted against the volume percentage concentration of acetone in the developing solvent (logarithmic scale). Saturated tanks. For abbreviations of compounds, see Table I.

For two polar solvents (methyl ethyl ketone and ethyl acetate), most of the dyes were also chromatographed in sandwich tanks in a quasi-column manner; the samples were spotted behind the solvent front when the front of solvent demixing (indicated by the flattened zone of azobenzene spotted on the dry layer) had left the chromatographic system (Figs. 3b and 4b). The R_F values of the dyes were determined using *trans*-azobenzene spotted just ahead of the samples as the marker of the flow-rate of the mobile phase. As demonstrated by column experiments, azobenzene migrates almost at the rate of the mobile phase ($R_F \approx 0.90$)* for concentrations of methyl ethyl ketone exceeding 20%. Only for more dilute solutions of the polar solvent would a noticeable error be introduced by assuming that the R_F value of azobenzene

* Even for non-adsorbed compounds of small molecular size there is some retention due to immobilization of part of the developing solvent by the adsorbent^{11,12}.

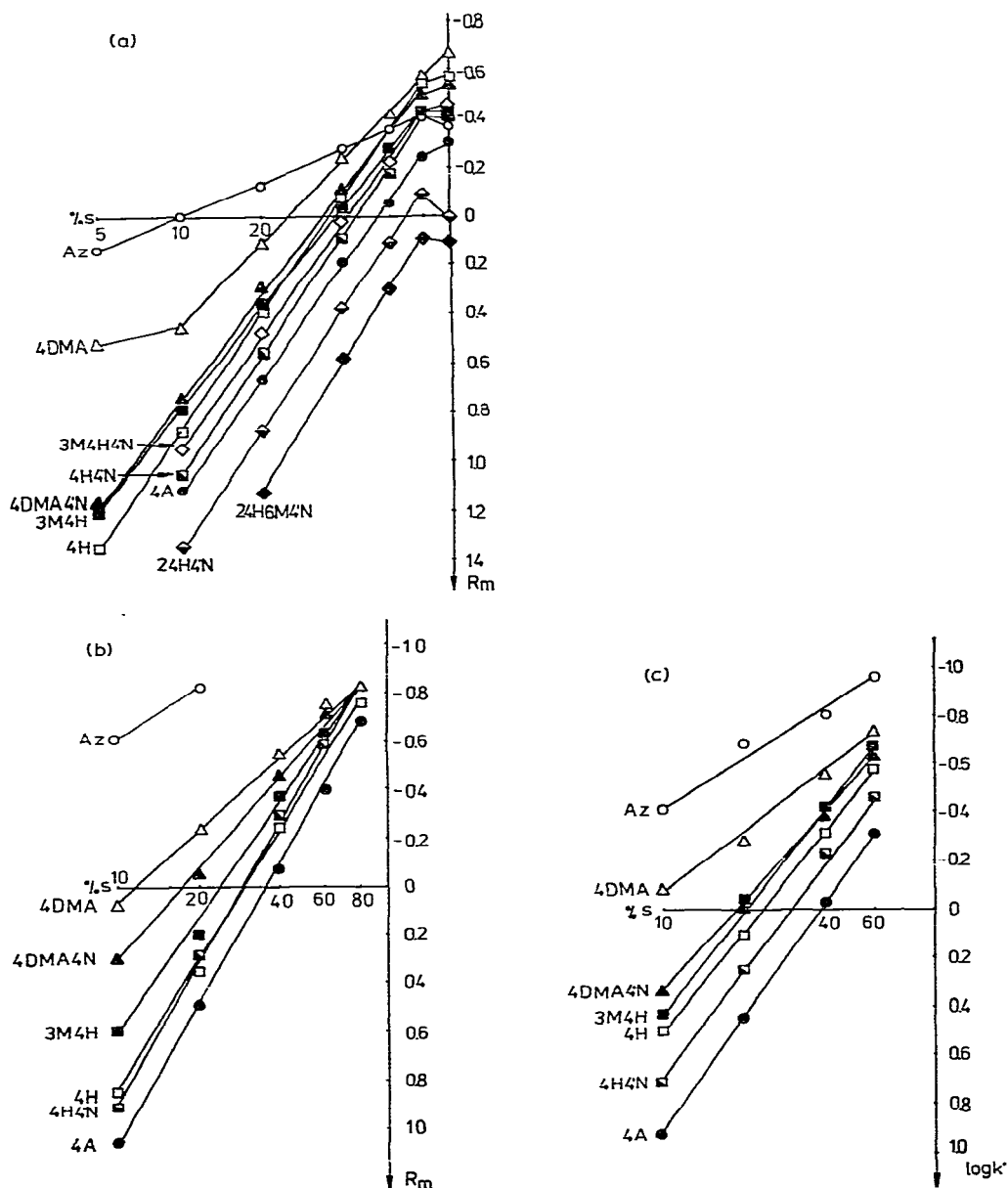


Fig. 3. (a) As in Fig. 2; polar component (S) of the developing solvent = methyl ethyl ketone. (b) Continuous sandwich development. (c) Column chromatography, plots of $\log k'$ versus \log [methyl ethyl ketone, %].

is 0.9; actually, the R_F values of azobenzene determined by sandwich TLC (using α -pinene and squalane as markers) for 5, 10 and 20% of ethyl acetate were 0.74, 0.79 and 0.85, respectively, and the corresponding values for methyl ethyl ketone systems were 0.69, 0.80 and 0.89, respectively. Therefore, the R_F values of the solutes

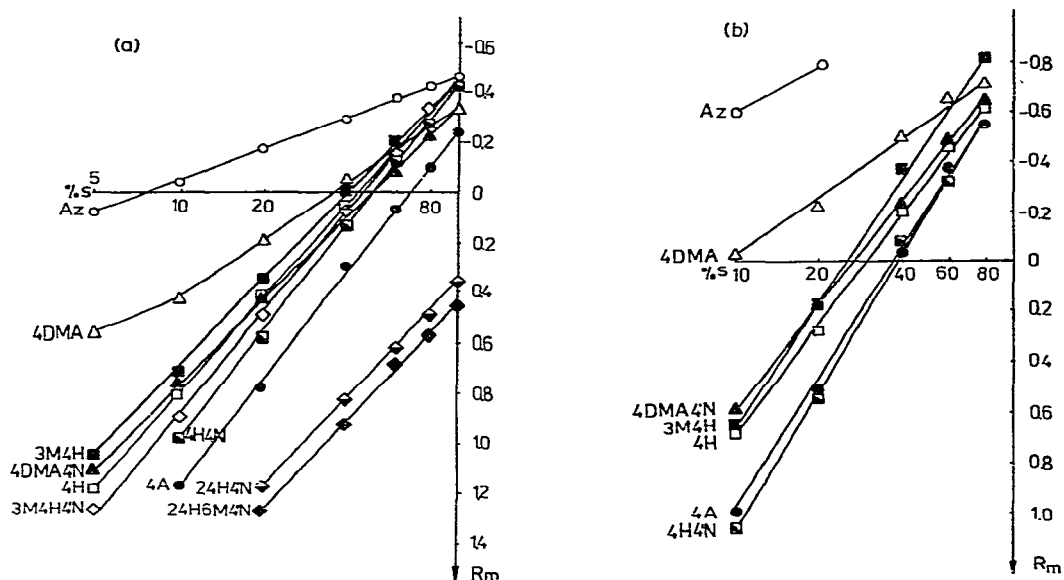


Fig. 4. (a) As in Fig. 2, polar solvent (S) = ethyl acetate. (b) Same system, sandwich development.

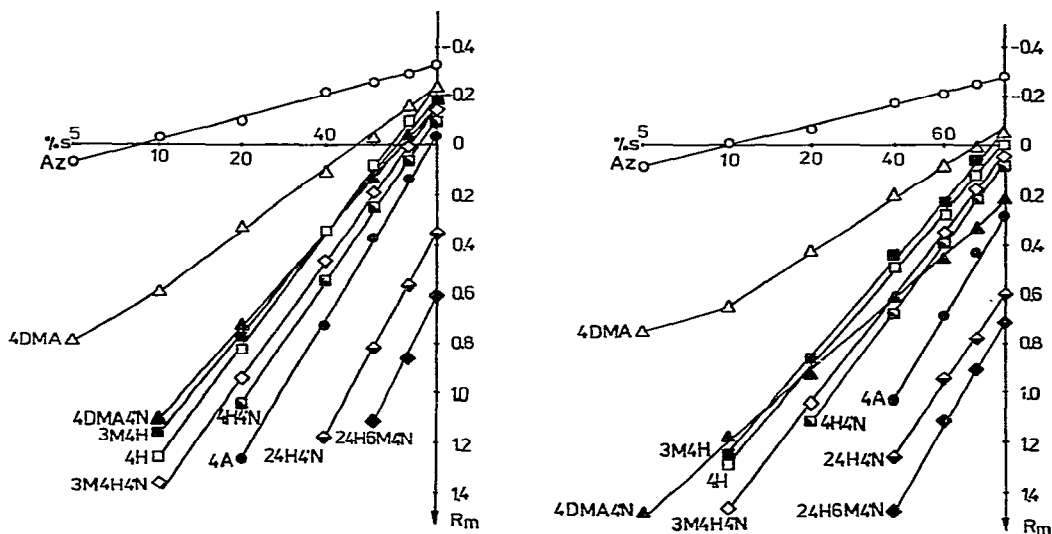


Fig. 5. As in Fig. 2, polar solvent (S) = diethyl ether.

Fig. 6. As in Fig. 2, polar solvent (S) = diisopropyl ether.

relative to azobenzene (R_{FX}/R_{FAZ}) were multiplied by 0.90 for higher concentrations of the polar solvents and, for more dilute solutions, by the actual R_F values of azobenzene determined by sandwich TLC relative to the non-polar terpenes.

High-performance liquid chromatographic (HPLC) data are presented as $\log k'$ versus \log % methyl ethyl ketone plots (Fig. 3c). The dead volume was deter-

TABLE I
SLOPES (t_1 , ABSOLUTE VALUES) OF R_{st} VERSUS COMPOSITION PLOTS IN FIGS. 2-6

No.	Substituents of azobenzene	Abbreviation	Dimethyl ketone		Methyl ethyl ketone		Ethyl acetate		Diethyl ether		Diisopropyl ether	
			sat.*	Col.*	Sat.*	Col.*	Sat.*	Sand.*	Sat.*	Sand.*	Sat.*	Sand.*
1	None (azobenzene)	Az	0.3	0.7	0.45	0.8	0.4	0.65	0.3	0.3	0.25	0.25
2	4-Dimethylamino	4DMA	0.7	0.85	1.15	1.0	0.8	0.8	0.8	0.8	0.7	0.7
3	4-Dimethylamino-4'-nitro	4DMA4'N	1.0	1.2	1.4	1.25	1.1	1.4	1.25	1.25	1.0	1.0
4	3-Methyl-4-hydroxy	3M4H	1.2	1.4	1.35	1.55	1.2	1.7	1.35	1.35	1.3	1.3
5	4-Hydroxy	4H	1.4	1.4	1.6	1.8	1.3	1.45	1.5	1.5	1.3	1.3
6	3-Methyl-4-hydroxy-4'-nitro	3M4H4'N	1.4	—	1.55	—	1.35	—	1.55	1.55	1.4	1.4
7	4-Hydroxy-4'-nitro	4H4'N	1.65	1.5	1.6	1.9	1.4	1.8	1.6	1.6	1.5	1.5
8	4-Amino	4A	1.65	1.6	1.55	1.9	1.4	1.7	1.9	1.9	1.9	1.9
9	2,4-Dihydroxy-4'-nitro	24H4'N	2.0	—	1.6	—	1.2	—	2.0	2.0	1.65	1.65
10	2,4-Dihydroxy-6-methyl-4'-nitro	24H6M4'N	2.0	—	1.75	—	1.2	—	2.1	2.1	1.9	1.9

* Sat. = TLC in saturated tanks; sand. = TLC in sandwich tanks; col. = column chromatography.

mined from the retention volume of azobenzene for development with pure methyl ethyl ketone.

The slopes of the plots are summarized in Table I.

RESULTS AND DISCUSSION

In accordance with the adsorption models based on the law of mass action^{2,13,14}, the R_M ($\log k'$) versus $\log \%S$ plots are linear (strictly, the concentration of the interacting solvent, S, should be expressed as a molar fraction; however, owing to the logarithmic scale and the comparable molar volumes of the solvents, the use of the more convenient volume percentage compositions, analogous to mole/dm³ concentrations, causes only insignificant deviations in the relationships^{15,16}. However, the slopes of the plots, significant for the investigations of the molecular mechanisms of adsorption, show some differences depending on the experimental technique used.

Comparison of Figs. 3a–3c shows that greatest discrepancies between the slopes determined by the three techniques are observed at higher R_F values and lower concentrations of the polar solvent, *i.e.*, for the less polar solutes (azobenzene and dimethylaminoazobenzene). Their plots were less steep and their R_F values much lower for the saturated tanks (Figs. 3a and 4a) than for sandwich quasi-column development (Figs. 3b and 4b), apparently indicating a diffuse profile of solvent demixing in the upper zone of the thin-layer chromatogram at lower concentrations of the polar solvent. On the other hand, at higher concentrations of the polar solvent the upper R_M versus $\log \%S$ lines obtained for saturated tanks converge owing to condensation of the solvent vapour above the solvent front (also during the pre-equilibration of the plates; *cf.*, ref. 4, pp. 177–188, Fig. 61, and ref. 17) so that for even the least polar solutes the R_F values do not exceed 0.8. The effect is even stronger for the more volatile solvent [diethyl ether (Fig. 5) and diisopropyl ether (Fig. 6)]. The results obtained by column chromatography (Fig. 3c) are much more similar in general appearance to those obtained with sandwich chambers (Fig. 3b) than with saturated tanks (Fig. 3a), indicating that the technique of continuous TLC development eliminates or at least reduces the effects that are specific to saturated tanks and is thus promising for physico-chemical investigations of chromatographic processes and reconnaissance experiments for HPLC. The plots obtained with sandwich TLC are in general steeper than those obtained with saturated tanks, although the differences in the slopes only rarely exceed 0.3 unit (compare Figs. 3a and 3b, and 4a and 4b and Table I).

The experimental results obtained in this and earlier investigations^{1,2,5} indicate that the semi-empirical equation

$$R_M = \text{constant} - n \log \%S$$

characteristic of adsorption models based on the application of the law of mass action to competitive adsorption of solutes and solvents^{2,13,14} is valid for numerous solute–solvent–adsorbent systems, especially for polar (hydrogen bonding) solvents and adsorbents with hydroxylated surfaces, such as silica^{5,18}, Florisil¹ and alumina¹⁹. In

spite of the complicating effects, the equation also holds for TLC in saturated tanks, although for column experiments much more regular relationships are obtained^{2,5,7,16*}.

The discrepancies between TLC and HPLC data apparently depend on several parameters and are more significant for very volatile polar solvents, adsorbents of high specific surface area (stronger effects of adsorption of the polar solvent above the front from the atmosphere — “Vorbedämpfung”^{4,17}) and less polar solutes, which tend to run close to the solvent front (gradient effects due to solvent demixing; their elimination by pre-equilibration leads to increased “Vorbedämpfung” effects). The activity of the adsorbent due to the presence of water vapour is probably less significant for developing solvents that contain polar electron-donor solvents, which can activate silica by desorption of water (ref. 13, p. 235); however, it is much more important for less polar solvents such as dichloroethylene²⁶. The analogies between TLC and HPLC are closer when the same type of silica is used for TLC adsorbents and column packings [e.g., SI-60, which is used for the preparation of both LiChrosorb for HPLC and silica gel for TLC (Merck)].

The possible distortions of R_M versus $\log \%S$ plots by phenomena specific to saturated TLC tanks indicate that more exact interpretations of TLC data should be preceded by repetition of some of the experiments using column chromatography or equilibrium quasi-column sandwich TLC. The latter technique is simpler and much less expensive than HPLC, but it still requires a detailed study of its capabilities.

The sequence of the solutes was similar for all solvent systems and techniques; only occasionally were minor changes in ΔR_M values and the sequence of adjacent spots observed. The azo group, as judged from the high R_F value of azobenzene, presumably plays a minor role in the adsorption mechanism of the remaining azo dyes whose polar groups are much more strongly adsorbed. Except for the dihydroxy derivatives, which can be attached by two-point adsorption (although internal hydrogen bonding of the 2-hydroxyl group to the vicinal azo nitrogen atom may decrease its adsorption affinity), the remaining azo dyes with a single hydroxyl or amino group are presumably adsorbed by single-point attachment (vertical orientation). Their slopes, which are greater than unity (*ca.* 1.5), are typical of the adsorption of monofunctional solutes of class AB from solvents of class B (ref. 2), presumably due to competitive solvation of the proton donor group²⁷; similar slopes were also obtained for simple phenols and anilines. The 4'-nitro group takes a more significant part in the adsorption mechanism only in the case of 4-N,N-dimethylamino-4'-nitroazobenzene, in which it has an adsorption energy similar to that of the N,N-dimethylamino group. However, adsorption of this compound in a flat orientation seems less probable as it would require the displacement of several solvent molecules from the surface of the adsorbent.

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* It should be noted that for hydrophobic adsorbents and aqueous solvent systems another relationship is often obtained: $R_M = \text{constant} + n \cdot \%H_2O$ (refs. 20–25).

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