

CHROM. 11,382

SOLVENT DEMIXING EFFECTS IN CONTINUOUS THIN-LAYER CHROMATOGRAPHY AND THEIR ELIMINATION

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(Received June 29th, 1978)

SUMMARY

Demixing effects in thin-layer chromatography have been investigated for frequently used binary solvents of various compositions, and methods of eliminating such effects are described. The simplest way of eliminating solvent demixing consists in presaturation of the plate in the vapours of the polar solvent component or predevelopment with its more concentrated solution. The sample should be spotted some distance behind the front of the developing solvent to secure steady-state conditions analogous to column development with an equilibrated mobile phase. For more concentrated polar solvents (*e.g.*, >20%) it is sufficient to spot the sample several centimetres behind the solvent front; the R_F values of solvent demixing are then high so that the presaturation or predevelopment step can be omitted. Procedures for the elimination of solvent demixing for typical solvent mixtures in the minimum time are given in terms of the distance of predevelopment.

INTRODUCTION

The development of high performance liquid chromatography (HPLC) has provided a new role for thin-layer chromatography (TLC) as a convenient pilot technique for the choice of suitable solvent systems and prediction of separation conditions¹⁻⁴.

Under appropriate experimental conditions, there is a good correlation between R_M (TLC) and $\log k'$ (HPLC) values obtained with the two techniques⁵⁻⁷. Some deviations are caused by specific features of TLC carried out in saturated tanks, such as composition gradients of the mixed solvent along the adsorbent layer, solvent demixing, evaporation and condensation of the solvent. Two approaches have been used to eliminate or at least reduce these effects in TLC, especially for mixtures of solvents having large differences in polarity: (1) conditioning in saturated tanks; (2) construction of flat tanks of the sandwich type having minimal volume of the atmosphere, and preequilibration of the adsorbent.

In the first case the effect of the solvent vapours is decisive and the duration of conditioning is important, as demonstrated by Stahl⁸, De Zeeuw⁹, Geiss¹⁰ and

other workers^{11,12}. It has been shown that the penetration of solvent vapours into the adsorbent layer is accelerated by adsorption which greatly reduces the solvent demixing observed in unsaturated tanks. However, the contact of the dry adsorbent with the solvent vapours leads to preadsorption ("Vorbedämpfung") effects (*cf.*, refs. 10 and 13, pp. 178 and 365) which are manifested by decreasing R_F values and flattening of $R_M(\log k')$ vs. $\log C$ relationships, especially at higher concentrations (C) of polar solvents comprising small molecules (*e.g.*, acetone). Preadsorption effects can often be corrected for by the use of the factor ξ (*cf.*, ref. 10, p. 178; ref. 6, Figs. 2 and 3):

$$R_F' = \xi R_{F(\text{obs.})}$$

The correction factor is usually in the range 1.0–1.6, depending on the developing solvent and experimental conditions.

The second approach consists in the use of tanks in which the adsorbent is presaturated with solvent vapours; in some techniques the sample can be spotted behind the solvent front, after attainment of equilibrium between the mobile and stationary phase. These investigations resulted in the construction of the KS-chamber by Geiss *et al.*¹⁴ and further modified as the so-called Vario-KS chamber. Later an apparatus for continuous circular development (U-chamber; Camag, Muttenz, Switzerland¹⁵) was constructed. Another modification of the KS-chamber by De Zeeuw¹⁶ permits the use of a vapour gradient (see also ref. 17). Marichy-Viricel and Lamotte¹⁸, applying the KS-chamber to the chromatographic analysis of alkyl phosphates, demonstrated its advantages over the traditional saturated tanks; for the former technique a good separation was obtained with only slight flattening of the R_M vs. $\log N$ relationships (N -mole fraction of the polar component), while, in saturated tanks, solvent demixing was observed with a resulting loss of selectivity. Siouffi *et al.*³ observed good agreement between column and TLC parameters when the thin layer was developed with the solvent before spotting the samples.

Further possibilities are offered by the use of sandwich tanks with continuous development (Brenner–Niederwieser chambers¹⁹). To secure preconditioning of the adsorbent, Hara and Mibe²⁰ constructed a sandwich tank with a parallel plate which produces an atmosphere saturated with the solvent vapours. The solutes are spotted on the dry layer ahead of the solvent front. Another modification of the BN-chamber was proposed by Soczewiński and co-workers^{21–23}; the layer is conditioned by the solvent itself and the sample is spotted behind the solvent front, a special glass distributor spreading the solvent evenly across the plate width so that the developing solvent can be delivered by a single capillary siphon from a small container. Two glass cover plates joining over the start line permit spotting of the sample both on the dry adsorbent and during development. However, development with mixed solvents in sandwich tanks is accompanied by pronounced solvent demixing, analogous to frontal analysis. The appearance of multiple demixing fronts has been extensively investigated by Brenner and co-workers (*cf.*, ref. 8, p. 118). The formation of steep solvent-composition gradients causes an accumulation of flattened zones and a loss of selectivity, and is especially disadvantageous in non-analytical experiments since the capacity factor is different within the separate zones and the chromatographic process is of the gradient-elution type. Snyder (ref. 13, p. 213) has derived a formula for the variation of concentration of the polar component along the layer, and

demonstrated that solvent demixing is dependent on the activity and specific surface area of the adsorbent, and on the elution strength of the solvent and its molecular area. To eliminate solvent demixing effects, Snyder suggests wetting the adsorbent with a suitable volume of the solvent (ref. 13, eqn. 8-11a).

Preequilibration of the adsorbent prior to sample spotting can easily be attained in the sandwich tank for quasi-column development^{22,23}. Therefore, systematic investigations on solvent demixing were carried out for binary solvents of various qualitative and quantitative compositions and on the most popular adsorbents, silica and alumina. The main goal was to determine, for various experimental conditions, the minimum volume of mobile phase necessary to equilibrate the system so that even the fastest solute cannot overtake the front of solvent demixing. In other words, a compromise was sought between the attainment of equilibrium and the duration of the chromatographic run.

THEORETICAL

Assuming idealized conditions of the chromatographic process (negligible loss of solvent at the front due to evaporation caused by thermal effects or holdup in the pores), the relationship between the R_F value of the solvent demixing front (R_{Fd} , applied here in the sense of the position on the chromatograms) and the volume of the developing solvent (expressed in units of dead volume corresponding to the migration of a non-sorbed marker dye from the start line to the end of the layer) can be represented as in Fig. 1 which permits a graphical determination of the volume of the mobile phase (or the distance behind the solvent front for sample spotting) necessary for equilibration of the system. The straight line OA represents the migration of the solvent front (α), and the line OB that of the delayed solvent demixing front which reaches $R_F = 1.0$ (the end of the layer) after more than one dead volume (V^0) of the mobile phase has migrated past the start line. The volume of the mobile phase corresponding to $R_{Fd} = 1.0$ is equal to $1/R_{Fd}$ and the excess volume is thus $1/R_{Fd} - 1$ (in V^0 units). It is evident that a non-sorbed solute spotted behind the solvent front will migrate with the solvent at a constant distance from the solvent front. To determine the minimum distance behind the solvent front for which the solute

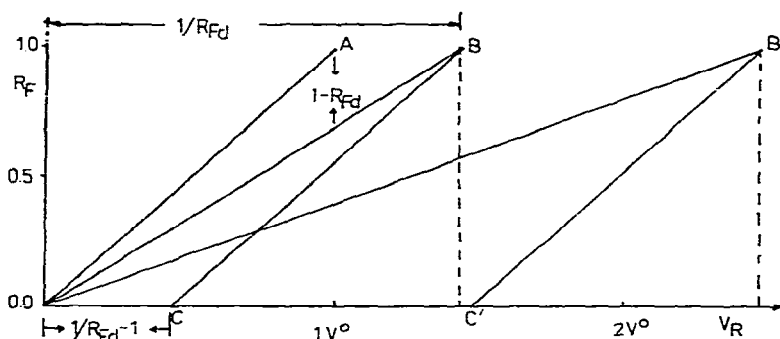


Fig. 1. Schematic representation of the migration of the apparent solvent front (OA), and the front of solvent demixing, $R_{Fd} = 0.7$ (OB) and 0.4 (OB'). Solutes spotted past the points C, C' cannot overtake the demixing fronts OB and OB', respectively.

(marker) cannot overtake the solvent demixing front R_{Fd} , a straight line is drawn parallel to OA from the point B to the intersection with the start line (C). It follows from the diagram that the minimum volume of the mobile phase necessary to eliminate solvent demixing corresponds to OC and is equal to $1/R_{Fd}-1$; solutes spotted on the start line past the point "C" cannot overtake the solvent demixing front.

The same conclusion can also be derived from the transformation of the equation for the retention volume of the solvent demixing front (V_{df}):

$$V_{df} = V^0(1 + k')$$

$$V_{df}/V^0 = 1 + k' = 1/R_{Fd}$$

For the idealized picture of solvent demixing, $1/R_{Fd}$ is thus equivalent to the total volume of mobile phase (in V^0 units) necessary to move the solvent demixing front to the end of the plate ($R_F = 1.0$). For developing solvents having low contents of the polar component the R_{Fd} values are lower so that larger volumes of the mobile phase are necessary to eliminate solvent demixing (in Fig. 1 the graphical relationships are given for R_{Fd} values of 0.7 and 0.4), and the equilibration requires prolonged development. Therefore, in the experimental studies, besides systematic investigations of solvent demixing effects, other modifications of preequilibration were tested in order to reduce the duration of analysis.

EXPERIMENTAL

0.25 mm layers of adsorbents (20×5 cm) were prepared and activated in the usual way; Quickfit spreader was used. The following adsorbents were purchased from E. Merck (Darmstadt, G.F.R.): silica gel G 60, aluminium oxide G Type E and neutral aluminium oxide Type T, as well as precoated plates, silica gel 60_{F254} and alumina foil-aluminium oxide 60_{F254}.

A number of dyes of different polarity was used to localize the demixing front: azulene, azo and anthraquinone dyes, and test mixtures supplied by Kavalier (Votice, Czechoslovakia, five dyes denoted in the text as 1-5 in the sequence obtained for development with benzene) and E. Merck (high-performance TLC test, 8 dyes).

All the solvents (Table I) were dried over molecular sieve 4A (Polish Reagents, Gliwice, Poland). The development was carried out in sandwich tanks for continuous development, the thickness of the distance plates being adapted to alufoils, precoated plates (1.25 m glass carrier plates) and 3.2 mm carrier plates (for details of the tank, see refs. 21 and 23).

The plates were prepared before use as follows. At one end of the plate the adsorbent was removed to form a 1.5 cm margin; on the long sides, 1 mm margins were produced to avoid contact of the adsorbent layer with the inner walls of the tank. The plates were activated for 2 h at 135°, cooled in a desiccator with wide-pore silica, then transferred to the tanks and immediately covered with the two cover plates. *Ca.* 2 μ l of 0.5% solutions of the test dyes were applied to the dry layer through the slit between the cover plates. The space under the distributor was filled with the solvent and the cover plates were adjusted to ensure contact of the solvent with the edge of the thin layer. The start line was *ca.* 1.5 cm from the edge so that the distance of development was 16-17 cm. The test dyes were so chosen that they were strongly

adsorbed from solutions in the non-polar diluent and displaced by the polar component; therefore their spots were flattened on the front of solvent demixing (Fig. 2). The development was stopped when the observed (apparent) solvent front reached the end line; the R_F of the front of solvent demixing, R_{Fd} , was calculated by measuring the distances from the edge of the layer. The experimental results are represented in the Table I and graphically as plots of R_F vs. % S (Figs. 3-5) (S = more polar component of the developing solvent) or R_M vs. \log (% S) (Fig. 6).

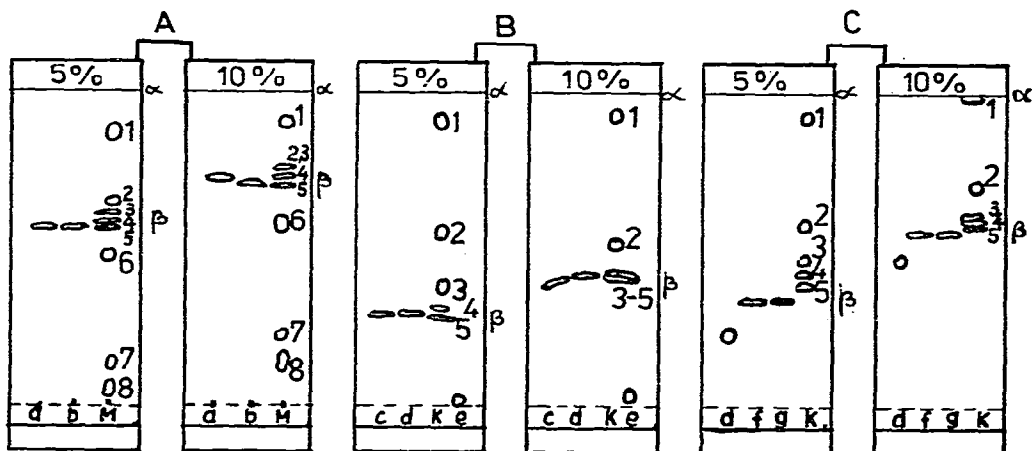


Fig. 2. Chromatograms of dyes demonstrating solvent demixing on silica in various binary systems. Polar solvent: A = diisopropyl ether; B = dimethyl sulphoxide; C = N,N-dimethylethanolamine. Diluent, benzene. Solutes: a = *p*-dimethylaminoazobenzene; b = 1-N-methylaminoanthraquinone; M = high-performance TLC test mixture (E. Merck, Darmstadt, G.F.R.); c = *p*-aminoazobenzene; d = *p*-hydroxyazobenzene; K = TLC test mixture (Kavalier, Votice, Czechoslovakia); e = methylene blue; f = Rhodamine B; g = Nile blue.

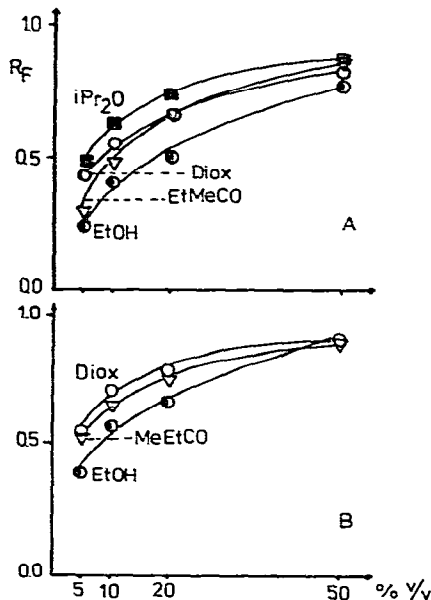


Fig. 3. R_F of solvent demixing (R_{Fd}) plotted against the concentration of the polar solvent. Diluent, heptane. Adsorbent: A = silica gel; B = alumina.

TABLE I

VALUES OF $R_{FD} \times 100$ ON SILICA AND ALUMINA AND FOR VARIOUS BINARY SOLVENTS

AcOEt = Ethyl acetate; iPr_2O = diisopropyl ether, MeEtCO = methyl ethyl ketone; MeBuCO = methyl butyl ketone; nAm₂O = di-*n*-amyl ether; EtOH = ethanol; Me₂CO = acetone; DMSO = dimethyl sulphoxide; DEA = diethylamine; DMAEtOH = N,N-dimethylethanolamine; Diox = dioxane.

Adsorbent (%, v/v)	Diluent													
	Heptane				Benzene				Acetone					
	AcOEt	iPr_2O	MeEtCO	Diox	nAm ₂ O	EtOH	iPr_2O	Me ₂ CO	Diox	DMAEtOH	DMSO	DEA	DMSO	DEA
Silica	5	48-50	29-34	41-43	--	24-30	60-63	56-61	58	39	32-34	46	60	57
	10	63-64	50-55	55	60	40	73-78	67-70	69	58	49	67	74	66
	20	75	66-68	67-70	76	50-53	88-91	80	85	86	60	81	88	85
	50	87	85	82	92	78	96	95	90	100	93	98	100	98
Alumina	AcOEt	iPr_2O	MeEtCO	Diox	MeBuCO	EtOH	MeEtCO	Diox	DMAEtOH	DMSO	DEA	DMSO	DEA	DEA
	5	85	53	55	56	38-40	76	71	55	51	65	67	76	76
	10	67	66	71	72	57	83	81	73	61	80	79	95	95
	20	75	77	79	81	64	94	88	89	76	94	92	100	100
	50	87	89	89	93	91	100	100	99	93	100	100	100	--

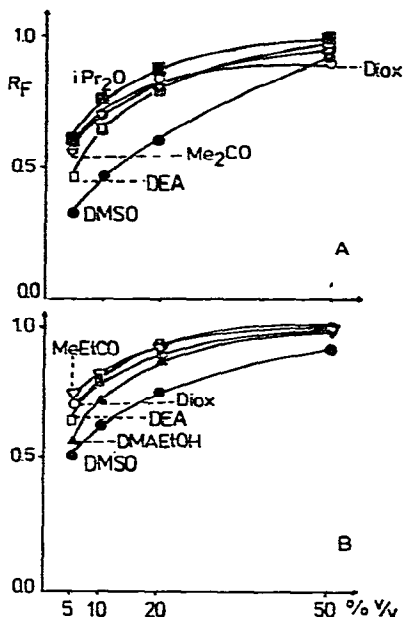


Fig. 4. Plot of R_f against concentration as in Fig. 3. Diluent, benzene.

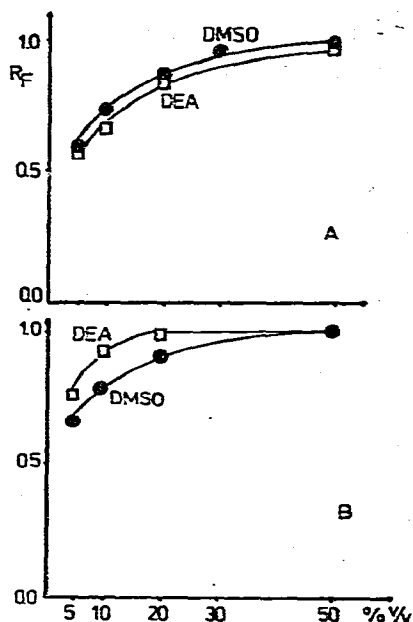


Fig. 5. Plot of R_f against concentration as in Fig. 3. Diluent, acetone.

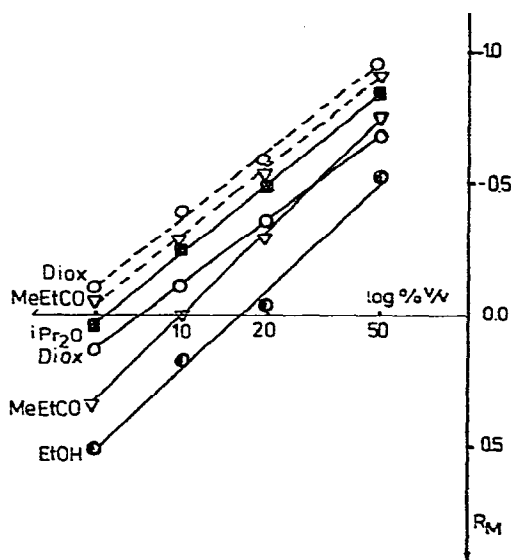


Fig. 6. Data of Fig. 3 plotted in the coordinate system R_M vs. $\log(\%S)$. Dashed lines, alumina; continuous lines, silica gel.

RESULTS AND DISCUSSION

The main goals of the investigations were concerned with the effect of the type and concentration of the polar component of the solvent and of the type of diluent on the formation of the solvent demixing front and its movement on the layers of

two common adsorbents, silica gel and alumina. As a practical consequence, in the second series of experiments, procedures leading to the elimination of solvent demixing effects were developed.

First, the behaviour of binary electron-donor solvents was investigated, ketones, ethers, esters and dimethyl sulphoxide, in concentrations ranging from 5 to 50% (v/v), the diluent being non-polar heptane having zero eluent strength, or the more polar benzene and acetone (*cf.*, ref. 13, Tables 8-1 and 8-2). Proton-donor solvents such as methylene chloride and chloroform did not form detectable demixing fronts owing to their weaker adsorption affinity. Of the most polar donor-acceptor solvents, diethylamine, NN-dimethylethanolamine, methanol and ethanol were investigated.

The experimental results given in Table I can be summarized as follows:

(1) For a given adsorbent and diluent, the R_F of the solvent demixing front (R_{Fd}) decreases with the solvent eluent strength of the polar component.

(2) The lowest values of R_{Fd} are obtained for dilute developing solvents; for more concentrated solvents, *e.g.*, >20%, high values of R_{Fd} are obtained irrespective of the diluent used.

(3) For more polar diluents higher values of R_{Fd} are obtained.

(4) Stronger demixing effects are obtained for silica than for alumina, which is presumably caused by differences in the geometry of active sites and not by differences in the adsorption energies (*cf.*, ref. 13, Fig. 8-2 and eqn. 8-6a) (Fig. 7A, B).

(5) Solvent demixing effects are less pronounced for adsorbents with lower specific surface areas (Fig. 7C, D).

Furthermore, the limited data seem to indicate that the molecular area of the polar solvents plays a more important role in demixing effects on alumina (see Table I); however, this conclusion requires verification for a greater number of systems. For dilute solvents the front of solvent demixing is more diffuse than for solvents containing higher concentrations of the polar component.

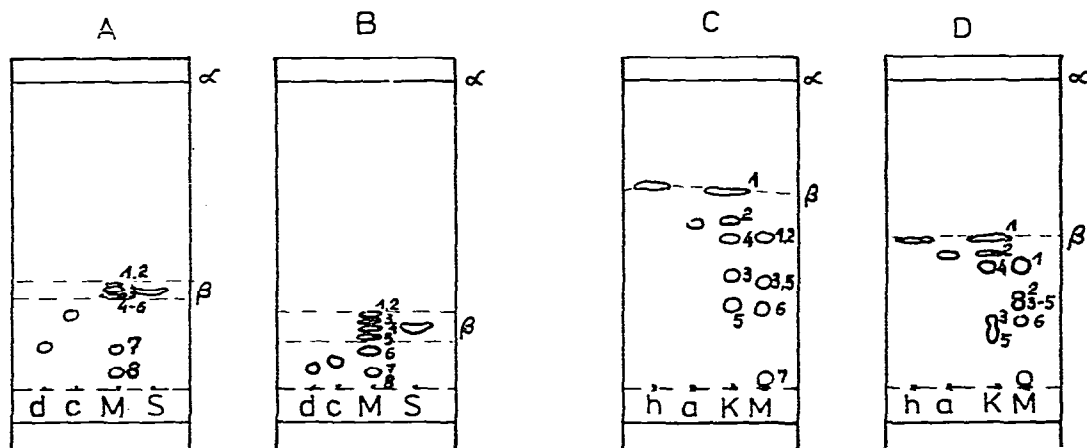


Fig. 7. Effect of specific surface area on solvent demixing. A, Alumina, 200 m²/g; B, silica gel 60, 500 m²/g; C, alumina Type T, 70 m²/g; D, alumina Type E, 200 m²/g. Mobile phase: A,B, 5% ethanol in heptane; C,D, 5% dioxane in heptane. Notation of solutes as in Fig. 2, and in addition s = Sudan red, h = azulene.

mine experimentally the minimum distance behind the solvent front which would preclude overtaking the front of solvent demixing by the weakly polar solutes, without unnecessary prolongation of development. The volume of the solvent necessary for saturation of the layer can be calculated, as mentioned above, from Snyder's formula. The following approximate estimation of the distance can also be made.

If the R_F of the solvent demixing front is equal to R_{Fd} , then $1/R_{Fd}$ dead volumes of the mobile phase are necessary for the front to leave the plate and then equilibration of the two phases extends over the whole plate. However, the samples can be spotted one dead volume earlier (measured by the passage of the marker solute from the start line to the end of the plate, *cf.*, ref. 24), since in that situation even the fastest solute cannot overtake the demixing front (Fig. 1). The minimum volume of the developing solvents which should migrate before the sample can be spotted is thus equal to $1/R_{Fd} - 1$. For higher R_{Fd} values the formula simplifies to $1 - R_{Fd}$. It follows, therefore, that the sample should be spotted when the solvent demixing front is in the position $1 - R_{Fd}$ on the plate. Thus, when R_{Fd} is 0.8 the samples can be spotted when the demixing front is in the position 0.2, the R_F of the observed front being 0.25 (since $0.2/0.25 = 0.8$, and $1/0.8 - 1 = 0.25$). For 20 cm plates this is equivalent to spotting the sample only 5 cm behind the observed front (4 cm behind the solvent demixing front). The rule was confirmed experimentally (Fig. 8D).

However, for dilute solutions of the polar solvent (*e.g.*, 5%; *cf.*, Table I) the preliminary development should be considerably longer. For instance, for $R_{Fd} = 0.33$, two dead volumes of the mobile phase are required to eliminate solvent demixing. For a 20 cm plate the flattened zone of the test dye marking the front of solvent demixing should be, at the moment of sample spotting, at a distance of $0.66 \times 20 = 13.2$ cm from the start line. The necessary passage of two dead volumes of the mobile phase along the plate requires *ca.* 3 h for typical solvents and plates (Fig. 9A). Although this calculation neglects partial evaporation of the solvent and its immobilization in the pores of the adsorbent, the experiments with the series of dyes confirmed the principle of calculations.

To reduce the relatively long preequilibration of the plates with dilute solutions of polar solvents, another modification of the procedure was investigated, consisting of preliminary equilibration of the adsorbent with more concentrated solutions, *e.g.*, 50 or 20% heptane solutions of methyl ethyl ketone (MeEtCO). After the solvent demixing front had passed the distance corresponding to $1 - R_{Fd}$ (*e.g.*, $1 - 0.9 = 0.1$ for 50% solution), the development was continued with 5% MeEtCO. Test dyes spotted just after the change of containers (Fig. 9B) were diffuse and poorly separated, with changes in sequence. The disturbance was due to a decreasing gradient of solvent concentration behind the leading, more concentrated, zone. However, spotting the sample with some delay, *e.g.*, *ca.* 6 cm behind the front of decreased concentration, eliminated this effect and the test dyes were separated as in the case of prolonged preequilibration with 5% solvent. The procedure of predevelopment with 50% MeEtCO reduced the time of preequilibration by half (Fig. 9B). A similar result was obtained for predevelopment with a zone of 20% MeEtCO (Fig. 9C). Further delay in spotting the sample of test dyes did not change the relative migration rates; also the solvent flow-rate becomes constant, as illustrated in Fig. 9D. Zones of Sudan IV spotted at the same place in equal time intervals migrated equal distances.

Attempts to estimate the volume of the diluted solvent which should follow

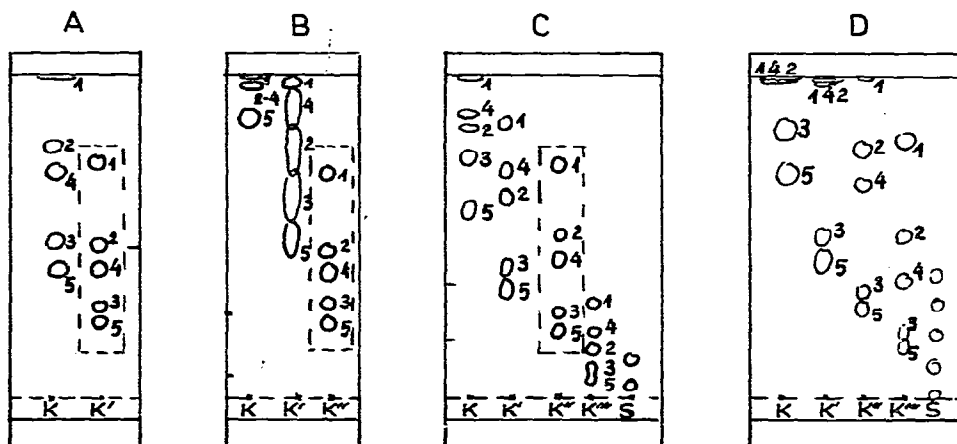


Fig. 9. Elimination of solvent demixing by predevelopment. A, Continuous development with 5% MeEtCO in heptane, samples spotted on the dry layer (K) and behind the solvent front. B, Predevelopment with 5% MeEtCO at a distance of 2.5 cm followed by development with 5% MeEtCO. Samples spotted on the dry layer (K), just after the change of developing solvent (K') and 6 cm behind the front of decreased concentration (K'). C, Predevelopment with 20% MeEtCO at a distance of 3 cm followed by development with 5% MeEtCO. Samples spotted on the dry layer (K), just after the change of solvent concentration (K'), 7 cm behind the front of decreased concentration (K'') and with further delay (K'''). D, Continuation of development of plate C, Sudan IV spotted in equal (2 cm) distances. Adsorbent, alumina.

the first, concentrated, zone lead to the conclusion that the sample can be spotted when the spot of the marker dye which shows the first solvent demixing front is at the distance of the arithmetical mean of $1 - R_{Fd}$ values of the dilute and concentrated solvent (Table II). The probable concentration profiles of the polar solvent for the

TABLE II

DISTANCE BEHIND THE SOLVENT FRONT (FOR 20 cm PLATES) SUITABLE FOR SPOTTING THE SAMPLE TO ELIMINATE SOLVENT DEMIXING EFFECTS

Adsorbents: silica and alumina (values in parentheses).

Mobile phase: polar solvent (S) + diluent	Distance (cm) corresponding to $1 - R_{Fd}$				Distance (cm) for 5% S after predevelopment with 50% S
	5% S	10% S	20% S	50% S	
Ketones + heptane	13.0-15.0 (10-11)	10.0 (6.6)	6.8 (4.8)	3.0-4.0 (3.0)	9.5 (6.7)
Ethers + heptane	11-12 (3.0)	7.5 (2.0)	5.0 (1.0)	2.6-3.0 (0.5)	7.5 (1.5)
Esters + heptane	13-14 (8-9)	9.6 (6.6)	7.0 (5.0)	4.0 (3.0)	9.0 (6.0)
Alcohols + heptane	14-15 (12-13)	12.0 (8.6)	10.0 (7.2)	4.5 (2-2.5)	10.0 (4.5)
Diethylamine + benzene	11-12 (7-8)	6.2 (4.0)	4.0 (2.0)	1.5 (0.5)	6.5 (4.5)
Dimethyl sulphoxide benzene	13-14 (10-11)	10.2 (7.8)	8.0 (3.0)	1.5-2.0 (1.5)	7.0 (6.0)
Ketones + benzenes	8-9 (5-6)	6.6 (3-4)	4.4 (2.0)	1.5 (0.5)	5.5 (3.5)

two-stage procedure are illustrated in Fig. 10. The empirical distance equal to the half the sum of the $1 - R_{Fd}$ values for the two concentrations corresponds to the equilibrium of the system in the proximity of the start line.

The procedure of preequilibration of the adsorbent with more concentrated developing solvents permits the use of dilute solutions of polar solvents which in ordinary saturated tanks frequently give unreproducible results quite incompatible with column separations. The procedure could be tested more precisely with column chromatography; however, it was necessary to investigate the behaviour of chromatographed solutes under conditions of continuous TLC in sandwich tanks. Even for the predevelopment procedures, the expenditure of mobile phase was very low, of the order of several millilitres only.

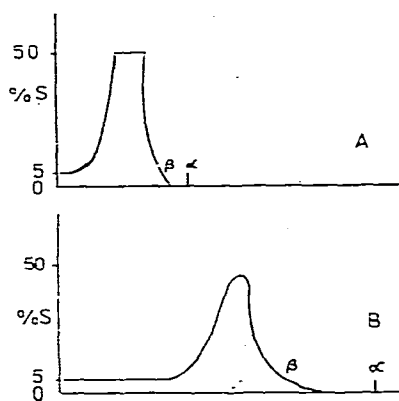


Fig. 10. Probable concentration profiles during predevelopment with 50% MeEtCO (A) followed by development with 5% MeEtCO (B). Tailing of the zone of higher concentration requires spotting of the sample with some delay, α . Observed front; β , diffuse front of solvent demixing.

ACKNOWLEDGEMENT

This research was financially supported by the Polish Academy of Sciences (Problem No. 03.10).

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