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PURITY CONTROL OF SOME *o*-HYDROXY-SUBSTITUTED THIAZOLYLAZO DYES BY THIN-LAYER CHROMATOGRAPHY

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SUMMARY

By using ascending chromatography on thin layers of Silica Gel G, some *o*-hydroxy-substituted monoazo dyes were separated from common impurities such as other azo dyes and free phenols. With the suggested solvents containing acetic acid, the azo dyes migrate as undissociated species and the formation of colour complexes with metal impurities from commercial silica gels (which occurred when chromatographing with neutral or basic solvents) was suppressed. Developing techniques with both equilibrated and unsaturated N-chambers proved to be suitable.

INTRODUCTION

With increasing use of the N-heterocyclic *o*-hydroxy-substituted monoazo dyes in analytical and coordination chemistry[1], a higher degree of purity of these reagents is required. For testing the purity of these and related azo dyes, paper[2–5] and thin-layer chromatography (TLC) on Silica Gel G [6] have been used with various basic, neutral and acidic developing solvents. However, these methods are oriented mainly to the separation and detection of those sample components which are either coloured themselves or give coloured complexes with metal salts as detecting reagents; no information is given in these papers concerning the migration of other possible (colourless) impurities such as starting compounds and by-products. As we observed when repeating certain of the above methods, some azo dyes migrate at the same rate as their passive phenolic components. Moreover, in the neutral and basic solvents, the chromatographed reagents react with trace metal impurities present in the common commercial silica gels and papers, and give crescent-shaped ghost spots of complexes that usually migrate before the dye spots. During the chromatography of the same reagent with some basic solvents, more coloured spots also appeared than with acidic or neutral solvents[5], but this phenomenon could be also due to the separate migration of the dye as its molecular and ionic species.

The aim of this work was to eliminate all these imperfections with regard to the nature of the system. Hence, for TLC on Silica Gel G, only acidic solvent systems were used with an acetic acid concentration capable of preventing the dissociation

of the phenolic hydroxyl group and of inhibiting a possible complexation of the dyes with metal impurities from the silica gel. The separate migration of the dyes examined and their possible impurities was studied as the decisive criterion for the choice of suitable solvent combinations.

EXPERIMENTAL

Chemicals and reagents

The azo dyes, their corresponding phenols, and aminothiazole were either commercial *pro analysi* preparations (Lachema, N.E., Brno, Czechoslovakia) or were synthesized in the Research Institute for Pure Chemicals (Brno) and in our Department from chemicals of *pro analysi* or pure quality by using conventional methods. If required, the preparations were recrystallized from ethanol. The solvents of the same degree of purity were distilled before use (except acetic acid). Chloroform contained 1% of ethanol as a conservation admixture. The detection reagents (compositions given in Table I) were prepared from *pro analysi* chemicals. All reagents and solvents used here were purchased from Lachema, N.E., Brno.

TABLE I

SENSITIVITY OF VARIOUS DETECTION METHODS (ON CHROMATOGRAMS DEVELOPED WITH SOLVENT I)

Spray solutions (in water, proportions by volume given) or procedure: D 1 = 0.1 *N* $KMnO_4$; D 2 = 0.1 *N* $AgNO_3$ + 5 *N* NH_4OH (1:1), heated for 5–10 min at 80–100°, this treatment being repeated four times; D 3 = 0.1 *N* $CuSO_4$ + 10% NH_3 (1:1); D 4 = quenching of the fluorescence of the inorganic fluorescent indicator (excitation wavelength 254 nm); D 5 = natural colouration, given for acid/base form in air and NH_3 atmospheres, respectively.

Compound	*Detection limits (μg)*				
	D 1	*D 2*	*D 3*	*D 4*	*D 5*
TAR[a]	0.2	0.05	0.02	0.2	0.1; 0.05
TAMH[a]	0.2	0.1	0.02	0.2	0.1; 0.02
Resorcinol	0.1	0.1	—	0.5	—
4-Methoxyphenol	0.2	0.1	—	1.0	—
Aminothiazole	0.1	0.02	—	0.1	—

[a] See Table II.

Procedures and techniques

Glass plates, 20 × 20 cm, were coated with MN-Silica Gel G for TLC with the standard spreading apparatus (Desaga, Heidelberg, G.F.R.) and procedure described by STAHL *et al.*[7]; the layer thickness was 0.25 mm. The coated plates were dried for 2–3 h in air and for 1.5 h at 105°; they were deactivated again before use by standing for 5–24 h in air at room temperature (20 ± 2°) and 50–60% relative humidity. For the fluorescence-quenching detection under the short-wave UV lamp (Fluotest; Haereus, Hanau, G.F.R.), 1% of Fluorescent Indicator Green (Woelm, Eschwege, G.F.R.) was added to the solid adsorbent before slurrying it.

The 0.3% sample solutions in ethanol were applied to the starting line in quantities of 1–10 μg of solid sample at each point. The plates were developed by

ascending chromatography up to a solvent front distance of 15 cm from the start with 50 ml of fresh solvent mixture in a standard large-volume N-chamber (Desaga, Heidelberg, G.F.R.), conditioned in the two following ways:

(A) Equilibrated chamber. The plates with applied samples were equilibrated for 30 min with solvent vapour in a chamber, the inner walls of which were lined with a U-shaped strip of filter-paper; the development was started without opening the chamber.

(B) Unsaturated chamber. The chromatography was started immediately after the introduction of the solvent and the plate into the chamber; no filter-paper was used.

The developed chromatograms were dried in a hot air stream and detected by spraying with the reagents given in Table I.

RESULTS AND DISCUSSION

The detection limits of the visualization methods used for some representative compounds are given in Table I. The azo dyes can be detected either on the basis of their own coloration or better as their more intensively coloured metal complexes. For the detection of phenols and/or aminothiazole, the reaction with an ammoniacal solution of silver nitrate was found to be more sensitive and that with permanganate more universal and rapid. The fluorescence-quenching method (D 4) is rather unreliable because of the occurrence of the complexation between metal ions released from the inorganic indicator and the azo dyes applied to the layer a certain time before starting the development.

The azo dyes, their passive phenolic components and aminothiazole were chromatographed in parallel in the equilibrated (A) and unsaturated (B) chambers. The solvents used together with the abbreviations used for the azo dyes and the R_F values for both developing techniques are given in Table II.

In general, the R_F values are higher and the separations of the pairs azo dye–corresponding phenol are more effective when developed in the unsaturated chamber. Of course, the R_F values (depending on the solvent nature, geometry of the arrangement and other factors) are less reproducible here than in the equilibrated chamber (see also Figs. 1 A and B). When a slight edge effect or other similar developing irregularities occurred (more frequently with solvent mixtures III–V, containing carbon tetrachloride), only the R_F values above the starting points situated nearer to the centre of the chromatogram were read and tabulated.

As a rule, lower and more reproducible R_F values should be obtained with saturated chambers. As the usual method of chamber saturation (without plate) with the solvent added not long before starting the chromatography[7], provided results that still fluctuated to some extent, we preferred to equilibrate the layers with solvent vapour for 30 min before developing. In this way, the minimum R_F deviations on the same plate were achieved, probably due to a more complete suppression of the concentration gradients of the solvent components in the gas phase and the layer as well. Although the chromatography in the equilibrated chambers took only two-thirds of the period required for the unsaturated chambers, the total times required were approximately equal for both developing techniques.

The first three solvent mixtures, I–III, are universal for the given group of

TABLE II

R_F VALUES OF THE AZO DYES AND SOME OF THEIR CONSTITUENTS

Developed in (A) equilibrated and (B) unsaturated chamber. Developing solvents: I = benzene–acetic acid (80:20); II = benzene–chloroform–acetic acid (25:5:6.2); III = carbon tetrachloride–acetic acid (30:8); IV = carbon tetrachloride–ethyl acetate–acetic acid (30:3:2); V = carbon tetrachloride–acetic acid (30:4).

Compound	*Abbreviation*	*R_F values for solvents*									
		I		*II*		*III*		*IV*		*V*	
		A	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*
2-(2-Thiazolylazo)-*p*-cresol	TAC	0.59	0.76	0.64	0.85	0.65	0.81	0.53	0.74	0.49	0.75
p-Cresol	—	0.47	0.70	0.49	0.77	0.47	0.68	0.39	0.61	0.29	0.51
2-(2-Thiazolylazo)hydroquinone	TAH	0.27	0.49	0.27	0.53	0.23	0.35	0.17–0.10	0.26–0.12	0.10–0.0	0.17–0.07
Hydroquinone	—	0.14	0.27	0.12	0.30	0.07	0.18	0.05	0.11	0.03	0.05
2-(2-Thiazolylazo)hydroquinone 4-methyl ether	TAMH	0.55	0.74	0.60	0.80	0.58	0.76	0.45	0.55	0.44	0.61
4-Methoxyphenol	—	0.41	0.63	0.41	0.69	0.35	0.57	0.29	0.42	0.20	0.33
2-(2-Thiazolylazo)hydroquinone 4-ethyl ether	TAEH	0.57	0.75	0.60	0.80	0.62	0.78	0.47	0.55	0.47	0.58
4-Ethoxyphenol	—	0.45	0.68	0.43	0.70	0.41	0.61	0.31	0.41	0.24	0.33
4-(2-Thiazolylazo)resorcinol	TAR	0.27	0.48	0.27	0.52	0.21	0.39	0.17	0.27	0.08	0.16
Resorcinol	R	0.15	0.30	0.13	0.32	0.08	0.20	0.07	0.13	0.03	0.06
2-(2-Thiazolylazo)resorcinol 5-methyl ether	TAMR	0.56	0.73	0.59	0.78	0.57	0.76	0.43	0.46	0.43	0.51
3-Methoxyphenol	—	0.43	0.61	0.42	0.69	0.38	0.61	0.31	0.38	0.21	0.29
4-(2-Thiazolylazo)-6-methylresorcinol	MeTAR	0.33	0.61	0.33	0.53	0.28	0.47	0.18	0.29	0.15	0.22
6-Methylresorcinol	MeR	0.21	0.42	0.18	0.35	0.15	0.26	0.09	0.17	0.03	0.11
4-(2-Thiazolylazo)-6-ethylresorcinol	EtTAR	0.36	0.62	0.35	0.55	0.31	0.48	0.21	0.29	0.15	0.20
6-Ethylresorcinol	EtR	0.28	0.52	0.21	0.40	0.21	0.33	0.13	0.23	0.07	0.13
4-(2-Thiazolylazo)-6-propylresorcinol	PrTAR	0.39	0.63	0.38	0.58	0.33	0.51	0.23	0.26	0.16	0.18
6-Propylresorcinol	PrR	0.25	0.47	0.21	0.41	0.24	0.32	0.13	0.16	0.07	0.09
4-(2-Thiazolylazo)-6-butylresorcinol	BuTAR	0.41	0.63	0.39	0.59	0.36	0.57	0.24	0.29	0.19	0.22
6-Butylresorcinol	BuR	—	—	—	—	—	—	—	—	—	—
4-(2-Thiazolylazo)-6-pentylresorcinol	PenTAR	0.43	0.65	0.41	0.61	0.38	0.62	0.25	0.33	0.21	0.25
6-Pentylresorcinol	PenR	—	—	—	—	—	—	—	—	—	—
4-(2-Thiazolylazo)-6-hexylresorcinol	HexTAR	0.45	0.65	0.43	0.62	0.41	0.68	0.25	0.37	0.21	0.30
6-Hexylresorcinol	HexR	0.36	0.58	0.31	0.53	0.28	0.56	0.18	0.29	0.12	0.20
4-(2-Thiazolylazo)orcinol	TAO	0.28	0.50	0.28	0.52	0.24	0.44	0.17	0.33	0.09	0.15
Orcinol	—	0.17	0.30	0.15	0.31	0.11	0.26	0.07	0.17	0.03	0.07
4-(2-Thiazolylazo)phloroglucinol	TAPh	0.15	0.34	0.15	0.31	0.10	0.23	0.08	0.12	0.03	0.06
Phloroglucinol	—	0.03	0.07	0.02	0.05	0.03	0.33	0.01	0.02	0	0
4-(2-Thiazolylazo)pyrogallol	TAP	0.17–0.0	0.34–0.0	0.18–0.07	0.25	0.15–0.0	0.30–0.19	0.07–0.0	0.10–0.0	0.05	0.09
Pyrogallol	—	0.10	0.20	0.10	0.22	0.07	0.15	0.04	0.05	0.03	0.04
2-Aminothiazole	—	0.04	0.09	0.04	0.08	0.05	0.08	0	0	0.02	0.03
Developing time (min)		55	85	60	90	95	120	75	120	80	125

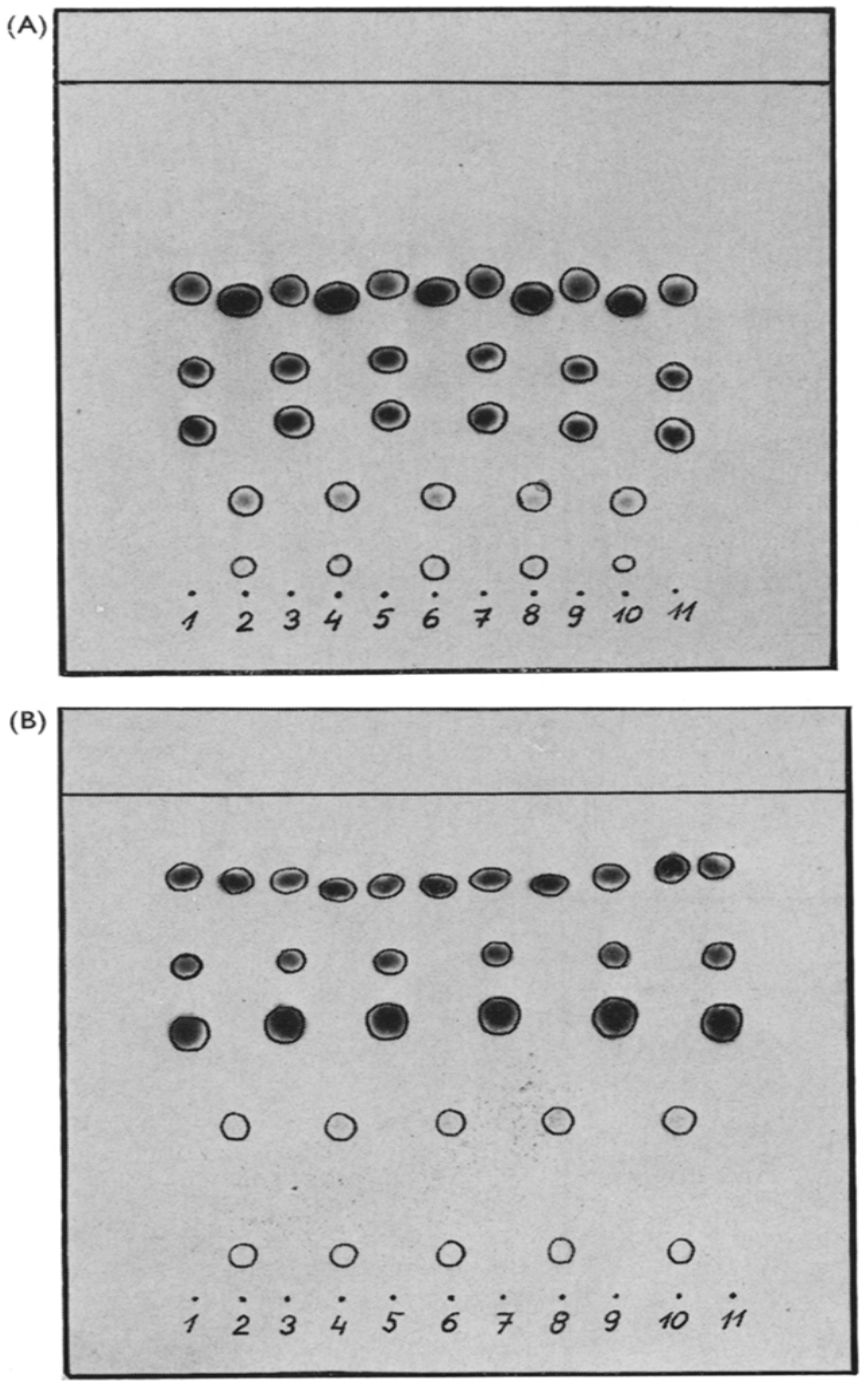

Fig. 1. TLC of some azo dyes and their constituents on a Silica Gel G layer with benzene–acetic acid (80:20, v/v) in (A) equilibrated and (B) unsaturated chamber. Spots (from top to bottom): odd numbers, TAC, HexTAR, TAR; even numbers, TAMR, R, aminothiazole.

compounds; by using them, almost all the azo dyes examined can be separated from their corresponding phenols and aminothiazole (Figs. 2 and 3). The mobilities of the azo dyes and phenols decrease with an increase in the number of polar substituents on the ring of the passive component. The separation of TAP from pyrogallol was not achieved; TAPh separated from phloroglucinol rather unsatisfactorily.

The relative differences in R_F values between an azo dye and its corresponding

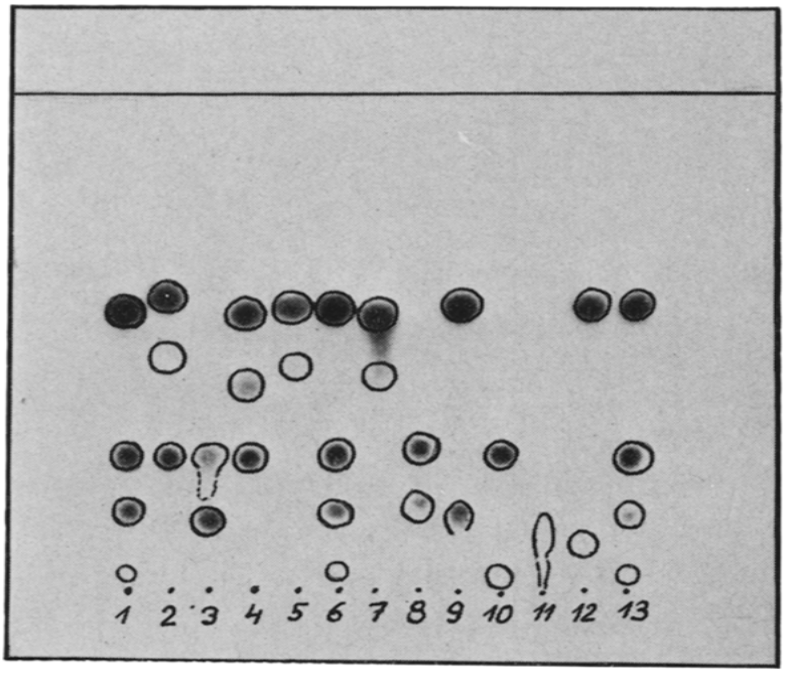

Fig. 2. Separation of some azo dyes from phenols. Developing solvent I, equilibrated chamber. Spots (from top to bottom): 1 = TAMR, TAR, R, aminothiazole; 2 = TAC, cresol, TAR; 3 = TAH, hydroquinone; 4 = TAMH, 4-methoxyphenol, TAR; 5 = TAEH, 4-ethoxyphenol; 6 = TAMR, TAR, R, aminothiazole; 7 = TAMR, 3-methoxyphenol; 8 = TAO, orcinol; 9 = TAMR, TAPh; 10 = TAR, phloroglucinol; 11 = TAP; 12 = TAMR, pyrogallol; 13 = TAMR, TAR, R, aminothiazole.

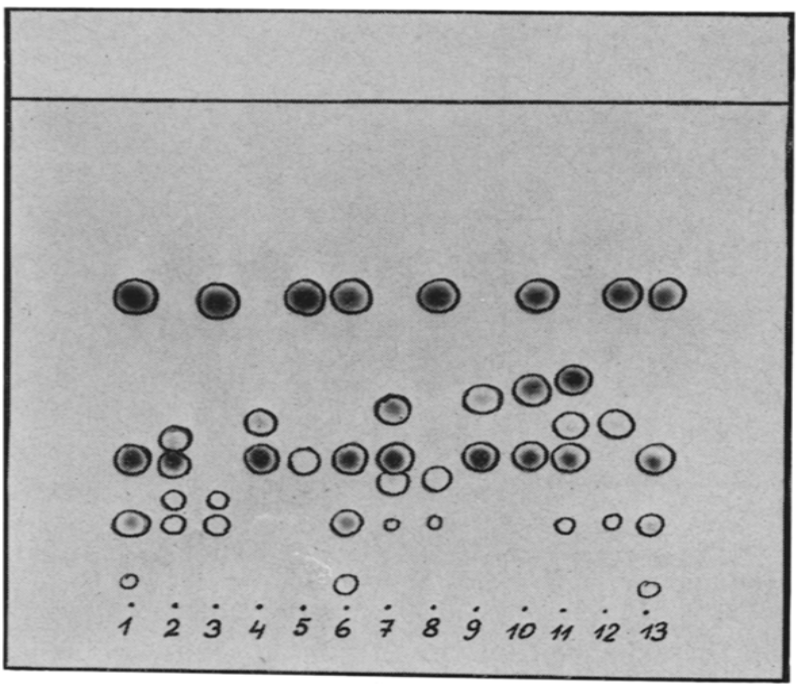

Fig. 3. Separation of *n*-alkylresorcinols from their azo dyes. Developing solvent I, equilibrated chamber. Spots (from top to bottom): 1 = TAMR, TAR, R, aminothiazole; 2 = MeTAR, TAR, MeR, R; 3 = TAMR, MeR, R; 4 = EtTAR, (TAR + EtR); 5 = TAMR, EtR; 6 = TAMR, TAR, R, aminothiazole; 7 = PrTAR, TAR, PrR, R; 8 = TAMR, PrR, R; 9 = BuTAR, TAR; 10 = TAMR, PenTAR, TAR; 11 = HexTAR, HexR, TAR, R; 12 = TAMR, HexR, R; 13 = TAMR, TAR, R, aminothiazole.

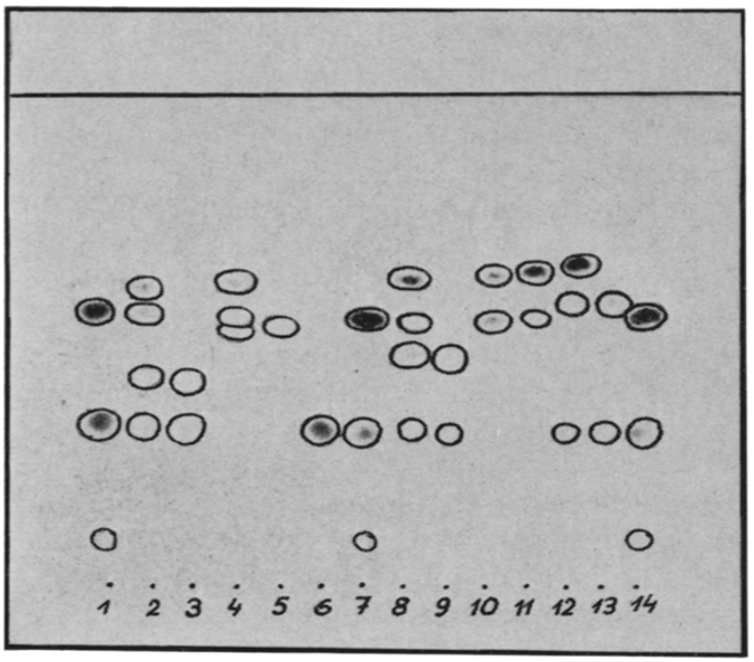

Fig. 4. Separation of *n*-alkyl-TARs from their azo dyes. Developing solvent I, unsaturated chamber. Spots (from top to bottom): 1 = TAR, R, aminothiazole; 2 = MeTAR, TAR, MeR, R; 3 = MeR, R; 4 = EtTAR, TAR, EtR; 5 = EtR; 6 = R; 7 = TAR, R, aminothiazole; 8 = PrTAR, TAR, PrR, R; 9 = PrR, R; 10 = BuTAR, TAR; 11 = PenTAR, TAR; 12 = HexTAR, HexR, R; 13 = HexR, R; 14 = TAR, R, aminothiazole.

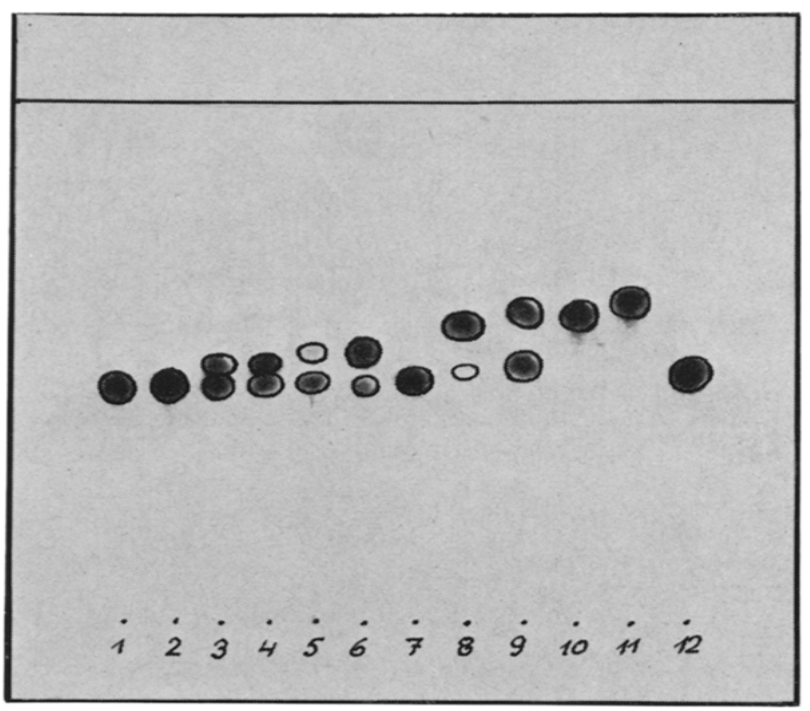

Fig. 5. TLC detection of TAR as an impurity in preparations of alkyl-TARs. Spots (from top to bottom): 1,2,7,12 = TAR; 3 and 4 = various preparations of MeTAR; 5, 6 = various preparations of EtTAR; 8 = PrTAR; 9 = PenTAR; 11 = HexTAR.

phenol in the equilibrated chambers are smaller while, on the contrary, the R_F differences in the homologous series of alkyl-TARs (derived from 6-alkylated resorcinols with n-alkyl = C_1–C_6) are greater than in the unsaturated chambers. This behaviour corresponds qualitatively to the probable shift of the separation mechanism from adsorption to partition caused by the foregoing equilibration. In the unsaturated chamber, however, TAR can also be separated from alkyl-TARs quite well with solvents I–III (Fig. 4).

Solvent mixtures IV and V are suitable for separating more rapidly migrating azo dyes from each other and from their corresponding phenols (including the pair TAR–resorcinol) in both equilibrated and unsaturated chambers. In particular, when solvent V is used, the greatest relative R_F differences for these compounds can be achieved. On the other hand, the separation of alkyl-TARs from each other is almost impossible.

The TLC methods described here have been used successfully for testing the purity of *o*-hydroxy-substituted thiazolylazo dyes, the complex equilibria and analytical reactions of which have been studied systematically in our department. By using a TLC test, for example, a very strong contamination of various preparations of alkyl-TARs with TAR was proved (see Fig. 5); in this instance, the starting 6-alkylresorcinols used for the coupling synthesis of the dyes evidently contained large quantities of resorcinol. Greater contamination of the azo dyes with free phenols was observed less frequently. If one considers the detection limit (*e.g.*, about 0.1 μg for resorcinol) and the average capacity of the method for separating the pairs azo dye–phenol, which is 10 μg of TAR for the single development and 30 μg of TAR for the double development from one starting point, the presence of more than 1.0% of free resorcinol in the sample analyzed for the single development and 0.3% for the double development can be identified by using this TLC test.

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