Journal of Chromatography, 161 (1978) 279–286

CHROM. 11,125

SEPARATION AND IDENTIFICATION OF WATER-SOLUBLE FOOD DYES BY ION-EXCHANGE AND SOAP THIN-LAYER CHROMATOGRAPHY

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(First received April 18th, 1978; revised manuscript received May 4th, 1978)

SUMMARY

Eighteen water-soluble food dyes have been studied by chromatography on thin layers of anion-exchange (AG 1-X4, DEAE-cellulose, PAB-cellulose and chitosan) and cation-exchange (Dowex 50-X4, Rexyn 102 and humic acid) materials; layers of silanised silica gel impregnated with cationic or anionic detergent were also used. Fourteen of the dyes were separated, but the two orange and the two black dyes were not. Some applications of the techniques to commercial products are reported.

INTRODUCTION

Differences of opinion about the toxicity of various food colours are reflected in the different lists of food dyes permitted in most countries. In Italy, according to E.E.C. directions, the number of synthetic organic dyes has recently been reduced from 17 to 10. We deemed it useful, therefore, to study the possibility of separating, by thin-layer chromatography (TLC), 18 dyes, including orcein and all the synthetic dyes now permitted in Italy or that have been used in the past.

On the basis of the results achieved in the separation of many classes of organic compounds[1–7], studies have been carried out on layers of weak or strong anion- and cation-exchangers with polystyrene, paraffinic or cellulosic matrices; soap TLC[8] was also used. This work complements that of other researchers on alumina[9], cellulose[10], silica gel[10–13] and polyamide[14] thin layers.

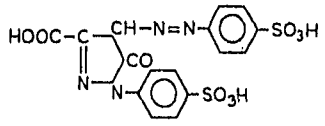
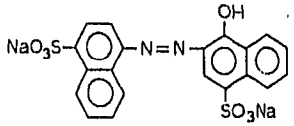
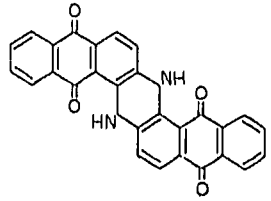
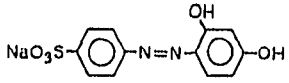
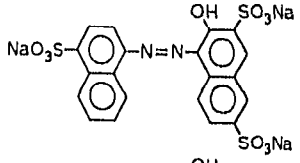
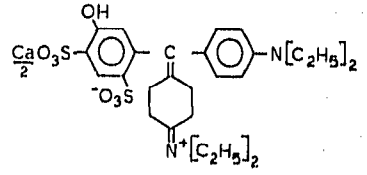
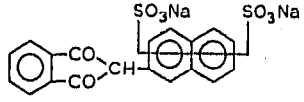
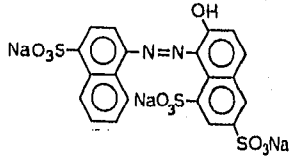
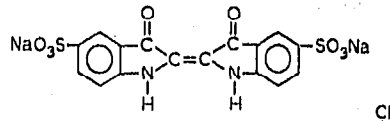
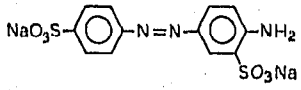
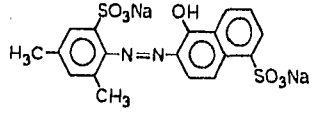
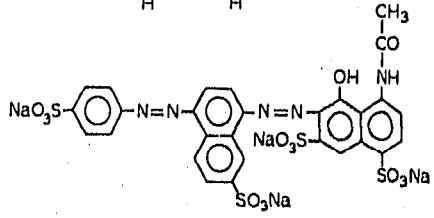
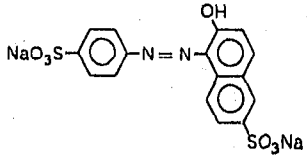
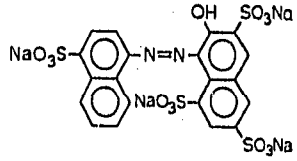
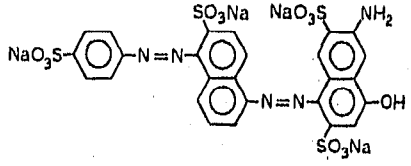
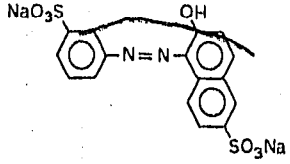
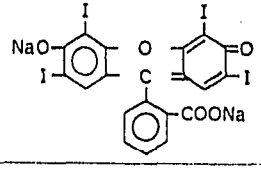
EXPERIMENTAL

Preparation of solutions

Standard solutions (2 mg/ml) were prepared in water–methanol (4:1, v/v); with indanthrene blue (E 130), a suspension was used. The amount of each dye on the layer varied with the type of exchanger and is shown in the tables. A fresh solution of

TABLE I

NUMBERS, COLOURS AND STRUCTURES OF DYES

Dye, C.I. number and colour	*Structure*	*Dye, C.I. number and colour*	*Structure*	*Dye, C.I. number and colour*	*Structure*
E 102 19140 Yellow		E 122 14720 Red		E 130 69800 Blue	
E 103 14270 Yellow		E 123 16185 Red		E 131 42051 Blue	
E 104 47005 Yellow		E 124 16255 Red		E 132 73015 Blue	
E 105 13015 Yellow		E 125 14815 Red		E 151 23440 Black	
E 110 15985 Orange		E 126 16290 Red		E 152 27755 Black	
E 111 15980 Orange		E 127 45430 Red			
E 121* — Red					

* Orcein, a natural extract obtained from *Rocella* and other lichens.

indigo carmine (E 132), which easily decomposes, was used in each experiment. Table I shows the chemical structures and the Colour Index numbers of the dyes.

Dyes were extracted from chocolate candies by dipping them in water until the superficial layer had dissolved[15]; the aqueous solution was then treated with an equal volume of ethanol, and the resulting suspension was filtered.

Preparation of layers

The layers (thickness 300 μm) were prepared, with use of a Chemetron apparatus, from the following mixtures. (a) DEAE-Cellulose (Bio-Rad): 9 g of the exchanger in 50 ml of water; (b) AG 1-X4 (Bio-Rad), Dowex 50-X4 (Dow), Rexyn 102 (Fisher), PAB-Cellulose (Serva), chitosan[16] or humic acid (Roth)[17]: 3 g of the exchanger and 9 g of microcrystalline cellulose (Merck) in 50 ml of water. The layers of silanised silica gel impregnated with anionic or cationic detergents were prepared as described elsewhere[8]. Before use, the exchangers (except for humic acid, chitosan and PAB-cellulose) were rinsed with water and methanol and dried at room temperature. Layers prepared by mixing humic acid with microcrystalline cellulose were grey in colour.

All measurements were carried out at 25 $\pm$ 0.5°, and the migration distance was 11 cm unless otherwise stated.

RESULTS AND DISCUSSION

Anion-exchangers

Table I shows that most of the dyes contain one or more sulphonic acids and/or carboxyl groups; the dyes should therefore behave like anions, except for orcein (E 121) and indanthrene blue. For this reason, their retention on anion-exchangers can be predicted.

AG 1-X4(CH_3COO^-). On this exchanger, the dyes are strongly retained and remain at the starting point during development with aqueous solutions of different pH values and concentrations and with aqueous–organic mixtures. This strong retention can be ascribed, as well as to the ion-exchange process, to adsorption of the dyes (which contain at least two aromatic nuclei) by the polystyrene matrix of the exchanger.

DEAE-cellulose (Cl^-). This cellulose-based strong anion-exchanger was chosen in order to limit adsorption by the matrix. Table II (column 2) shows the R_F values of the 18 dyes on development with 0.6 *M* ammonium chloride in water–methanol (7:3, v/v). From these data, strong retention of most dyes is indicated; however, some separations can be effected, such as that of the three blues (E 130, E 131 and E 132) and that of E 131 from all the other dyes. Also, the two orange compounds (E 110 and E 111) can be separated from the other dyes (except E 121, which gives an elongated spot starting from the application point). With E 104, two spots are observed owing to the presence of monosulphonate by-products of the dye.

The high mobility of E 131 in this system can be ascribed to the presence of a positive charge in the molecule, which reduces ionic interaction with the functional groups of the exchanger.

PAB-cellulose. This cellulose is a weak anion-exchanger and therefore the behaviour of the dyes could be studied both in the presence and in the absence of the

TABLE II

R_F VALUES OF WATER-SOLUBLE FOOD DYES ON LAYERS OF (a) DEAE-CELLULOSE, (b) PAB-CELLULOSE, (c) CHITOSAN AND (d) MICROCRYSTALLINE CELLULOSE

E.S. = Elongated spot.

Dye	*Mobile phase*				*Amount (μg)*
	0.6 M NH_4Cl in H_2O–methanol (7:3, v/v)	*0.1 M NaH_2PO_4*	*H_2O–ethanolamine (14:1, v/v)*		
	(a)	*(b)*	*(c)*	*(d)*	
E 102	0.10	0.47	0.89	0.90	1.0
E 103	0.21	0.38	0.68	0.72	1.0
E 104	0.05*	0.04***	0.35**	0.40**	1.0
	0.11**	0.19***	0.45**	0.51**	
			0.69*	0.76*	
E 105	0.25	0.65	0.75	0.79	1.0
E 110	0.33	0.78	0.81	0.87	1.0
E 111	0.33	0.78	0.81	0.87	1.0
E 121	E.S.	E.S.	(0.93)§	E.S.	1.5
E 122	0.03	0.11	0.44	0.54	1.0
E 123	0.10	0.20	0.59	0.63	1.0
E 124	0.10	0.57	0.74	0.78	1.5
E 125	0.20	0.45	0.91	0.91	1.5
E 126	0.10	0.23	0.54	0.58	1.0
E 127	0.03	0.05	0.27	0.37	1.0
E 130	0.00	0.00	0.00	0.00	1.5
E 131	0.81	E.S.	0.89	0.89	0.5
E 132	0.08	E.S.	E.S.	E.S.	2.0
E 151	0.00	0.01	(0.43)§	0.57	1.5
E 152	0.00	0.01	(0.43)§	0.57	1.5

* Main spot.
** Secondary spot.
*** Two spots of similar intensity.
§ Parentheses indicates streaking.

ion-exchange process. In fact, with acid mobile phases, it behaves like an anion-exchanger: under such conditions, the dyes, as might be expected, are strongly retained. With neutral and alkaline solutions, the functional groups of the exchanger change to the free-base form so that ionic interactions between them and the sulphonic acid and/or carboxyl groups of the dyes disappear and there is a general increase in R_F values.

Table II (column 3) shows that, in this system, the four yellow dyes (E 102, E 103, E 104 and E 105) can be separated with 0.1 *M* NaH_2PO_4 at pH 7 as mobile phase. The two orange dyes (E 110 and E 111) can be separated from all the other compounds, including E 121 (which gives a less elongated spot than on DEAE-cellulose). Among the red dyes, the good separation between the isomers E 123 and E 124 is noteworthy.

With aqueous solutions at pH > 7 as mobile phase, no remarkable differences were observed in the behaviour of most dyes as compared with use of 0.1 *M* NaH_2PO_4. Thus, the functional groups of the exchanger must already be in free-base form at pH 7.

Chitosan. This cellulose-based exchanger was used only with alkaline mobile phases, as with acid and neutral solutions it behaved like an anion-exchanger[16]. The best results were obtained with water–ethanolamine (see column 4 of Table II). In the same table (column 5) are reported values obtained on microcrystalline cellulose with similar mixtures for development. Comparison of these results shows that (a) the R_F sequences on the two layers are similar and (b) most of the dyes are retained to a greater extent on chitosan than on microcrystalline cellulose. This stronger retention on chitosan can be ascribed to non-ionic interactions between the dyes and the functional groups of the exchanger.

The differences in the chromatographic behaviour of the dyes on the two layers are important analytically, as, on microcrystalline cellulose, some separations on chitosan cannot be effected. For example, those of E 102, E 103, E 104, E 105 and E 110 (or E 111) and E 122, E 123, E 124, E 125, E 126 and E 127. Also, E 121 can be identified, as it gives an elongated spot different in colour from that of E 125.

Cation-exchangers

Owing to the anionic character of most dyes, their chromatographic behaviour can be studied in the absence of the ion-exchange process on cation-exchanger layers.

Dowex 50-X4(H^+). The polystyrene matrix of this exchanger exhibits a remarkable affinity towards the dyes. On development with aqueous solutions, the compounds are strongly retained, giving diffuse spots. With aqueous–organic solvents, however, the influence of the matrix is decreased and more compact spots are obtained.

In Table III (column 2) are reported the R_F values of the dyes with a mobile phase of 0.1 *M* HCl in water–methanol (7:3, v/v).

The R_F sequence of the yellow and the orange dyes is different from that observed, under different migration conditions, on strong or on weak anion-exchangers; the sequence of the red dyes is similar to that obtained on PAB-cellulose. The use of layers of Dowex 50-X4(H^+) does not offer any improvement in the separation of the dyes, since, on this exchanger, a difference of 0.10 in the R_F values is necessary to achieve good separation.

Rexyn 102(H^+). With this paraffin-based exchanger, separations better than those on polystyrene or cellulose-based exchangers can be attained (see Table III, column 3). On Rexyn 102(H^+), in fact, as well as the separation of the four yellow dyes, and of the three blue dyes, and of these from the two black dyes, the seven red dyes can be identified. E 127, which remains at the application point, can be visualised by its deep red colour, despite the presence of the pink secondary spot of E 121.

As regards the differences between the behaviour of the dyes on Rexyn 102 and on Dowex 50-X4, the sequence reversal of E 123 and E 126 and the levelling-out of R_F values of the two orange dyes and E 105 should be noted. The influence of structure on the chromatographic behaviour of the dyes cannot be easily evaluated, as it is difficult to compare compounds with different structures. When such comparison is possible, it is found that the position and/or the number of the sulphonic acid groups greatly affects the chromatographic behaviour (see, for example, E 122, E 123, E 124 and E 126).

Humic acid. The chemical structure of humic acid has not been completely established[17]; to our knowledge, it has never been used in column chromatography

TABLE III

R_F VALUES OF WATER-SOLUBLE FOOD DYES ON LAYERS OF (a) DOWEX 50-X4(H^+), (b) REXYN 102(H^+), (c) HUMIC ACID, (d) SILANISED SILICA GEL AND (e) SILANISED SILICA GEL IMPREGNATED WITH 4% OF DBS

E.S. = Elongated spot.

Dye	*Mobile phase*					*Amount (μg)*
	0.1 M HCl in H_2O–CH_3OH (7:3)			*H_2O–CH_3OH–acetic acid (64.3:30:5.7)*		
	(a)	*(b)*	*(c)*	*(d)*	*(e)*	
E 102	0.65	0.62	0.66	0.95	0.95	1.0
E 103	0.40	0.29	0.29	0.57	0.73	1.0
E 104	0.22**	0.28***	0.28**	0.72**	0.81**	1.5
	0.42*	0.38***	0.44*	0.87*	0.88*	
	0.65**		0.68**	0.97**	0.97**	
E 105	0.82	0.75	0.75	0.93	0.88	1.0
E 110	0.93	0.75	0.78	0.80	0.85	1.0
E 111	0.93	0.75	0.78	0.82	0.85	1.0
E 121	(0.02)§	0.00**	0.00	E.S.	(0.02)§	1.5
		0.09*				
E 122	0.23	0.18	0.29	0.55	0.77	1.0
E 123	0.42	0.48	0.55	0.95	0.95	1.0
E 124	0.82	0.83	0.77	0.95	0.95	1.0
E 125	0.70	0.55	0.73	0.69	0.83	1.0
E 126	0.51	0.30	0.37	0.54	0.74	1.0
E 127	0.00	0.00	0.00	0.01	0.01	1.0
E 130	0.00	0.00	0.00	0.00	0.00	1.5
E 131	E.S.	0.59	0.97	0.19	0.27	1.0
E 132	E.S.	0.39	(0.55)§	0.95	0.95	1.5
E 151	(0.06)§	(0.13)§	(0.25)§	0.94	0.93	1.0
E 152	(0.06)§	(0.13)§	(0.25)§	0.95	0.93	1.0

* Main spot.
** Secondary spot.
*** Two spots of same intensity.
§ Parentheses indicate streaking.

or in TLC. With this last technique, its use is restricted owing to the difficulty in detecting compounds after migration (because of the dark colour of the layer). In our work, no such difficulty existed and such layers could be used.

Aqueous and aqueous–organic solutions at different pH values were used as mobile phases, as the layer disintegrated with weakly alkaline phases (*i.e.*, 0.1 *M* $NaHCO_3$). In Table III (column 4) are reported the R_F values under the same experimental conditions as with Dowex 50-X4 and Rexyn 102.

On the basis of the close similarity in behaviour of the dyes on such layers, it would appear that humic acid behaves like a cation exchanger. From the analytical standpoint, many separations that can be effected on the other two exchangers can also be obtained on humic acid. The amount of dye deposited on the layer was 2 μg for the yellow and for two red (E 126 and E 127) colours.

Soap TLC

We employed both anionic (DBS = triethanolamine dodecylbenzenesulphon-

ate) and cationic (DPC = *n*-dodecylpyridinium chloride) detergents in order to compare the behaviour of the dyes on such layers and on anion- and cation-exchanger layers. Some dyes have already been studied by paper chromatography, with mobile phases of aqueous–organic mixtures containing anionic, cationic and non-ionic detergents and on papers impregnated with such surface-active agents[18].

Silanised silica gel impregnated with DBS. In contrast to what was observed with other compounds[8], the presence of DBS did not lead to stronger retention of the dyes compared with that on the silanised silica gel alone. Sometimes, higher R_F values were obtained, as is shown in columns 5 and 6 of Table III, which refer to development with 1 *M* acetic acid in an aqueous–organic mixture containing 30% of methanol. This agrees with observations on cation-exchangers and confirms that the behaviour of the sulphonated dyes is correlated with the position and/or number of sulphonic acid groups rather than with the number of aromatic nuclei.

Silanised silica gel impregnated with DPC. In the presence of the cationic detergent, all the dyes remained at the point of application when development was with the above-mentioned aqueous–organic mixtures; such behaviour can be ascribed to an anion-exchange process. In fact, as shown on the chromatogram in Fig. 1 (obtained on layers impregnated with 2% of DPC), in the presence of 1 *M* hydrochloric acid, an increase in R_F is observed also for those compounds (such as E 123, E 111 and E 124) that are not affected by the activity of the mobile phase owing to the absence of basic groups from their molecules.

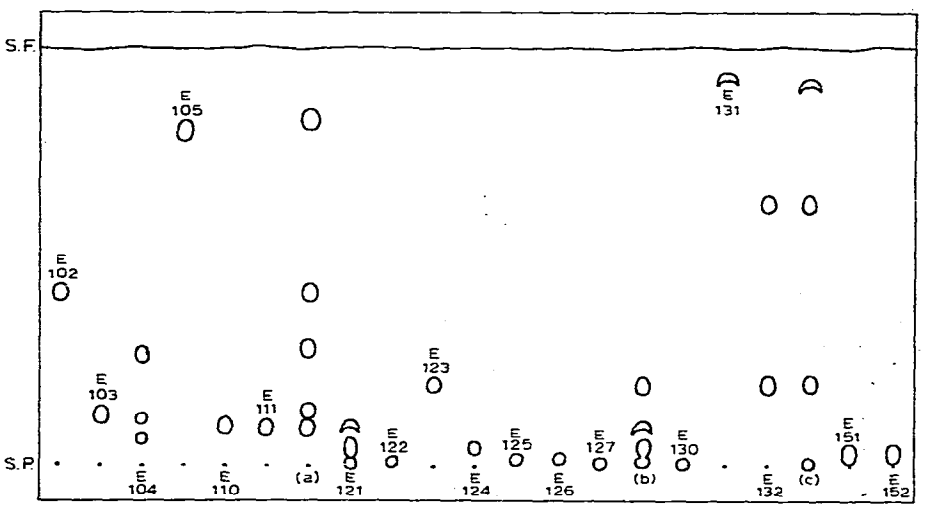


Fig. 1. Thin-layer chromatogram of water-soluble food dyes on silanised silica gel impregnated with 2% of DPC. Mobile phase: 1 *M* hydrochloric acid in water–30% methanol–5.7% acetic acid. S.P. = Starting point; S.F. = solvent front. (a) = Mixture of E 102, E 103, E 104, E 105 and E 111; (b) = mixture of E 121, E 122, E 123, E 124, E 125, E 126 and E 127; (c) = mixture of E 130, E 131 and E 132.

Very compact spots are obtained, so that the amount of dye on the layer can be reduced to half of that used with anion-exchangers and the separations shown in Fig. 1 can be achieved. With silanised silica gel impregnated with cationic detergent in lower concentration (*i.e.*, 0.5%), R_F values are higher, but the spots are less compact.

As the percentage of methanol in the mobile phase is increased, with the concentrations of acetic acid and hydrochloric acid being kept constant at 1 *M*, a marked increase in R_F values is observed, similar to that noted for other compounds on silica gel impregnated with DBS[8] and in agreement with the characteristics of reversed-phase chromatography.

With 60% of methanol, E 130 (which remains at the starting point) and E 127 ($R_F = 0.34$) can be separated from all the other dyes (whose R_F values range from 0.45 to 0.77).

As the acetic acid concentration in the mobile phase is increased, similar results are obtained, except that the increase in R_F values is more marked than that achieved by changing the percentage of methanol.

Without hydrochloric acid, an increase in the acetic acid concentration produces a levelling-out of, rather than an increase in, R_F values. For instance, on development with 4 *M* acetic acid in an aqueous–organic mixture containing 30% of methanol, most of the dyes exhibit R_F values between 0.50 and 0.59; however, E 130 ($R_F = 0.00$), E 127 ($R_F = 0.33$) and E 121 ($R_F = 0.87$) can be separated from all the other dyes in this system.

Analysis of food colours

We studied the dyes extracted from two commercial products ["Lenti", manufactured by Perugina (Perugia, Italy), and "Smarties" manufactured by Rowntree Mackintosh (York, Great Britain)] on Rexyn 102(H^+) and/or silanised silica gel impregnated with 2% of DPC, with 0.1 *M* HCl in water–methanol (2:1, v/v) and 1 *M* HCl in water–30% methanol–5.7% acetic acid, respectively, as mobile phases. The following dyes were identified (the colour named in parentheses refers to that of the chocolate candies) in "Lenti", E 127 (red); E 102 (yellow); and E 102, E 110, E 124 and E 132 (brown), and in "Smarties", E 102 (yellow); E 122 (red); E 132 and E 102 (green); E 132 and E 127 (violet); and E 151, E 102 and E 110 (pale brown).

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