Journal of Chromatography, 93 (1974) 243–247

CHROM. 7407

Note

The thin-layer chromatographic separation of water-soluble food dyes on silica gel layers

H. DE CLERCQ and D. L. MASSART

Farmaceutisch Instituut, Vrije Universiteit Brussel, Paardenstraat 67, B-1640 Sint Genesius Rhode (Belgium)

(Received December 10th, 1973)

Several TLC separations of water-soluble food dyes on silica gel layers have been published, and the literature has been summarized by Schweppe[1]. As water-soluble food dyes can be separated only with polar solvents, the use of several of these as eluting agents, particularly dimethylsulphoxide, has been investigated for the separation of the water-soluble dyes permitted in the six original E.E.C. countries.

EXPERIMENTAL

The following reagents were used and were all of reagent grade: dimethyl sulphoxide (DMSO, Uvasol, Merck, Darmstadt, G.F.R.) for spectroscopy; silica gel G according to Stahl, Type 60 (Merck); and reference dyes (obtained from P. Entrop, Machelen, Belgium), used as 0.2% (w/v) solutions in ethanol at 60°.

A Vario KS development chamber (Camag, Muttenz, Switzerland) was used. Silica gel layers (300 μm) were prepared according to standard practice[2]. Spots of 2.5 μl volume were applied.

Before development, the plate was equilibrated in the Vario KS chamber containing concentrated sulphuric acid (sp.gr. 1.84) for 45 min. The solvent was allowed to develop for a distance of about 12 cm at room temperature.

RESULTS AND DISCUSSION

Preliminary experiments with Cochenille Red A (C.I. 16255) as a test dye and a variety of solvents with widely differing polarity (light petroleum, benzene, chloroform, pyridine, dioxane, DMSO, methanol, ethanol, isopropanol and diethylamine) showed that complete migration ($R_F = 1$) was obtained with DMSO, methanol, ethanol, dimethylformamide and ethylene glycol. Isopropanol gave R_F values of ± 0.1.

The high R_F values with DMSO are not surprising as it has a high dielectric constant and is miscible with water and a good solvent for salts. However, it is also miscible with most organic, apolar solvents. According to Martin *et al.*[3], it is also a good solvent for dyes and it has been applied in our laboratory with good results for the direct extraction of dyes from toilet paper[4]. Until now, this solvent has been tried

only once for the TLC of dyes[5]. R_F values between 0.9 and 1.0 were found, so that no separations were obtained.

It was thought that by proper adjustment of the polarity of the eluting agent, it would be possible to obtain TLC separations of food dyes with a solvent incorporating DMSO. Separations of dyes on silica gel are usually not very good, owing to tailing and streaking, and it was hoped that the solvent properties of DMSO would lead to better shaped spots. By varying the DMSO:isopropanol:acetic acid ratio from 10:90:1 to 90:10:1, and by varying the concentration of acetic acid and isopropanol in the DMSO–isopropanol–acetic acid mixture from 30:69:1 to 30:63:7, an optimal ratio for the separation of the food dyes of DMSO:isopropanol:acetic acid of 30:69:1 was found. The acetic acid was added in order to ensure that the dyes were present essentially in their acidic, non-dissociated forms. The results are given in Table I.

TABLE I
APPROXIMATE R_F VALUES OF SYNTHETIC FOOD DYES ON SILICA GEL

Dye	*Colour Index No.*	*Solvent 1**	*Solvent 2***
Permitted by E.E.C. countries			
Tartrazine	19140 (E 102)	0.1	0.1
Chrysoine S	14270 (E 103)	0.8	0.5
Quinoline yellow	47005 (E 104)	0.3	0.3
Acid yellow	13015 (E 105)	0.3	0.3
Sunset yellow	15985 (E 110)	0.3	0.3
Orange GGN	15980 (E 111)	0.3	0.3
Carmoisine	14720 (E 122)	0.3	0.4
Amaranth	16185 (E 123)	0.1	0.2
Cochenille Red A	16255 (E 124)	0.1	0.3
Scarlet GN	14815 (E 125)	0.1	0.3
Ponceau 6R	16290 (E 126)	0.1	0.2
Patent blue	42051 (E 131)	0.3	0.4
Indigo carmine	73015 (E 132)	0.2	0.3
Brilliant Black BN	28440 (E 151)	0.3	0.2
Black 7984	27755 (E 152)	0.1	0.1
Erythrosine	45430	1	0.9
Other red, yellow and orange dyes			
Bordeaux B	16180	0.1	0.1
Eosine	45380	1	1
Fast Red E	16045	0.3	0.4
Ponceau MX	16150	0.2	0.3
Ponceau SX	14700	0.2	0.4
Red 2G	18050	0.2	0.3
Red 6B	18055	0.2	0.2
Red 10B	17200	0.2	0.3
Red FB	14780	0.2	0.2
Rhodamine B	45170	0.7	0.7
Auramine	41000	0.8	0.8
Chrysoidine	11270	1	1
Naphtol Yellow S	10316	0.2	0.3
Orange G	16230	0.2	0.3
Orange I	14600	0.8	0.8

* 1% Cu (II) sulphate in DMSO–isopropanol (70:30).
** DMSO–isopropanol–acetic acid (30:69:1).

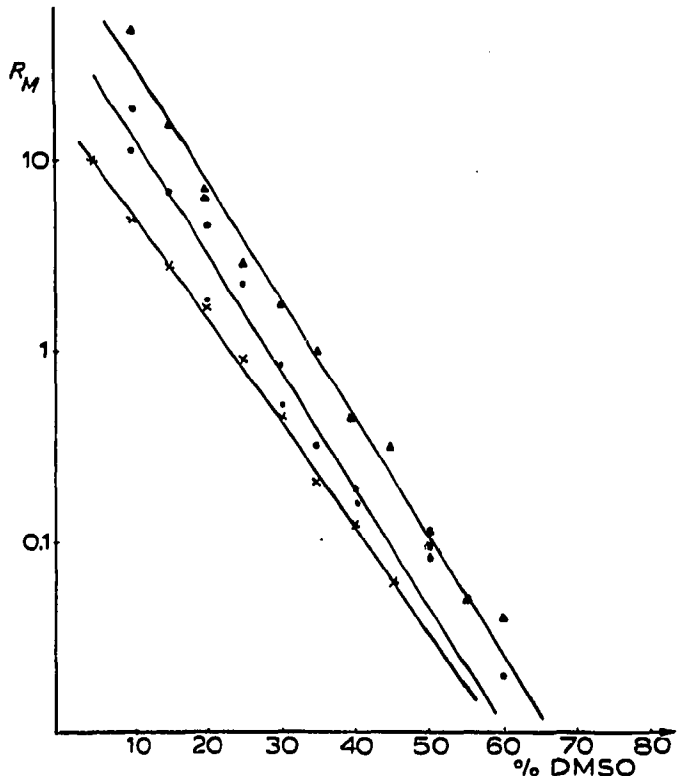

Fig. 1. R_M as a function of the composition of the solvent for sunset yellow (×), acid yellow (●) and Chrysoine S (▲).

Fig. 1 gives R_M values ($R_M = \log (1/R_F - 1)$) as a function of the composition of the solvent. It was found that the R_M value is usually a linear function of the DMSO content of the solvent for R_F values between 0.10 and 0.85, as could be expected from theory. Exceptions are erythrosine (C.I. 45430), which has high R_F values in all the solvent combinations, and Brilliant Black B (C.I. 28440) and Black 7984, which are very subject to tailing.

In general, circular spots were obtained. When this was not the case, R_F values were calculated using the leading front of the streak. In order to obtain better spots, other separation systems were sought. One of the techniques that is often applied when the substances to be separated show undesirable separation characteristics is to convert them into other substances and to subject these to chromatography. As dyes are often used as reagents for complexing and extracting metal ions, it occurred to us that one way of achieving a separation of chemically converted dyes might be to chromatograph their complexes with a metal, and we therefore investigated the separation of water-soluble food dyes as their copper complexes.

Excellent results were obtained with the copper complexes of some sulphonated diazo dyes (*e.g.*, amaranth, Black 7984 and cochenille red), the tailing effect which occurs with the first solvent of Table I being completely eliminated (Fig. 2). By varying the Cu^{2+} content (as a copper(II) sulphate solution in the DMSO phase)

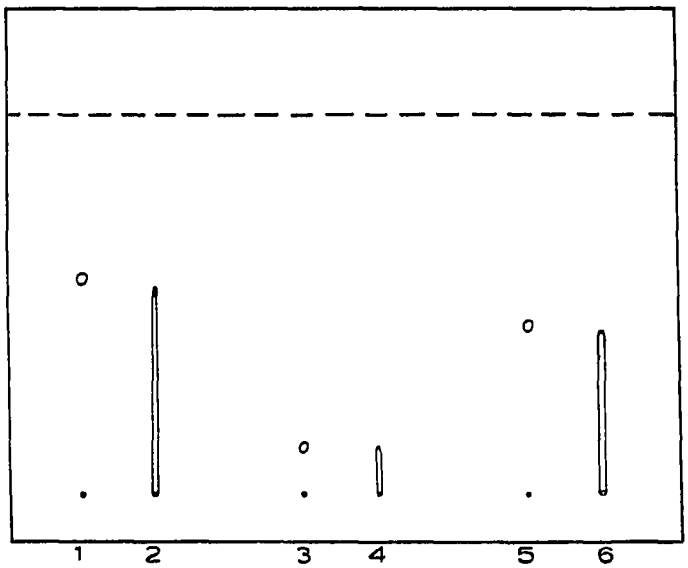

Fig. 2. TLC of (1) amaranth with solvent 2, (2) amaranth with solvent 1, (3) Black 7984 with solvent 2, (4) Black 7984 with solvent 1, (5) Cochenille Red A with solvent 2 and (6) Cochenille Red A with solvent 1. Solvent numbers as in Table I.

from 0.1 to 1.6% an optimal proportion of 1% copper(II) sulphate was found in a 30:70 DMSO–isopropanol solvent. R_F values are given in Table I.

It can be concluded that TLC with DMSO–isopropanol–Cu^{2+} is a valuable alternative for the separation of food dyes. This is shown in Table II were the information which can be obtained as expressed by the information content (for definition, see ref. 6), of this solvent–stationary phase system is compared with the information contents of the solvents described by Hoodless *et al.*[7]. It follows from Table II that the system in question is the third best choice of the ten which are compared. As it is

TABLE II

INFORMATION CONTENT (*I*) OF DIFFERENT SYSTEMS USED FOR THE TLC SEPARATION OF RED, ORANGE AND YELLOW FOOD DYES

For the first eight solvents, R_F values from ref. 7 are used. Two dyes are considered to be separated when the R_F difference is 0.1.

Solvent	*I (bit)*
Trisodium citrate (2 g), water (85 ml), 0.88 ammonia (15 ml)/cellulose	3.14
tert.-Butanol–propanoic acid–water (50:12:38)/cellulose	2.67
Trisodium citrate (2 g), hexamine (5 g), water (50 ml), methanol (50 ml)/cellulose	2.60
2-Methylpropan-1-ol–water–ethanol–0.88 ammonia (25:25:50:2)/cellulose	2.47
Propan-1-ol–ethyl acetate–water (6:1:3)/cellulose	2.86
Butan-1-ol–water–glacial acetic acid (20:12:10)/cellulose	2.71
Butan-1-ol–water–pyridine–ethanol (4:4:2:2)/cellulose	2.01
Propan-2-ol–0.88 ammonia (4:1)/silica gel	2.28
Solvent 1 (Table I)	2.37
Solvent 2 (Table I)	2.81

not unusual for several systems to be used in parallel in food colour identification procedures (Hoodless *et al.*[7] used four), it seems logical to include the DMSO–isopropanol–Cu^{2+}–silica gel system in such procedures.

ACKNOWLEDGEMENTS

The authors thank A. Schoonjans-Verstraeten for technical assistance and Paul Entrop, Machelen, for supplying standards.

REFERENCES

1 H. Schweppe, in E. Stahl (Editor), *Thin Layer Chromatography—A Laboratory Handbook*, Springer, Berlin, Heidelberg, New York, 1969, pp. 624–626.
2 E. Stahl, in E. Stahl (Editor), *Thin Layer Chromatography—A Laboratory Handbook*, Springer, Berlin, Heidelberg, New York, 1969, pp. 52–65.
3 D. Martin, A. Weise and H. J. Niclas, *Angew. Chem., Int. Ed.*, 6 (1967) 318.
4 M. Ponet and D. L. Massart, *Kleurstoffen in Toiletpapier, Thesis, Vrije Universiteit Brussel*, 1973.
5 W. P. Hayes, N. Y. Nyaker and D. T. Burns, *J. Chromatogr.*, 71 (1972) 585.
6 D. L. Massart, *J. Chromatogr.*, 79 (1973) 157.
7 R. A. Hoodless, K. G. Pitman, T. E. Stewart, J. Thomson and J. E. Arnold, *J. Chromatogr.*, 54 (1971) 393.