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The partition chromatography of food dyes on polycarbonate-coated foils

The thin-layer chromatography of permitted British food dyes[1] on layers of silica gel has been reported [2,3]. The use of laboratory-coated plates necessitated time-consuming air-drying, activation and cooling procedures before the layers were ready for use, though the first of these procedures was eliminated by the use of precoated silica gel layers[3].

The separation of various chemical species, including water-soluble dyes, on precoated polycarbonate foils has been reported[4]. In this report the suggestion that this substrate could be used in place of paper, with any of the mobile phases normally used for paper chromatography, was made.

Here, we report the separation of permitted British food dyes by partition chromatography on polycarbonate layers, our objective being to combine the advantages of thin-layer chromatography with the simplicity of paper chromatography. In particular, we were seeking a system in which recourse to tedious activation and cooling procedures was avoided.

Experimental

Aqueous solutions of the dyestuffs (1 μl of 0.1 % w/v) were applied to the precoated polycarbonate layers (10 × 10 cm) using to a multispotting technique[5]. The spots were dried and the chromatograms were developed using a number of mobile phases, including

(a) the seven solvent systems recommended for the paper partition chromatography of food dyes[6],
(b) *n*-butanol,
(c) *n*-butanol–hydrochloric acid (96:4)[4],
(d) *n*-butanol–hydrochloric acid (99:1),
(e) *n*-butanol–ammonia (sp.gr. 0.088) (96:4)[4],
(f) *n*-butanol–ammonia (sp.gr. 0.088) (98:2),
(g) *n*-butanol–ammonia (sp.gr. 0.088) (99:1).

The spotted plates were placed in tanks, the atmosphere of which had been preconditioned with the vapours of the mobile phase for 30 min, and developed until the solvent front had travelled 5 cm from the point of application of the dyes. The chromatograms were removed from the tanks and dried in an air oven to remove the mobile phases. The R_F values, computed from the layers, are given in Table I.

Discussion

Solvent systems

In six of the seven solvent systems recommended for the paper partition chromatography of permitted food dyes[6] it was found that the dyes were not retarded by the polycarbonate layers. In the seventh case (*n*-butanol–water–glacial acetic acid, 20:12:5) the dyes were retarded, some with streaking, but they were not resolved because they all had an R_F value of 0.70. This proved the generalisation that, for the separation of the same species in the same solvent system, polycarbonate could replace paper was unfounded for food dyes.

TABLE I

MEAN R_F VALUES[a] OF FOOD DYES CHROMATOGRAPHED ON POLYCARBONATE THIN LAYERS

Mobile phase: *n*-butanol–ammonia (sp.gr. 0.088) (99:1).

Dyestuff[b]	R_F *value*[c]
Chocolate Brown FB	0.00
Chocolate Brown HT	0.00
Brown FK	0.57 (1) 0.73 (2)
Black PN	0.08
Violet BNP	0.20
Indigo Carmine	0.00
Blue VRS*	0.15
Green S	0.00
Yellow RY*	0.00
Yellow 2G	0.00 (1) 0.56 (2)
Tartrazine	0.30
Sunset Yellow	0.55
Orange G	0.55
Yellow RFS*	0.62
Naphthol Yellow*	0.62
Orange RN	0.71
Ponceau 4R	0.13
Amaranth	0.20
Ponceau MX	0.23 (1) 0.73 (2)
Ponceau 3R*	0.25
Red 6B	0.33
Red 2G	0.37
Red 10B	0.48
Ponceau SX*	0.49 (1) 0.73 (2)
Fast Red E	0.57
Carmoisene	0.61
Red FB	0.73
Erythrosine BS	0.76

[a] Mean of five values reproducible to ± 0.04 R_F units.

[b] * indicates non-permitted dyes[7].

[c] (1) = lower spot of double spot from a single dye; (2) = upper spot of double spot from a single dye.

Because systems (c) and (e) have been used successfully for the separation of water-soluble dyes[4], it was decided to explore systematically the suitability of solvent systems based on *n*-butanol for the separation of these compounds.

Using *n*-butanol alone (system (b))none of the dyes moved. In the acidic systems (c) and (d) they all moved with the solvent front.

Not unexpectedly, because of the acidic nature of the dyes, more success was obtained when basic solvent systems ((e), (f) and (g)) were used. However, the amount of ammonia (sp.gr. 0.088) in the mobile phase appeared to be critical, too much ammonia resulting in the dyes remaining at the point of application though a few of the dyes were made to migrate in systems (e) and (f). It was, however, with system (g) that the greatest spread of R_F values, and hence some worthwhile separations were obtained (Table I).

Separation of food dyes in system (g)

For convenience, the dyes are classified into the following groups: (1) brown dyes, (2) dyes with a bluish tinge (including Black PN and Green S), (3) yellow and orange dyes, and (4) red dyes.

(1) *Brown dyes.* Though Brown FK is split into two spots, each spot is clearly separated from the Chocolate Brown dyes, neither of which moved. This system therefore represents no improvement over the separation of the members of this group reported by us for adsorption thin-layer chromatography[3].

(2) *"Blue" dyes.* The separation of the members of this group on polycarbonate thin layers is less efficient than is their separation on silica gel thin layers because only Blue VRS and Violet BNP move in the system reported here. Of importance, however, is the fact that not only can they be separated from the other "Blue" dyes but they can be separated from each other. This latter separation, which could not be done using adsorption systems, is now of importance because Blue VRS is no longer a permitted dye[7].

(3) *Yellow and orange dyes.* The original eight water-soluble dyes of this group[1] has now been reduced to five with the removal of Yellow RY, Yellow RFS and Naphthol Yellow from the list[7]. Notwithstanding this, we have chromatographed the original eight dyes in system (g) and obtained the following order of separation: Yellow RY = Yellow $2G_1$ < Tartrazine < Sunset Yellow = Orange G = Yellow $2G_2$ < Yellow RFS = Naphthol Yellow < Orange RN.

Thus it is possible to separate unambiguously two of the three non-permitted yellow dyes (Yellow RFS and Naphthol Yellow) from the remaining members of the group whereas in the adsorption system[3], Yellow RFS overlapped the spots of Orange G and Sunset Yellow. In the polycarbonate system, Yellow RY can be separated from all the other yellow dyes except the lower of the two spots obtained from Yellow 2G. However, these two compounds can be resolved on our adsorption system[3].

(4) *Red dyes.* This constitutes the largest group of permitted food dyes even though the original twelve members of this group[1] have been reduced to ten by the deletion of Ponceau 3R and Ponceau SX from the list[7]. The removal of this latter compound from the list should greatly simplify the problem of identifying the permitted colours because, as is shown in Table I and as we have previously shown for adsorption systems[3], this compound gave multiple spots on the chromatograms. The order in which these dyes chromatographed is: Ponceau 4R < Amaranth ≤ Ponceau MX_1 ≤ Ponceau 3R < Red 6B < Red 2G < Red 10B = Ponceau SX_1 < Fast Red E < Carmoisene < Red FB = Ponceau MX_2 = Ponceau SX_2 ≤ Erythrosine BS.

Thus the twelve dyes (or fourteen if the double spots are included) can be divided into eight groups, five of which will represent the pure dyes, whilst three will be mixtures.

Some further resolution of two of the three groups of mixed spots is possible if authentic dyes are chromatographed alongside the unknown mixtures. Thus the R_F values of the three dyes Amaranth, Ponceau MX_1 and Ponceau 3R are sufficiently different for Amaranth and Ponceau 3R just to be separable with Ponceau MX_1 overlapping the upper end of the former and the lower end of the latter. A mixture of these three therefore appears as a somewhat diffuse zone on the chromatogram, rather than as a sharp spot, so that the presence of more than one of these dyes can be inferred even if a positive identification of the individual dyes cannot be made. Re-chromatographing

the mixture with separate spots of the authentic dyes alongside may given a positive identification of the dyes present in the mixture. Under these circumstances (*i.e.* re-chromatography of the mixture scrapped from the layer) one may prove the existence of Ponceau MX in the mixture by virtue of the presence of a second spot for this compound.

The existence of Erythrosine in a mixture with Red 10B, Ponceau MX_2 and Ponceau SX_2 can be similarly inferred.

Overall, for red dyes, the separations reported here are better for these compounds when they are chromatographed in our adsorption system[3].

Conclusion

Thin-layer chromatography of food dyes on polycarbonate thin layers is a useful rapid method of qualitative assay of these compounds. However, it lacks the degree of reproducibility of R_F values which we have previously reported for adsorption systems[3].

We have shown that in some cases, but not in all, better, though less reproducible, separations can be obtained in the partition system than in the adsorption system. We recommend, however, that the two systems should compliment each other rather than that either system be used exclusively.

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1 *Colouring Matter in Food Regulations*, H.M.S.O., London, 1957.
2 G. J. DICKES, *J. Assoc. Public Analysts*, 3 (1965) 49.
3 R. J. T. GRAHAM AND A. E. NYA, *Symp. Intern. Chromatog. Electrophorèse, V, 1968 P. A. E., Brussels*, 1969, p. 486.
4 A. LESTIENNE, E. P. PRZYBLOWICZ, W. J. STAUDENMAYER, E. S. PERRY, A. D. BAITSHOLTS AND T. N. TISCHER, *Symp. Intern. Chromatog. Electrophorèse, III, 1964*, Soc. Belge Sci. Pharm., Brussels, 1965, p. 233.
5 L. S. BARK, R. J. T. GRAHAM AND D. MCCORMICK, *Talanta*, 12 (1965) 122.
6 *Separation and Identification of Food Colours Permitted by the Colouring Matter in Food Regulations, 1957*, Association of Public Analysts, London, 1960.
7 *Food Standards Committee Report on Colouring Matters*, H.M.S.O., London, 1964.

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