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Note

Liberation of acid dyes from their quaternary ammonium salts on chromatograms

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Quaternary ammonium compounds that are sufficiently bulky can be used for the isolation of acid dyes from food¹⁻⁵ and some cosmetic preparations⁶. The extracted compounds cannot be directly chromatographed on paper⁴ until the starting line is impregnated with alkyl arylsulphonates⁵, which liberate the dyes from their quaternary ammonium salts. Unfortunately, the satisfactory liberation of some less hydrophilic cosmetic dyes can be achieved only on silica layers⁷. On the other hand, mobile phases that contain various salts dissolved in water, *e.g.*, a 2% solution of sodium citrate in 5% ammonia⁸, cannot be used in such impregnation⁵ because of the distortions of the solvent front and spot shapes caused by the impregnation. In order to find an impregnant which would not distort the chromatograms, a number of compounds were tested and their activities in liberating the dyes were determined.

EXPERIMENTAL

Dyes

C.I. 15 630, 16 290 and 42 051 were commercial products for cosmetic use. To 10 ml of the 0.05 *M* solution or suspension of the dye in water were added 1 ml of saturated Na₂HPO₄ solution and 10 ml of a 0.03 *M* aqueous solution of benzalkonium chloride. The mixture was allowed to stand for 10 min, then 50 ml of chloroform were added and the mixture was shaken for 1 min. The layers were allowed to separate and the chloroform phase was retained for further use.

Liberating compounds

Sodium or ammonium salts of the following benzene derivatives were used as 0.05 *M* solutions in 65% aqueous methanol: blank, *N,N*-dimethylaniline, phenol, 2-methylphenol, 2-chlorophenol, pyrocatechol, trichlorophenol, tribromophenol, hexachlorophene (HCP), benzoic acid, 2-methylbenzoic acid, 2-chlorobenzoic acid, 2-methoxybenzoic acid, salicylic acid^{6,7}, *n*-butyl salicylate, anthranilic acid, phthalic acid, phenyl-*N*-nitrosohydroxylamine⁴, phenyl-*N*-benzoylhydroxylamine, benzenesulphonic acid, benzenesulphonic acid, 4-toluenesulphonic acid, tetrapropylenebenzenesulphonic acid (TPBS), phenylphosphoric acid and sodium tetraphenylboron (BPh₄).

Determination of the activity of the liberating compounds

A 30- μ l volume of a solution of the liberating compound was spread on Whatman No. 1 paper. After air-drying for 4 min, 10 μ l of the dye solution in chloroform was applied to the centre of the spot and the average diameter of the coloured spot was measured. Then, 20 μ l of chloroform was applied to the centre of the spot and the average diameter of the spot was measured again. The sum of both average diameters is an inverse measure of the activity of the compound.

RESULTS AND DISCUSSION

The three dyes used were selected for the following reasons. C.I. 16 290 is a tetrasulphonated naphthalene-azo-2-naphthol, which is readily extracted quantitatively under the test conditions and is also easily liberated from its benzalkonium salt. C.I. 15 630, on the other hand, is an *ortho*-monosulphonated naphthalene-azo-2-naphthol, which is one of the dyes least readily liberated from its benzalkonium salt⁷. C.I. 42 051 was chosen because it is a triphenylmethane dye and it behaves differently in some respects from azo dyes.

The liberating compounds tested are fully active only on humid adsorbents. The activity of the monosubstituted benzene derivatives in liberating the dyes was found to increase in the following series (the sum of the average diameters is given in parentheses):

(a) dye C.I. 16 290: dimethylaniline, phosphate and sulphinate (inactive) < carboxylate (24 mm) < phenolate (19 mm) < N-nitrosohydroxylamine (11 mm) < sulphonate (9 mm);

(b) dye C.I. 42 051: dimethylaniline, phosphate and sulphinate (inactive) < N-nitrosohydroxylamine (33 mm) < carboxylate (31 mm) \approx sulphonate (31 mm) < phenolate (30 mm).

The influence of *ortho*-substituents was as follows. The methyl group reduced the activity of phenol and benzoic acid, while the chloro group and especially the hydroxyl group enhanced it. Salicylic acid was in all instances more active than anthranilic acid, phthalic acid, *o*-methoxybenzoic acid and *n*-butyl salicylate.

Only three compounds liberated quantitatively all three dyes (Table I). The most suitable was sodium tetraphenylboron, which was used as a solution of 2 g in

TABLE I

SUM OF THE AVERAGE SPOT DIAMETERS (mm) OF THE DYES LIBERATED BY THE MOST ACTIVE LIBERATING COMPOUNDS AS THE INVERSE MEASURE OF THE ACTIVITY OF THE LIBERATING COMPOUND

Liberating compound	Dye		
	C.I. 15 630	C.I. 16 290	C.I. 42 051
TPBS	28	9 (29*)	13 (28*)
HCP	22	13	12
BPh ₄	17	9	11
Blank	35	30	35

* Faintly coloured part of the spot.

a mixture of 100 ml methanol or ethanol, 2 ml of water and 0.2 ml of glycerol for the 1–2 cm wide impregnation of the starting line of the chromatogram. It liberates all acid (food and cosmetic) dyes⁹ and causes no distortions due to surface activity. The liberated dyes were concentrated as small and compact spots regardless of their concentration until it exceeded the exchange capacity of the impregnation.

The applied spots must be washed off with chloroform, if water-rich mobile phases are to be used. For this reason, 10 μ l of chloroform were first applied to the centre of each spot and the starting line was then washed off with a light stream of chloroform. The chromatogram could also be bent and dipped into chloroform so that the starting line remained over the solvent surface. The chloroform ascended and washed the benzalkonium tetraphenylboron and other interfering lipophilic materials to the lower margin of the chromatogram, where they were cut off. Sometimes it was advantageous to insert the chloroform-wet chromatogram into the mobile phase.

Ammonium and potassium salts cause spreading of triphenylmethane dyes so that lithium or sodium salts should be used. Ethanolamine or ethylenediamine is recommended instead of ammonia. Some typical mobile phases are the following solutions: 2 g of sodium or lithium citrate, sulphate, hydrogen phosphate or carbonate or EDTA dissolved in a solution of 5 ml of ethanolamine in 95 ml of water, or in a solution of 2 ml of ethylenediamine in 98 ml of water. Solutions prepared by mixing 100 parts of 2% lithium or sodium carbonate solution with 5 parts of *tert.*-butanol, acetonitrile, dimethyl sulphoxide or other lower alcohols or amides are also good mobile phases.

Mobile phases rich in organic solvents (also if they contain ammonia) can be used on cellulose or silica gel without first washing off the spots with chloroform. The following mixtures are recommended for food dyes: nitromethane–acetonitrile–water (1:8:3), nitromethane–acetonitrile–formic (or acetic) acid–water (10:80:1:30) and triethylamine–acetonitrile–water (5:1:2).

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