

CHROM. 6520

Note

Separation and identification of certain xanthene and other red basic dyes

In the course of investigation of basic dyes as spectrophotometric reagents for the determination of anionic species it has become apparent that in certain cases the dye purity is a significant factor in the extent of anion extraction and in the overall reliability of procedures¹⁻⁴. Recent studies have been particularly concerned with xanthene dyes. It has been necessary to check the identity of materials, due to the large number of names in use for certain compounds¹ and because particular names are used for more than one compound⁵, and also to check the purity of samples of known structure. Many of the dyes examined are of histochemical interest⁵⁻⁸.

Materials and method

Chromatographic solvents. Mixtures were freshly prepared according to the following compositions: (1) ethanol-(0.88) ammonia-water (2:2:1); (2) ethanol-methanol (4:1)⁹; (3) isopropanol-(0.88) ammonia-water (10:1:1); (4) *n*-butanol-1.5 *N* ammonia (1:1); (5) glacial acetic acid-water (1:9); (6) glacial acetic acid-water (15:85); (7) glacial acetic acid-water(1:4); (8) 1 *N* hydrochloric acid; (9) (0.8) ammonia-ethanol-water (5:10:85).

Paper. Whatman No. 1 (25 × 25 cm) which was thoroughly predamped by a fine water spray when used in solvent 8 and impregnated with 5% cetyl alcohol in ethanol¹⁰ before use in solvent 1. In all other cases it was used as supplied. Sample spots were placed 3 cm from base and developed upwards for 15 cm (45-60 min). With acidic solvents spots were developed by exposure to ammonia vapour.

Results

Useful results were only obtained with solvent systems 8 and 9 and these are given in Table I. R_F values were calculated by measuring to the leading edge of a spot; values for minor components are shown in parentheses. With solvent systems 2, 3 and 5 dyes travelled in the solvent front. With systems 1, 4, 6 and 7 only slight separations were achieved.

Discussion

The identity of samples of Basic Red 14 and of Basic Red 18 from two sources was confirmed as was that of three samples of Basic Violet 10. Rhodamine 3 B, Basic Violet 11, was found to be contaminated with Rhodamine B, Basic Violet 10. The predamping of the paper before use with solvent 8 was found to reduce tailing considerably. With TAYLOR'S⁹ solvent 9, tailing was further reduced in most cases. This solvent was less satisfactory for our purposes in that it did not resolve ethyl and butyl rhodamines although the separation of Rhodamine 3B and Rhodamine B was better than that reported earlier by HOROBN AND MUEGFROYD⁸. Solvent

TABLE I
APPROXIMATE R_F VALUES

Trade name	Colour index ¹² name and number	Type	R_F value	
			Solvent 8	Solvent 9
Maxilon Red BL	Basic Red 22	azo	0.68 (0.79)	0.41
Maxilon Red GRL	Basic Red 49	monoazo	0.58 (0.64)	0.14 (0.33)
Sevron Bordeaux G	Basic Red 19	triarylmethane	0.93 (0.69; 0.42)	0.84
Sevron B Red D	Basic Red 19	xanthene	0.48	0.25
Sevron B Red B	Basic Red 15	methine	0.40	0.17
Seyron Red GL	Basic Red 18; 11085	cyanine	0.32	0.11
Sevron B Red 4G	Basic Red 14	cyanine	0.31	0.16
Deorlene B Red R	Basic Red 27	methine	0.25	0.15
Deorlene B Red 3B	Basic Red 26	triarylmethane	0.81	0.68
Deorlene B Red 4G	Basic Red 14	cyanine	0.31	0.16
Deorlene F Red 2GL	Basic Red 54	cyanine	0.33	0.11
Rhodamine S	Basic Red 11; 45950	succinim	0.32	0.23
Rhodamine 6G	Basic Red 11; 45160	xanthene	0.45	0.29 (0.49)
Rhodamine 6GD	Basic Red 11; 45160	xanthene	0.44	0.29
Rhodamine 3B	Basic Violet 11; 45175	xanthene	0.59 (0.59)	0.28 (0.65)
Rhodamine B	Basic Violet 10; 45170	xanthene	0.59	0.66
Rhodamine 3G	Basic Red 31; 45210	xanthene	0.49	0.25 (0.54)
Butyl Rhodamine B	Basic Red 31; 45210	xanthene	0.34	0.25

9) was used successfully, however, for the purification of Rhodamine 3B on a preparative scale using a cellulose column. Improved resolution in the Deorlene series of red dyestuffs has also been achieved. It should be noted that for Deorlene Brilliant Red 3B (Basic Red 26) spots only appear slowly when the paper has almost dried (30 min).

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