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Note

Thin-layer chromatography of Sudan dyes

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The Sudan dyes are widely used for the histological demonstration of fats[1]. However, few rapid and effective methods for the quality control of these dyes appear to have been published. Most analytical methods have been paper chromatographic ones[2–12] which are extremely slow and sometimes of low resolution. By and large, rapid analytical thin-layer chromatographic (TLC) methods have been devised primarily to distinguish between the various Sudan dyes and not to separate the impurities of individual dyes[5,13–17]. The majority of these methods are therefore unsuitable for the quality control of individual dyes.

In this paper a rapid, highly effective TLC system for the quality control of Sudan dyes is described. Analytical data on commercial samples of various dyes are also presented.

MATERIALS AND METHODS

Reagents and equipment

Acetone, benzene and chloroform of AnalaR grade were used. The various Sudan dye samples studied are listed in Tables I–V.

Aluminium-backed, silica gel TLC sheets were obtained from Merck (type 5553, 200 × 200 mm without fluorescent indicator, adsorbent thickness 0.25 mm). Disposable micropipettes (Microcaps®, Drummond, Broomall, Pa., U.S.A.) or a special applicator (serum applicator 51225, Gelman, Ann Arbor, Mich., U.S.A.) was used for sample application. Chromatograms were developed in a sandwich tank (Eastman Chromagram developing apparatus 6071).

Chromatography

Two microlitres of dye solutions (about 4 mg/ml in acetone) were applied to unactivated TLC sheets as bands 20 mm in length. The developing solvent consisted of benzene–chloroform (10:1, v/v). Development was performed in darkness.

RESULTS AND DISCUSSION

Several reports, *e.g.* those of Schweppe[16], Walker and Beroza[17] and Jordan[18,19], indicate that good separations of Sudan dyes may be obtained on thin layers of silica

TABLE I

TLC DATA ON COMMERCIAL SAMPLES OF OIL BLUE N (CI 61555), SUDAN BLUE (NO CI NO.), SUDAN BLUE GA (CI 61525) AND SUDAN GREEN (CI 62545)

m = Major component; t = trace; i = intermediate amount. Data on components: (no., mean R_F value, colour): 1 = immobile, grey; 2 = immobile, brown; 3 = 0.05, mauve; 4 = 0.09, purple; 5 = 0.10, blue; 6 = 0.13, mauve; 7 = 0.17, blue; 8 = 0.26, blue; 9 = 0.31, blue; 10 = 0.32, pink; 11 = 0.39, blue; 12 = 0.44, mauve; 13 = 0.46, mauve; 14 = 0.46, yellow; 15 = 0.51, orange; 16 = 0.62, mauve; 17 = 0.64, blue; 18 = 0.68, yellow.

Dye, supplier and batch no.	*Component*																	
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*
Oil Blue N																		
Difco, 1358								t			m							
Gurr, 23619	t		t	i		t	m	m			m		t			t		
R. A. Lamb, 0572	t			t		t	m	m	t		m							
Sudan Blue																		
Difco, 1040		i						m				i			t			
Gurr, 0886		t								i	m							
Sudan Blue GA																		
R. A. Lamb, 0486	t				t			m				t						
Sudan Green																		
Difco, 1041	t							m							t		t	m
Gurr, 1372	t							m							t			m
R. A. Lamb, 0488	i				t			m				t		t	t			m

TABLE II

TLC DATA ON COMMERCIAL SAMPLES OF OIL RED O (CI 26125), SUDAN II (CI 12140), SUDAN III (CI 26100) AND SCARLET R (MICHAELIS) (CI 26105)

m = Major component; t = trace; i = intermediate amount. Data on components (no., mean R_F value, colour): 1 = immobile, orange; 2 = immobile, brown; 3 = 0.06, brown; 4 = 0.08, brown; 5 = 0.12, brown; 6 = 0.19, brown; 7 = 0.31, yellow; 8 = 0.40, maroon; 9 = 0.41, yellow; 10 = 0.51, maroon; 11 = 0.56, orange; 12 = 0.59, pink; 13 = 0.62, amber; 14 = 0.65, yellow; 15 = 0.68, orange; 16 = 0.73, pink.

Dye, supplier and batch no.	*Component*															
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*
Oil Red O																
Difco, 0210		t			t	t		i		m	i	i				
Gurr, 0770		t						t		m						
R.A. Lamb, 3682		t			t	t	t	i		m		i				
Sudan II																
Gurr, no batch no.											m		i		m	
R. A. Lamb, no batch no.	i		m													
Sudan III																
Difco, 2374									t		m			t		t
Gurr, 71427		t						i		m	m		i			
R. A. Lamb, 4308									t		m					t
Scarlet R (Michaelis)																
Difco, 0686		i		t		t		m		m	m	m				
Gurr, no batch no.		i		t		t		m		m	m	m				
R. A. Lamb, 3626		i		t		t		m		m	m	m				

TABLE III

TLC DATA ON COMMERCIAL SAMPLES OF SUDAN BLACK B (CI 26150) AND ACETYLATED SUDAN BLACK B (NO CI NO.)

m = Major component; t = trace; i = intermediate amount. Data on components (no., mean R_F value, colour): 1 = immobile, brown; 2 = 0.02, brown; 3 = 0.04, brown; 4 = 0.08, brown; 5 = 0.11, black; 6 = 0.29, brown; 7 = 0.37, brown; 8 = 0.45, brown; 9 = 0.49, blue-black; 10 = 0.52, grey; 11 = 0.57, grey; 12 = 0.90, grey-green; 13 = 0.96, pink.

Dye, supplier and batch no.	*Component*												
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*
Sudan Black B													
Difco, 1870	i	i	i	t	m				m	t	t	t	t
Gurr, 80167	i	i	i	t	m				m	t	t	t	t
R. A. Lamb, 2715	i	i	i	t	m	t	i	i	m	t	t	t	t
Acetylated Sudan Black B													
Difco, 1549	i	i	i	t	m	t	i	i	m	t	t	t	t
Gurr, MN 1058	i	i	i	t	m	t	i	i	m	t	t	t	t
R. A. Lamb, 1994	i	i	i	t	m	t	i	i	m	t	t	t	t

TABLE IV

TLC DATA ON COMMERCIAL SAMPLES OF SUDAN BROWN (CI 12020)

m = Major component; t = trace; i = intermediate amount. Data on components (no., mean R_F value, colour): 1 = immobile, brown; 2 = 0.04, brown-orange; 3 = 0.15, brown; 4 = 0.25, brown-red; 5 = 0.31, brown-red; 6 = 0.50, orange; 7 = 0.62, red; 8 = 0.74, brown; 9 = 0.86, pink; 10 = 0.92, grey; 11 = 0.97, green-yellow.

Supplier and batch no.	*Component*										
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*
Difco, 0206	m	i	m	t	t	m	m	t	i	i	t
Gurr, 14517	m	i	m	t	t	m	m	t	i	i	t
R. A. Lamb, 0487	m	i	m	t	t		m		i	i	t

TABLE V

TLC DATA ON COMMERCIAL SAMPLES OF SUDAN YELLOW (CI 12055)

m = Major component; t = trace; i = intermediate amount. Data on components (no., mean R_F value, colour): 1 = immobile, cerise; 2 = 0.46, orange; 3 = 0.55, mauve; 4 = 0.60, orange; 5 = 0.65, yellow; 6 = 0.77, orange.

Supplier and batch no.	*Component*					
	1	*2*	*3*	*4*	*5*	*6*
Difco, 0267	i	m	t	t	t	t
Gurr, 23813	t	m				t
R. A. Lamb, 3660		m				

using benzene as the developing solvent. In the author's hands a mixture of benzene and chloroform proved much more effective.

Chromatographic data on commercial Sudan dyes are shown in Tables I–V, where "m" indicates a major component, "t" a trace one and "i" one present in an intermediate amount. A developed chromatogram is shown in Fig. 1.

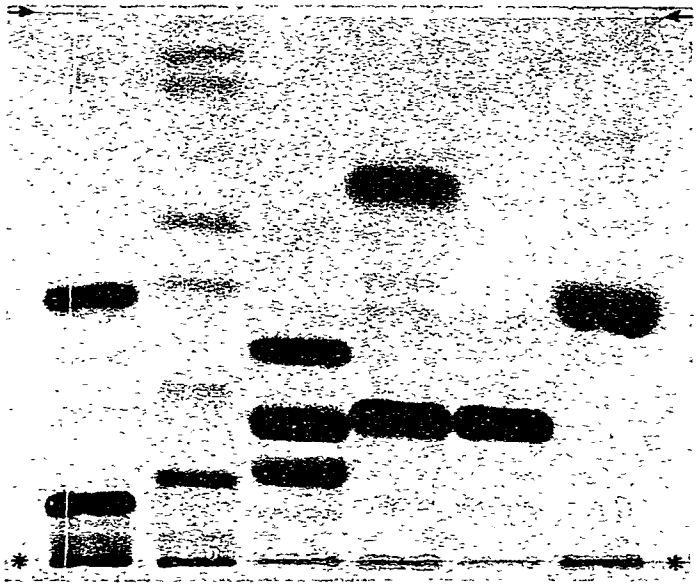

Fig. 1. Thin-layer chromatogram of commercial samples of Sudan dyes. Samples are from left to right: Sudan Black B (R. A. Lamb, 2715); Sudan Brown (Gurr, 14517); Oil Blue N (Gurr, 23619); Sudan Green (R. A. Lamb, 0488); Sudan Blue GA (R. A. Lamb, 0486) and Sudan Yellow (Difco 0267). Asterisks indicate the origin, arrows the solvent front. The chromatogram has been somewhat overloaded in order to demonstrate as many components as possible. In spite of this, certain trace components may not be clearly visible.

Oil Blue N, Sudan Blue, Sudan Blue GA and Sudan Green (Table I)

Most of these dyes were extremely heterogeneous. With the samples of Oil Blue N, Sudan Blue and Sudan Blue GA it is impossible to decide which, if any, of the major components correspond to the nominal ones. The samples of Sudan Green do not correspond to the Colour Index[20] formulation since they contain no green dye. They are, in fact, merely mixtures of a blue Sudan dye with an unidentified yellow component.

Oil Red O, Sudan II, Sudan III and Scarlet R (Michaelis) (Table II)

The majority of the samples of these dyes were complex mixtures. Those of Oil Red O contained a single major component, which was also identified in samples of Scarlet R (Michaelis). Coloured impurities in Oil Red O have previously been detected using paper chromatography[5,8] and TLC[5,21]. The two samples of Sudan II examined were completely dissimilar; one contained a large proportion of an orange component identified in the samples of Sudan III and Scarlet R (Michaelis). Previous authors have detected coloured impurities in Sudan II by paper chromatography[6] and TLC[16,22]. Sudan III and Scarlet R (Michaelis) contained several common components. Indeed, it is alleged that some commercial samples of Sudan III are mixtures of the nominal dye and Scarlet R (Michaelis)[20]. Impurities in these dyes have been identified previously by both paper chromatography[2,3,5,6,11] and TLC[5,16].

Sudan Black B and Acetylated Sudan Black B (Table III)

Sudan Black B is an extremely heterogeneous product. The three samples contained the same pair of major components. Ten components were common to all samples and these may correspond to the fractions isolated by column chromatography[23]. Side reactions occuring during the synthesis of Sudan Black B may produce as many as seven contaminants[24]. The number of components in fact detected may perhaps indicate the presence of *cis*- and *trans*-isomers.

Coloured impurities in this dye have been detected by many authors using gel-filtration chromatography[25], paper chromatography[7,10] and TLC[5,16,18,19].

Surprisingly, the samples of Acetylated Sudan Black B contained no component that was not present in the parent dye.

Sudan Brown (Table IV)

Samples of this dye contained up to eleven coloured components.

Sudan Yellow (Table V)

All samples of this dye contained the same major component, possibly the nominal one. The sample from R. A. Lamb was exceptional amongst those of the Sudan dyes examined in that it was free from detectable coloured contaminants.

REFERENCES

1 R. D. Lillie, *Histopathologic Technic and Practical Histochemistry*, McGraw-Hill, New York, 3rd ed., 1965, p. 454.
2 J. F. Christman and R. H. Trubey, *Stain Technol.*, 27 (1952) 53.
3 J. F. Christman and H. J. Werner, *Stain Technol.*, 28 (1953) 259.
4 J. Gasparič and M. Matrka, *Collect. Czech. Chem. Commun.*, 25 (1960) 1969.
5 R. W. Horobin and L. B. Murgatroyd, *Histochemie*, 11 (1967) 141.
6 H. Kutt, D. Lockwood and F. McDowell, *Stain Technol.*, 34 (1959) 197.
7 H. Kutt, D. Lockwood and F. McDowell, *Stain Technol.*, 34 (1959) 203.
8 H. Kutt and T. T. Tsaltas, *Clin. Chem.*, 5 (1959) 149.
9 W. Lindberg, *Z. Lebensm.-Unters.-Forsch.*, 103 (1956) 1.
10 S. I. Rosenthal, H. Puchtler and F. Sweat, *Arch. Pathol.*, 80 (1965) 190.
11 R. H. Trubey and J. F. Christman, *Stain Technol.*, 27 (1952) 87.
12 M. R. Verma and R. Dass, *J. Sci. Res. (Hardwar, India)*, 16B (1957) 131.
13 J. W. Copius-Peereboom and H. W. Beekes, *J. Chromatogr.*, 20 (1965) 43.
14 J. Davídek and G. Janíček, *J. Chromatogr.*, 15 (1964) 542.
15 R. A. Hoodless, J. Thomson and J. E. Arnold, *J. Chromatogr.*, 56 (1971) 332.
16 H. Schweppe, in E. Stahl (Editor), *Thin-layer Chromatography, A Laboratory Handbook*, Springer, Berlin, Heidelberg, New York, 2nd ed., 1969, p. 612.
17 K. C. Walker and M. Beroza, *J. Ass. Offic. Agr. Chem.*, 46 (1963) 250.
18 D. M. Jordan, *J. Chromatogr.*, 57 (1971) 427.
19 D. M. Jordan, *J. Chromatogr.*, 63 (1971) 442.
20 *The Colour Index*, Society of Dyers and Colourists, Bradford, 2nd ed., 1956.
21 E. V. Truter, *J. Chromatogr.*, 14 (1964) 57.
22 J. G. Kirchner, *J. Chromatogr.*, 63 (1971) 45.
23 E. W. Bermes and H. J. McDonald, *Arch. Biochem. Biophys.*, 70 (1957) 49.
24 J. Y. Terner, J. Schnur and J. Gurland, *Lab. Invest.*, 12 (1963) 405.
25 R. W. Horobin, *Stain Technol.*, 46 (1971) 297.