THIN-LAYER CHROMATOGRAPHY OF DYESTUFFS ON POLYAMIDE AND "SILVER NITRATE" LAYERS

J. W. COPIUS-PEEREBOOM AND HENNY W. BEEKES
Government Dairy Station, Leiden (The Netherlands)*
(Received March 11th, 1965)

Fat-soluble dyestuffs have in the past been used for the colouring of fats and fat products. In most countries such dyestuffs are no longer allowed as food additives, because of their potential toxicological (carcinogenic) hazards. In a few countries some special fat-soluble dyestuffs, such as Yellow AB, Yellow OB, Yellow XP, Ceres Orange GN, are, however, still on the list of approved food colours.

Table I presents a survey of the countries in which fat-soluble dyestuffs are still allowed as food colours. The "positive" lists of approved food colours of FAO, the Food-Drug-Cosmetic list (U.S.A.) and the European Common Market list do not contain any of these colouring materials.

TABLE I

LIST OF FAT-SOLUBLE DYESTUFFS APPROVED AS FOOD COLOUR IN SOME COUNTRIES*

Dyestuff	*Colour Index 1956 No.*	*Countries*
Sudan II	12140	Japan, Nicaragua, Dominican R., Cuba, Peru
Ceres Red G	12150	Yugoslavia, Poland
Ceres Orange GN	11920	Great Britain, Ireland, South Africa, Yugoslavia, Egypt, Poland
Yellow AB	11380	Japan, Thailand, Cuba, Dominican R., Nicaragua, Peru
Yellow OB	11390	Japan, Thailand, Cuba, Dominican R., Nicaragua, Peru
Yellow XP	12740	Great Britain, Ireland, Denmark
Orange SS	12100	South Africa, Japan, Cuba, Dominican R., Nicaragua, Peru, Venezuela (?)

* This list was compiled from the latest information up to 1963/1964 about food legislation, taken for example from the Current Food additives legislation CFAL of the FAO of the United Nations and from the list given by NIEMAN[1].

On the basis of this situation, the detection and identification of fat-soluble dyestuffs in fats and fat products has been studied in our institute. In the period before thin-layer chromatography was introduced the separation of these dyestuffs was performed by column adsorption chromatography (*e.g.* JAX[2]) and reversed-phase paper chromatography (*e.g.* LINDBERG[3], VERMA[4], MARK[5] and GASPARIC[6]). After the introduction of thin-layer chromatography (TLC) by STAHL, we investigated the migration rate of fat-soluble dyestuffs on gypsum-bound layers of silica gel G, kieselguhr G and aluminium oxide G. An extended survey of the results we obtained in

* Vreewijkstraat 12b, Leiden, The Netherlands.

TABLE II

R_F VALUES OF FAT-SOLUBLE DYESTUFFS IN SOME TLC SYSTEMS*

Dyestuff	*Chemical structure*
Sudan I, Oil orange E	Aminobenzene → 2-hydroxynaphthalene
Sudan II	2,4-Dimethyl-1-aminobenzene → 2-hydroxynaphthalene
Sudan III	4-Amino-azobenzene → 2-hydroxynaphthalene
Sudan IV	*o*-Amino-azotoluene → 2-hydroxynaphthalene
Bixin	
Martius yellow	Ammonium and sodium salts of 2,4-dinitro-1-hydroxynaphthalene
Chlorophyll	
β-Carotene	
Butteryellow, dimethylazobenzene	Aminobenzene → dimethyl-aminobenzene
Ceres red G	2-Anisidine → 2-hydroxynaphthalene
Ceres orange GN,	Aminobenzene → 1,3-dihydroxybenzene
Oil yellow G	
Ceres yellow 3G,	Aminobenzene → 1-phenyl-3-methyl-5-pyrazolone
Sudan yellow	
Oil yellow XP	2,4-Dimethyl-1-aminobenzene → 1-phenyl-3-methyl-5-pyrazolone
Yellow OB	2-Methyl-1-aminobenzene → 2-aminonaphthalene
Yellow AB	Aminobenzene → 2-aminonaphthalene
Orange SS	2-Methyl-1-aminobenzene → 2-hydroxynaphthalene
β-Apo-8′-carotinal	

* R_S value calculated with R_F of Ceres red G ≡ 1.00.

1961[7] is given in Table II. With these TLC systems it is possible to identify the fat-soluble dyestuffs present in a sample of fat. Similar results were obtained by DAVÍDEK[8], using so-called "loose layers" of aluminium oxide.

Later, the TLC of such dyestuffs was also studied by MONTAG[9] amongst others. Recently, DAVÍDEK[10] has described some carefully elaborated systems of reversed-phase TLC on starch layers impregnated with paraffin oil, which are applied for identification purposes.

Since the separation of some pairs of dyestuffs, *e.g.* Oil Yellow AB (aminobenzene → 2-aminonaphthalene) (I) and Oil Yellow OB (2-methyl-1-aminobenzene → 2-aminonaphthalene) (II), in the systems given in Table II was found to be difficult, we examined their behaviour on some other support materials, *viz.* layers of polyamide and of silver nitrate-impregnated silica gel G. On such layers, which possess special properties, some remarkable separations in the group of fat-soluble dyestuffs were achieved. Table III gives the relative R_S values (calculated with R_S Ceres Red G = 1.00) of the dyestuffs on polyamide and silver nitrate–silica gel G layers in different solvents. The following solvent mixtures were used:

polyamide, apolar, with:	A.	chloroform–methanol–water (5:15:1)
	B.	acetone–ethanol–water (6:3:1)
	C.	methanol–acetone–acetic acid (6:2:2)
polyamide, polar, with:	D.	light petroleum (b.p. 80–100°)–benzene–acetic acid (1:1:0.5)
silver nitrate–silica gel G	E.	chloroform–light petroleum (b.p. 60–80°)–acetic acid (75:25:0.5)
	F.	chloroform–carbon tetrachloride–acetic acid (80:20:0.2)
	G.	benzene–ether–acetic acid (90:10:0.1)

Colour Index 1956 *No.*	*Silica gel G*					*Kieselguhr G/ cyclohexane*	*Aluminium oxide G/ hexane–ethyl acetate (98:2)*
	Hexane–ethyl acetate (90:10)	*Chloroform*	*Light petroleum–ether–acetic acid (70:30:1)*	*Light petroleum–ether–ammonia (70:30:1)*	*Multiple (4×) development, trichloroethylene R_S**		
12055	0.68	0.60	0.77	0.70	0.24	0.63	0.56
12140	0.72	0.58	0.78	0.67	0.23	0.44	0.62
26100	0.56	0.52	0.68	0.61	0.13	0.15	0.41
26105	0.56	0.53	0.68	0.61	0.17	0.15	0.38
75120	0	0	0.23	0	0	0	0
10315	0	0	0.28	0	0	0	0
75810	0	0.08	0.21	0	0	0	0
75130	0.88	0.92	1.00	1.00	0.48	1.00	1.00
11020	0.68	0.62	0.68	0.57	0.28	0.85	0.59
12150	0.18	0.46	0.30	0.36	1.00	0.16	0.19
11920	0.14	0.24	0.36	0.37	0	0	0
12700	0.54	0.61	0.74	0.75	0.19	0.54	0.56
12740	0.60	0.64	0.81	0.80	0.23	0.40	0.68
11390	0.27	0.82	0.50	0.49	0.51	0.87	0.27
11380	0.25	0.80	0.46	0.41	0.48	0.88	0.22
12100	—	—	—	—	0.24	—	—
	—	—	—	—	0.67	—	—

The polyamide layers were prepared as follows: 12 g of polyamide powder, MN (Macherey and Nagel) is mixed with 55 ml methanol with the addition of 5 ml of 10% aqueous solution of starch (Riedel de Haan). This mixture is spread on chromatoplates. The layers are dried overnight.

The silver nitrate–silica gel layers are prepared from 13 g silver nitrate dissolved in 60 ml water and mixed with 30 g silica gel G.

When comparing the migration rates in the aqueous solvent mixtures A and B with those in the reversed-phase systems of paper chromatography (*e.g.* LINDBERG[3]) and TLC (DAVÍDEK[10]), the separation process in these polyamide systems can be considered to rest on *reversed-phase partition chromatography*[11]. Apparently the polyamide layer acts as a kind of *apolar* stationary phase. As a consequence the lipophilic compound β-carotene exhibits quite a low migration rate in these systems, whereas high R_F values (~ 0.8–1.0) were found on the adsorbents of Table II. On the other hand, bixin, which hardly migrates on polar adsorbents (see Table II), displays an R_S value of 0.40 in the polyamide systems A and B. In the solvents A, B and C, Sudan I is clearly separated from Sudan II, which has two extra CH_3 groups in the molecule. Consequently, Sudan II has a lower R_S value than Sudan I*. Oil Yellow OB (II) likewise has an extra CH_3 group as compared with Yellow AB (I) and consequently displays a lower migration rate in the systems A, B and C. On the other

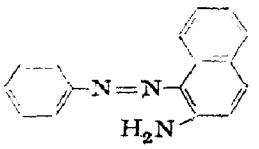
* Sudan III and IV were separated on normal silica gel G layers by multiple development with trichloroethylene (Table II).

TABLE III

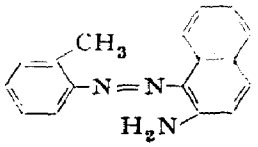
R_S (S = CERES RED G) VALUE OF FAT-SOLUBLE DYESTUFFS ON LAYERS OF POLYAMIDE (MN) AND SILVER NITRATE–SILICA GEL G LAYERS

Adsorbent	*Polyamide (MN)*				*Silver nitrate–silica gel G*		
Solvent	*A*	*B*	*C*	*D*	*E*	*F*	*G*
Dyestuffs	*Chloroform–methanol–water (5:15:1)*	*Acetone–ethanol–water (6:3:1)*	*Methanol–acetone–acetic acid (6:2:2)*	*Light petroleum–benzene–acetic acid (1:1:0.5)*	*Chloroform–light petroleum–acetic acid (75:25:0.5)*	*Chloroform–carbon tetrachloride–acetic acid (80:20:0.2)*	*Benzene–ether–acetic acid (90:10:0.1)*
Sudan I	1.15	1.09	1.17	1.13	1.29	1.34	1.19
Sudan II	0.65	0.62	0.68	1.11	1.37	1.39	1.23
Sudan III	0.11	0.10	0.11	0.90	0.61	0.47	1.09
Sudan IV	0.12	0.10	0.13	0.91	0.59	0.47	1.08
Bixin	0.37	0.39	0.39	0.82	0.23	0.21	0.18
Martius yellow	0	0	0.07	0.74	0.33	0.22	0.16
Chlorophyll	—	—	—	1.28	0.33	0.21	—
β-Carotene	0.24	0.37	0.40	1.14	0.36	—	—
Butter yellow	1.65	1.86	1.78	1.19	0.28	0.36	0.55
Ceres red G	≡1.00	1.00	1.00	1.00	1.00	1.00	1.00
Ceres orange GN	1.19	1.28	1.20	0.25	0.29 (0.39)	0.22 (0.27)	0.36 (0.15)
Ceres yellow 3G	1.68	1.50	1.57	1.23	0.29	0.23	0.17
Oil yellow XP	1.12	0.84	0.99	1.15	0.35	0.32	0.42
Yellow OB	0.63	0.83	0.71	0.73	0.71	0.73	0.95
Yellow AB	0.76	1.14	0.87	0.73	0.54	0.58	0.79
Orange SS	1.03	0.90	0.99	1.00	—	—	—
β-Apo-8′-carotinal	0.86	0.86	0.97	1.00	—	—	—

hand, on polar adsorbents (see Table II) Yellow OB was found to have a higher R_F value than Yellow AB.

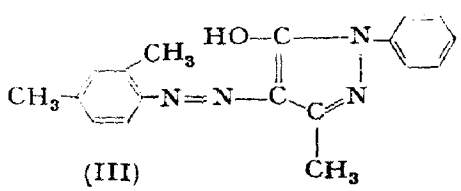


(I)

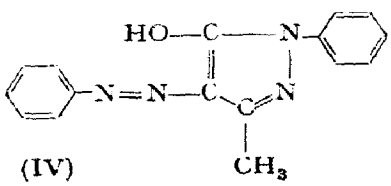


(II)

Analogously, the pyrazolone derivative Oil Yellow XP (2,4-dimethyl-1-aminobenzene → 1-phenyl-3-methyl-5-pyrazolone) (III), allowed as food colour in Great Britain and Denmark amongst other countries, contains two extra CH_3 groups, as compared with Ceres Yellow 3G (aminobenzene → 1-phenyl-3-methyl-5-pyrazolone) (IV), and consequently has a lower R_F value in the polyamide systems A, B and C.



(III)



(IV)

Quite a different chromatographic pattern was found in system D with the solvent light petroleum–benzene–acetic acid (1:1:0.5). This system was applied by the authors, for instance to the separation of fat antioxidants, and was supposed to belong to the type of *polar-phase partition chromatography*[11,12]. In this system an imbibed polyamide–acetic acid complex might act as *polar* stationary phase in conjunction with the *apolar* mobile phase. Consequently, β-carotene has a high R_S value in system D. The pairs Sudan I–Sudan II, Yellow AB–Yellow OB and Ceres Yellow 3G–Oil Yellow XP, which only differ in the number of CH_3 groups, are *not* separated in this system. The system D was found to have outstanding properties in the separation of various groups of compounds.

On silver nitrate-impregnated silica gel G layers the chromatographic behaviour was found to be quite different, allowing special separations to be made (see Table III).

The above systems were applied in practice to the detection and identification of fat-soluble dyestuffs in fats and fat products. They are also applicable to the separation of complex mixtures of carotenoids, xanthophylls, bixins, apocarotinals etc.

ACKNOWLEDGEMENT

The authors thank Dr. J. G. VAN GINKEL, Director of the Government Dairy Station (Rijkszuivelstation) for his permission to publish this paper.

SUMMARY

The chromatographic behaviour of fat-soluble dyestuffs in thin-layer chromatography has been studied. Various types of adsorbents such as silica gel G, Kieselguhr G, aluminium oxide G, polyamide and silver nitrate-impregnated silica gel were tested.

When using layers of polyamide and aqueous solvents (mixtures of alcohols, acetone, water, etc.) as mobile phase some dyestuffs are separated according to the principle of reversed-phase partition chromatography. By using the solvent mixture light petroleum–benzene–acetic acid (1:1:0.5) a polar-phase partition chromatography was obtained.

REFERENCES

1 C. NIEMAN, *Food Colours Recently Authorized in 43 Countries*, Consudel, Amsterdam, 1961.
2 P. JAX AND H. AUST, *Milchwissensch. Ber.*, 3 (1953) 145.
3 W. LINDBERG, *Z. Lebensm. Untersuch. Forsch.*, 103 (1956) 1.
4 M. R. VERMA AND R. DASS, *J. Sci. Ind. Res. (India)*, 16B (1957) 131.
5 E. MARK AND G. G. MCKEOWN, *J. Assoc. Offic. Agric. Chemists*, 42 (1959) 213.
6 J. GASPARIC AND M. MATRKA, *Collection Czech. Chem. Commun.*, 25 (1960) 1969.
7 J. W. COPIUS-PEEREBOOM, *Chem. Weekblad*, 57 (1961) 629.
8 J. DAVÍDEK, J. POKORNÝ AND G. JANÍCEK, *Z. Lebensm. Untersuch. Forsch.*, 116 (1961) 13.
9 A. MONTAG, *Z. Lebensm. Untersuch. Forsch.*, 116 (1962) 413.
10 J. DAVÍDEK AND G. JANÍCEK, *J. Chromatog.*, 15 (1964) 542.
11 J. W. COPIUS-PEEREBOOM, *Nature*, 204 (1964) 748.
12 J. W. COPIUS-PEEREBOOM, in K. MACEK AND I. M. HAIS (Editors), *Stationary Phase in Paper and Thin-layer Chromatography, Proc. 2nd Symp., Liblice, 1964*, Publishing House Czech. Acad. Sci., 1965, p. 134; see also H. ENDRES, *ibid.*, p. 125, and Discussion on pp. 150–152.