chromatography (120° start and 10° per min). Isothermal chromatography at 188°, 220° and 240° gave good separation of the isomers. The sharpest peaks were obtained at a temperature of 240°.

A flame ionization detector with the Carbowax 20 M column gave the best separation of isomeric bithienyls with a number of substrates studied (tricresyl phosphate, silicone gum rubber SE30 and Apiezon L grease). The Carbowax column gave excellent separation and identification of low concentrations of the isomeric bithienyls produced in the radiation chemistry of thiophene. The column material should be useful in the separation and identification of other isomeric heterocyclic compounds.

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Chromatography of fat-soluble food dyes on thin starch layers with stationary non-polar phases

Of the fat-soluble food dyes only the natural ones are permitted in moist countries. The use of synthetic fat-soluble dyes became restricted since Japanese research workers found them to be carcinogenic, especially Butter Yellow[1,2]. For this reason colouring of foods with these synthetics was prohibited in several countries, including Czechoslovakia[3]. The strict restriction, or even prohibition of these substances calls for a suitable, and above all, rapid analytical method of detection, or as the case may be, even for determination, of these substances in food inspection and research work.

Chromatographic methods based on adsorption[4] or on partition[5–7] are mostly used for the detection of fat-soluble food dyes. A classical system of identification of lipophilic food dyes has been described by THALLER AND SCHELLER[8] and JAX AND AUST[9]. These methods are based on pre-separation into groups by adsorption column chromatography; the components are then identified by means of partition paper chromatography. As the adsorption material alumina, various clays, etc., were employed. The use of partition paper chromatography which a stationary non-polar phase (paraffin oil) was first described by LINDBERG[5]. For the stationary phase other substances such as *n*-lauryl alcohol, oleic acid, diacetylethylene glycol monostearate[10],

and the like were also used; paraffin oil, however, found the widest application. The disadvantage of paper partition chromatography is that it is rather time consuming.

This drawback has been obviated to a certain extent by using thin-layer chromatography. For the partition of synthetic dyes this method was used first by MOTTIER AND POTTERAT[11,12], LAGONI AND WORTMANN[13], and by DAVÍDEK, POKORNÝ AND JANÍČEK[14,15]. All these methods make use of the adsorption ability of alumina or other adsorbents. Therefore the usefulness of the method is somewhat limited as it can only be applied to substances showing substantially different adsorption affinities. In some mixtures it was impossible to identify individual dyes by this method. In such cases a combination of the method with partition paper chromatography appeared necessary.

In the present paper the chromatographic separation of fat-soluble food dyes using partition chromatography on thin layers of starch with a stationary nonpolar phase is described. This method allows the principle of partition chromatography to be used even with the thin layer modification and so perfect separations of the said dyes could be obtained.

Experimental

The following dyes were studied: Yellow AB, Yellow OB, Orange SS, Sudan I, II, III, IV, and GN, Sudan Red G, and Butter Yellow. For the chromatographic partition 0.05–0.5 % dye solutions in ethanol, according to their solubility and coloring intensity, were used (see Table I). The chromatograms were developed with the following chromatographic solvent systems: aqueous methanol of various concentrations (50–70 %); a mixture of methanol 80 %, glacial acetic acid 5 % and water 15 %; a mixture of methanol 80 %, glacial acetic acid 10 % and water 10 %; mixtures of dioxan–water (3:2), and dioxan–water–ammonia (15:4:3).

Preparation of chromatographic plates. The chromatographic plates are prepared in the usual manner in wet state. To a 10 % solution of paraffin oil in petroleum ether, starch is added so as to obtain a fairly spreadable suspension (with about 10 g starch). This suspension is used for the preparation of the plate. After application on the glass plate and evaporation of the solvent, the plate is ready for use for chromatographic partition.

TABLE I

CONCENTRATION OF DYES USED

Dye	*Concentration mg/100 ml*
Yellow OB	2.00
Yellow AB	2.00
Orange SS	0.62
Oil Red OS	0.69
Sudan I	1.22
Sudan II	0.67
Sudan III	0.56
Sudan IV	0.65
Sudan Red G	0.64
Sudan Yellow 3G	1.13
Butter Yellow	1.19
Sudan GN	3.00

It appeared necessary first to establish the time required for evaporation of the solvent after the layer had been applied. The plates were prepared according to the procedure described above and dried in air for various time intervals; thereupon standards of dyes were applied and subjected to partition by means of the solvent system: methanol 80 %, water 15 %, and glacial acetic acid 5 %. The results are given in Table II.

TABLE II

INFLUENCE OF DRYING TIME OF THE PLATE UPON R_F VALUES

Drying time	R_F		
	Yellow OB	*Yellow AB*	*Orange SS*
20 min	0.63	0.73	0.33
30 min	0.60	0.73	0.32
90 min	0.58	0.68	0.30
150 min	0.59	0.71	0.28
180 min	0.58	0.72	0.30
210 min	0.57	0.70	0.27
270 min	0.58	0.72	0.28
7 h	0.56	0.70	0.26
24 h	0.53	0.68	0.28
48 h	0.50	0.61	0.19

As shown in the table, for perfect removal of the solvent a drying time of 20 min at room temperature is sufficient. Longer drying does not substantially influence the partition properties of the plate.

It was then necessary to establish the optimum concentration of paraffin oil for impregnating the thin layer. The preparation procedure was the same as before. For impregnation solutions of 1–25 % paraffin oil in petroleum ether were used. The plates were dried for 30 min at room temperature, and it was found that the optimum concentration of paraffin oil is about 5–10 % (Table III). After the optimum conditions had thus been established, the partition of lipophilic synthetic dyes was examined first in model mixtures. The results of these experiments are shown in Table IV. The solvent systems methanol 80 %, water 15 % and glacial acetic acid 5 %, and methanol 80 %, water 10 % and glacial acetic acid 10 % proved best. In these solvent systems even multi-component mixtures, such as are encountered in foodstuffs, can be successfully separated.

The dyes were usually isolated from the fat by acid extraction, adsorption on alumina, or extraction after saponification. As we have found before[14], for quantitative

TABLE III

INFLUENCE OF PARAFFIN OIL CONCENTRATION UPON R_F VALUES

Oil concentration %	R_F		
	Yellow OB	*Yellow AB*	*Orange SS*
1	0.88	0.91	0.67
5	0.70	0.74	0.34
10	0.57	0.71	0.29
20	0.44	0.58	0.14
25	0.32	0.42	0.10

TABLE IV

R_F VALUES OF THE INDIVIDUAL DYES

Dye	R_F in the solvent system				
	1	2	3	4	5
Yellow OB	0.04	0.29	0.59	0.57	0.61
Yellow AB	0.06	0.43	0.61	0.71	0.71
Orange SS	0.01	0.08	0.41	0.29	0.38
Oil red OS	0.00	0.00	0.10	0.03	0.04
Sudan I	0.02	0.11	0.40	0.36	0.41
Sudan II	0.01	0.04	0.32	0.19	0.28
Sudan III	0.00	0.03	0.27	0.10	0.24
Sudan IV	0.00	0.02	0.18	0.04	0.10
Sudan red G	0.04	0.26	0.54	0.53	0.58
Sudan yellow 3 G	0.02	0.14	0.48	0.33	0.48
Butter yellow	0.04	0.23	0.57	0.59	0.66
Sudan GN		0.48	0.63	0.64	0.74
		0.75	0.95	0.94	0.98

* Solvent systems: (1) 50% methanol.
(2) 70% methanol.
(3) 100% methanol.
(4) methanol–water–glacial acetic acid (16:3:1).
(5) methanol–water–glacial acetic acid (8:1:1).

isolation of the dye only extraction after saponification may be applied, and it is this method that we employed to isolate the dyes from model fat samples.

Method. 25 g of the coloured fat was weighed into a flask, and after addition of 200 ml of 50% alcoholic potassium hydroxide saponified by refluxing on a boiling water-bath for 30 min. Thereupon 160 ml water was added to the reaction mixture, and it was extracted with three separate portions of 50 ml of pentane. The extract was washed and dried and the solvent distilled off *in vacuo*; the residue was then dissolved in 2 ml ethanol. This solution was used for the chromatographic procedure.

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