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Polyamide–Silica Gel Layer Chromatography of Yellow Food Dyes

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Abstract □ The separation of five yellow food dyes and three toxic yellow dyes by mixed polyamide–silica gel thin-layer is described. The method shows good separation and sharp spots. For comparison, the thin-layer chromatography (TLC) on only polyamide and on only kieselguhr is also carried out.

Keyphrases □ Yellow dyes—analysis □ Dyes, yellow—separation, identification □ Polyamide–silica gel chromatography—analysis

The separation of synthetic food dyes on a thin-layer of cellulose (1), silica gel (2), aluminum oxide (3), starch (4), polyamide (5), and paper chromatography (6) has been reported, but none of these techniques gave entirely satisfactory results. Recently, the separation of 11 red food dyes on polyamide (12%)–silica gel G (88%) mixed thin-layers has been successfully applied by Chiang (7). Therefore, further application of this method was tried. In this note, the separation of five yellow food dyes and three toxic yellow dyes (auramine, metanil yellow, and picric acid) by mixed polyamide–silica gel TLC is described. For comparison, the TLC of only polyamide and of only silica gel is also reported.

EXPERIMENTAL

Material—The solvents and chemicals are the reagent grade of Wako Pure Chemical Industries, Ltd., Osaka, Japan.

Preparation of Polyamide–Silica Gel Mixed Layer—Eight grams of polyamide chip (Nylon 6,[1] type 1022B of UBE Industrial Ltd., Osaka, Japan) was dissolved in 80 ml. of 90% formic acid, and then 20 ml. of distilled water was added. After gentle warming (below 40°) and stirring, a homogeneous solution was obtained; this was cooled to the room temperature and 52 g. of silica gel G (E. Merck) was added. Two hundred milliliters of the above-mentioned solution was poured into a dish (14.5 × 19.5 × 2.5 cm.) and a glass plate (12 × 14 × 0.1 cm.) was dipped into it. Both sides of the glass were covered homogeneously. The glass was placed over the dish for 2 min. to let the excess solution drain back. It was then air dried for 3 hr. and heated at 100° for 30 min.

[1] U. S. manufacturer: American Enka Corp.

Table I—Chromatographic Data

No.	Dyes	Solvent I[a] P–S[c]	Solvent I[a] S[d]	Solvent I[a] P[e]	Solvent II[b] P–S	Solvent II[b] S	Solvent II[b] P
1	Naphthol yellow S	0.48	(0.95,	0.37[f])	0.10	(0.74,[f]	0.02)
2	Yellow AB	0.14	(0.62,	0.22)	0.74	(0.97,	0.48)
3	Yellow OB	0.10	(0.52,	0.22)	0.72	(0.97,	0.45)
4	Tartrazine	0.63	(0.98,	0.85)	0.01	(0.37,[f]	0.01)
5	Sunset yellow FCF	0.56	(0.98,	0.66)	0.14	(0.56,[f]	0.08)
6	Metanil yellow	0.28	(0.80,	0.14)	0.47	(0.83,	0.23)
7	Auramine	0.37	(0.71,	0.36)	0.62	(0.71,	0.89)
8	Picric acid	0.40	(0.97,	0.22)	0.53	(0.84,	0.22)
Time required,[g] (min.)		50	30	200	90	70	600

[a] Solvent I: methanol–23% ammonium chloride solution–chloroform (30:20:1.3). [b] Solvent II: isobutanol–ethanol–0.45% sodium chloride solution (3:5:1). [c] P-S, R_f value on polyamide-silica gel mixed layer. [d] S, on silica gel layer. [e] P, on polyamide layer. [f] Tailing. [g] Time required to ascend 10 cm. from origin.

Preparation of Silica Gel Layer—Dilute slurries of silica gel G (45 g. in 100 ml. of water) were sprayed at 2 kg./cm.^{3} pressure from a distance of 20 cm. onto eight sheets of glass plates (12 × 14 cm.) in a horizontal position, then dried at 100° for 30 min. The thickness of the layers was about 250 μ.

Chromatographic Procedure—One microliter of 0.3% alcoholic solution of yellow AB, yellow OB, and auramine, and 0.3% water solution of other dyes was applied to the start line 1.5 cm. from the bottom of the layer, and the plate was developed by ascending techniques. The chamber had been equilibrated with the respective solvent for 30 min. before use.

RESULTS AND DISCUSSION

R_f values obtained with two solvent systems are given in Table I. It has been found that the results obtained by the mixed polyamide–silica gel layers show better separation and sharper spots than that obtained by polyamide and silica gel layers. Also the time required to ascend 10 cm. from origin for the mixed layers is shorter than that for the polyamide layers. Separation mechanism on the mixed layers is based on the formation of hydrogen bonds between

the CONH group of polyamide and the sample and adsorption or partition between the silica gel and the sample. In the mixed layer, polyamide also serves as a strong binder and makes the layer very durable and easy to handle. Also the layer did not crack or peel and could be stored easily. Both sides of the glass are independent of each other and chromatography can be performed simultaneously on both sides. The addition of a small amount of salt (about 0.05% sodium chloride or 0.4% ammonium chloride) in the solvent mixture is essential to break hydrogen bonding between the polyamide and the dyes. Oil-soluble dyes of yellow AB and yellow OB are rather difficult to separate because of the close similarity of their structures (different only in one methyl group). The content of polyamide (13.6%) in this mixed layer was above that of the previous report (12%) (7) in order to obtain a more durable layer.

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Modified Granuloma Pouch Procedure for the Evaluation of Topically Applied Anti-inflammatory Steroids

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Abstract □ A laboratory procedure for evaluating topically applied anti-inflammatory preparations has been described. A modified croton oil-induced granuloma pouch served as the site of drug application and also as a physical barrier to avoid drug ingestion. Increasing concentrations and/or doses of test compounds are correlated with thymolysis, catabolism, and reduced exudate formation. The procedure can differentiate steroidal modifications, percutaneous absorption, and alterations of the vehicle. However, it is difficult to ascertain how much of the action of the test compound is due to its local and how much to its systemic properties. The assay also evaluated test compounds as pharmaceutical preparations. The order of increasing activity of the test substances can be listed as hydrocortisone acetate (1% cream) < methylprednisolone acetate (0.25% cream) < betamethasone 17-valerate (0.025% cream) < triamcinolone 16,17-acetonide (0.025% cream) < betamethasone (0.2% cream) < fluocinolone acetonide (0.025% alcoholic gel or cream).

Keyphrases □ Steroids, anti-inflammatory—topical activity determination □ Granuloma pouch test procedure—topical steroid activity □ Croton oil-air—granuloma pouch formation

Potential anti-inflammatory substances, topical or systemic, have been evaluated in a variety of procedures which apparently represent different modes of actions and/or different stages of the inflammatory process. Some of these procedures include: cotton pellet, granuloma pouch, paw edema, adjuvant-induced polyarthritis, cell cultures, uncoupling of mitochondrial oxidative phosphorylation, plasma protein changes, ear test, lung inflammation, and inhibition of erythema (1–4). It is evident that each test procedure has some merit; however, one cannot avoid nor explain discrepancies which occur between the various animal *in vitro* and clinical procedures. Nevertheless, most clinically efficacious drugs are active in most procedures, although in some assays a divergence may occur between steroidal and nonsteroidal drugs. Avoidance of ingestion of topically applied drugs in animals has presented some perplexing problems. Alternatives appear to introduce other problems; for example the use of occlusive dressings may alter absorption whereas the use of collars and other restraining devices produces stress which elaborates endogenous adrenocorticosteroids. The use of an air pouch which serves as the site of drug application and a physical barrier to avoid ingestion has been tried by the authors. They have also tried to observe whether or not the modified granuloma pouch procedure will distinguish steroidal modifications and/or alterations of the vehicle. It is known that these changes will affect drug absorption, retention, and biological activity (5, 6). It should also be mentioned that most animal (*in vivo* and/or *in vitro*) procedures require a solubilized test compound which requires organic solvents. The present study also evaluated drugs as pharmaceutical preparations.

MATERIALS AND METHODS

The method used was a modification of Selye's procedure (7). Male rats weighing 150–170 g. were individually housed and arranged in groups as indicated in the tables. The dorsal surface of the animal was shaved with animal clippers and the pouches were formed with the subcutaneous injection of 25 ml. of air followed by an injection of 0.5 ml. of a 1% solution of croton oil in sesame oil directly into the pouch. The test compounds included the commercial preparations: fluocinolone acetonide (Synalar cream, Syntex Laboratories Inc.); betamethasone (Celestone cream, Schering Corp.); methylprednisolone acetate (Medrol acetate cream, The Upjohn Co.); triamcinolone 16,17-acetonide (Kenalog cream, E. R. Squibb & Sons, Inc.); and also these laboratory preparations: hydrocortisone acetate in a cream base; betamethasone 17-valerate in a cream base; triamcinolone in a cream base; triamcinolone 16,17-acetonide in a cream base; and fluocinolone acetonide in an alcoholic gel base.