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Thudichum Psychiatric Research Laboratory, Galesburg State Research Hospital, Galesburg, Ill. 61401 (U.S.A.)

N. NARASIMHACHARI
J. PLAUT

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Separation and detection of basic dyes by polyamide thin-layer chromatography

Basic dyes are not permissible for use as food additives now, but an analytical method for the compounds is necessary because there is the danger of them being used or mixed in foods and pharmaceutical formulations either erroneously or illegally. Thin-layer chromatography (TLC) using only silica gel plates has previously been applied to the analysis of basic dyes[1]. No results however, have proved entirely satisfactory. In this paper, therefore, an attempt at analysis was made using polyamide layers, which are frequently used for separation and identification of water and oil-soluble dyes[2–6].

Materials and methods

Adsorbent. Polyamide powder (obtained from E. Merck, Darmstadt, G.F.R.) was used as the adsorbent. Before use, it was washed with a volume of methanol equal to 3–5 times the volume of polyamide powder. After drying in air, it was further dried in vacuum in a desiccator containing calcium chloride.

Dyes. Each test solution of the basic dyes listed in Table I was prepared by dissolving 10 mg of each of them in 10 ml of 60% ethanol solution. Aliquots (μl) of this solution were used for TLC.

Developing solvents. (a) Benzene; (b) carbon tetrachloride; (c) methanol; (d) ethanol; (e) 28% ammonia–methanol (1:8); (f) benzene–methanol (5:1); and (g) carbon tetrachloride–methanol (4:1). All the solvents were of analytical grade.

Preparation of the thin layers. Glass plates (20 × 20 cm) were coated with a homogeneous slurry composed of 15 g polyamide powder and 50 ml of isopropanol by means of an applicator giving a thin layer approximately 250 μ in thickness. The coated plates were dried in air for 15 min and then at 60° for 30 min. They were kept in a desiccator over silica gel until required.

TABLE I

BASIC DYES USED IN THIS STUDY

Group	*Dyes*
Triphenyl methane compounds	Fuchsine Base Crystal Violet Ethyl Violet Malachite Green Night Blue
Xanthene compounds	Pyronine G Rhodamine 6G Rhodamine B
Phenazine compounds	Neutral Red Acridine Orange Safranine O
Phenothiazine compounds	Methylene Blue New Methylene Blue
Azo compounds	Bismarck Brown
Phenoxazine compounds	Nile Blue

Development and detection. 0.5–2 μl of each test solution was spotted with a micropipette on the starting line 2 cm from the lower edge of the plates. After equilibrating the chamber (25 × 13 × 26 cm) with the respective developing solvent for 20–30 min, the plates were developed by an ascending technique until the solvent front had travelled to a distance of 10 cm from the starting line. After development, the plates were removed from the chamber and air dried. Each spot was detected under long-wavelength UV light (3650 Å) in addition to ordinary illumination.

Results and discussion

Fifteen basic dyes consisting of three xanthene compounds, three phenazine compounds, five triphenylmethane compounds, two phenothiazine compounds, one azo compound and one phenoxazine compound were used for chromatographic analysis on the polyamide thin layers. The R_F values of the 15 dyes obtained with each of the seven solvent systems are summarized in Table II. The values represent main spots of each of the basic dyes. A few spots of minor impurities in addition to the main spots occurring in each test solution of the basic dyes were clearly separated and detected under UV light when developed with some chromatographic solvents, especially the combined solvents. None of the main spots was affected by the presence of the minor spots.

These dyes were not separated in such solvents as benzene or carbon tetrachloride. When developed with ethanol or methanol, for the most part, the spots were not separated because of their proximity to the solvent front. On the other hand, development with 28% ammonia–methanol (1:8) gave a good separation of all the colours with slight tailing and diffusion of the spots of triphenyl methane compounds.

In development with benzene–methanol (5:1), although small and sharp spots were obtained they were not well separated. When developed with carbon tetrachloride–methanol (4:1), the dyes were almost all completely separated as circular,

TABLE II

R_F VALUES OF BASIC DYES ON POLYAMIDE THIN-LAYER PLATES USING SOLVENT SYSTEMS a–g

Chromatographic solvents: (a) benzene; (b) carbon tetrachloride; (c) methanol; (d) ethanol; (e) 28 % ammonia–methanol (1 : 8); (f) benzene–methanol (5 : 1); (g) carbon tetrachloride–methanol (4 : 1). t = tailing; d = dispersion; sf = solvent front.

Dyestuff	*Chromatographic solvent*						
	a	*b*	*c*	*d*	*e*	*f*	*g*
Fuchsine Base	0.00	0.00	0.92	0.94	0.81 t,d	0.13	0.23
Crystal Violet	0.00	0.00	0.96	— sf	0.67 t,d	0.43	0.54
Ethyl Violet	0.00	0.02 t	0.97	— sf	0.47 t.d	0.44	0.56
Malachite Green	— t	0.00	0.95	— sf	0.66 t,d	0.42	0.91
Night Blue	0.14	0.00	0.93	— sf	0.35 t,d	0.39	0.49
Bismarck Brown	0.02	0.00	0.43	0.42	0.39	0.30	0.17
Neutral Red	0.01	0.00	0.81	0.55	0.26	0.54	0.58
Safranine O	0.00	0.00	0.91	0.92	0.15	0.22	0.33
Acridine Orange	0.00	0.00	0.88	0.85	0.16	0.45	0.42
Methylene Blue	0.00	0.00	0.91	0.92	0.20	0.33	0.31
New Methylene Blue	0.00	0.00	0.82	0.85	0.07	0.21	0.30
Nile Blue	0.08 t	0.01 t	0.89	0.89	0.09	0.43	0.45
Pyronine G	0.00	0.00	0.92	0.93	0.26	0.41	0.43
Rhodamine 6G	0.00	0.00	0.95	— sf	0.41	0.39	0.50
Rhodamine B	— t	0.34 t	0.90	0.93	0.83	0.80	0.88
Time required (min)[a]	35	55	30	80	60	35	70

[a] Time required to ascend 10 cm from origin.

small, and sharp spots, although it was not easy to get a distinct separation among the six groups. A typical chromatogram is shown in Fig. 1.

It was observed that an increase in the carbon tetrachloride concentration in this solvent system effects a decrease of R_F values; for the best distribution and sharpness of the spots development with carbon tetrachloride–methanol (5–6 : 1) is re-

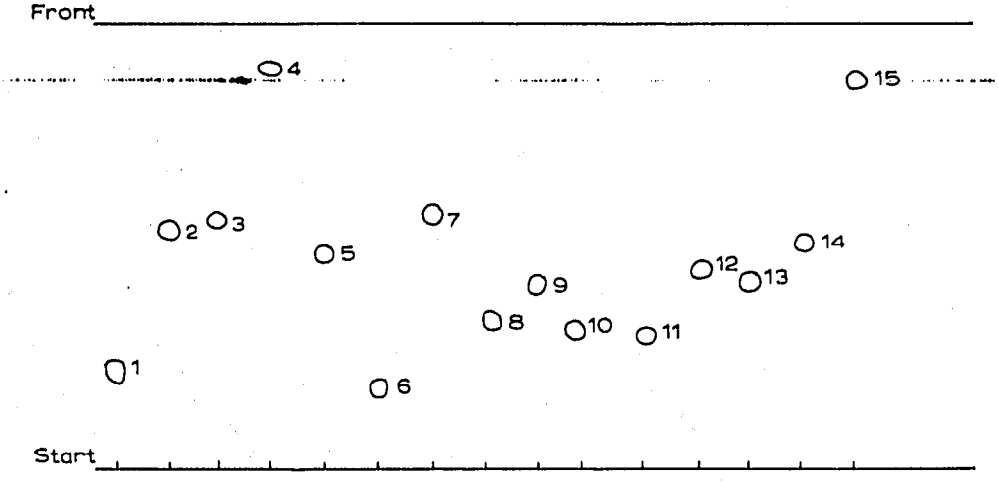

Fig. 1. Chromatogram of basic dyes on polyamide thin layers. Solvent system, carbon tetrachloride–methanol (4 : 1); developing time, 70 min; temperature, 20 ± 1°. Spot No.: 1 = Fuchsine Base; 2 = Crystal Violet; 3 = Ethyl Violet; 4 = Malachite Green; 5 = Night Blue; 6 = Bismarck Brown; 7 = Neutral Red; 8 = Safranine O; 9 = Acridine Orange; 10 = Methylene Blue; 11 = New Methylene Blue; 12 = Nile Blue; 13 = Pyronine G; 14 = Rhodamine 6G; 15 = Rhodamine B.

commended for the triphenyl methane group of compounds. The solvent system carbon tetrachloride–methanol (3–4:1) was found to be the most useful for separating the other compounds.

The running time in the solvent system carbon tetrachloride–methanol was variable depending on the volume of carbon tetrachloride. The time required was 70–90 min at 20 ± 1°.

The polyamide layer used was very durable and was easy to handle. Furthermore, the layer did not crack and peel after development with the chromatographic solvents. From the results obtained in this experiment, this polyamide thin layers were found to be more suitable for separating basic dyes than silica gel layers.

The Institute of Public Health,
6-1, Shirokanedai 4-chome,
Minato-ku, Tokyo (Japan)

RYUZO TAKESHITA
NOZOMU ITOH
YONEJI SAKAGAMI

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