

can using polyamide layers. The content (16%) of polyamide in this polyamide-silica mixed layer is higher than that in the previous report (12%)<sup>4</sup> for getting a more stable layer.

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First received May 12th, 1969; revised manuscript received July 1st, 1969

*J. Chromatog.*, 44 (1969) 201-203

HRM. 4284

### **Polyamide-kieselguhr thin-layer chromatography of yellow food dyes**

The thin-layer chromatography of food dyes has been studied by numerous investigators. The separation of synthetic food dyes by thin layers of cellulose<sup>1</sup>, starch<sup>2</sup>, silica gel<sup>3</sup>, aluminum oxide<sup>4</sup> and polyamide<sup>5</sup> has been reported. Recently, a better separation of red food dyes was obtained by CHIANG<sup>6</sup> with a mixed polyamide-silica gel thin layer. Therefore, further modification of this method was tried. In this note, the separation of five yellow food dyes and three harmful yellow dyes (auramine, metanil yellow and picric acid) by mixed polyamide-kieselguhr thin-layer chromatography is described. For comparison, the thin-layer chromatography on only polyamide and on only kieselguhr is also described.

#### *Experimental*

*Preparation of polyamide-kieselguhr mixed layer.* Ten grams of polyamide chip (Nylon 6, type 1022B of UBE Industrial Ltd., Osaka, Japan) were dissolved in 80 ml of 90% formic acid; then 20 ml of distilled water were added. After warming (below 60°) and stirring, a homogeneous solution was obtained. It was then cooled to room temperature, and 40 grams of Kieselguhr G (E. Merck) were added. Of the previous solution 200 ml were poured into a dish (14.5 × 19.5 × 2.5 cm) into which a glass plate (12 × 14 × 0.1 cm) was dipped. Both sides of the glass were covered homogeneously. The glass was hung for 2 min over the dish to let the excess solution drain off. It was then air dried for 3 h and heated at 100° for 30 min.

*Preparation of polyamide layer.* Instead of 10 g, 20 g of polyamide were dissolved before proceeding as described in the previous method, but without adding Kieselguhr G.

*Preparation of kieselguhr layer.* Dilute slurries of Kieselguhr G (45 g in 100 ml of water) were sprayed at 2 kg/cm<sup>3</sup> pressure from a distance of 20 cm onto 8 horizontal glass plates (12 × 14 cm) which were then dried at 100° for 30 min. The thickness of the layers was about 250 μ.

*J. Chromatog.*, 44 (1969) 203-204

TABLE I

## CHROMATOGRAPHIC DATA

Solvent I: methanol-acetone-water-30% sodium acetate solution-ethylenediamine (10:10:20:5:2); solvent II: ethanol-water-ether-5% NH<sub>4</sub>Cl solution-ethylenediamine (15:15:10:5:2). a, *R<sub>F</sub>* value obtained on polyamide-kieselguhr layer; b, kieselguhr layer; c, polyamide layer.

No. Dyes	Solvent I			Solvent II		
	a	b	c	a	b	c
1 Naphthol yellow S	0.66	0.97	0.45	0.69	0.98	0.54
2 Yellow AB	0.11	0.82	0.02	0.35	0.88	0.10
3 Yellow OB	0.05	0.79 <sup>a</sup>	0.01	0.23	0.84	0.10
4 Tartrazine	0.91	0.97	0.84	0.88	0.85	0.80
5 Sunset yellow FCF	0.38	0.98	0.59	0.81	0.94	0.78
6 Metanil yellow	0.31	0.88	0.13	0.53	0.92	0.43
7 Auramine	0.73	0.96	0.35 <sup>a</sup>	0.76	0.96	0.49
8 Picric acid	0.53	0.98	0.77 <sup>a</sup>	0.60	0.95	0.52
Time required (min) <sup>b</sup>	75	30	300	150	90	600

<sup>a</sup> Tailing.

<sup>b</sup> Time required to ascend 10 cm from origin.

*Chromatographic procedure.* A 0.5% alcoholic solution of Yellow AB, Yellow OB and auramine and a 0.5% water solution of other dyes were applied to the starting 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*

The *R<sub>F</sub>* values obtained with two solvent systems are given in Table I. It has been found that the results show better separation and sharp spots with polyamide-kieselguhr mixed layers than with polyamide and kieselguhr layers. Also a 10-cm ascent from the origin is more rapid using the mixed layers than when polyamide layers are employed. In the mixed layer, polyamide serves as a strong binder and makes the layers very durable and easy to handle. 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 and ethylenediamine in the solvent mixture is essential to break hydrogen bonding between polyamide and dyes. Oil-soluble dyes of Yellow AB and Yellow OB are rather difficult to separate because of the close similarity of their structures.

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First received June 9th, 1969; revised manuscript received July 11th, 1969