Results

The results obtained are presented in Figs. 1 and 2 and indicate that ATP is contaminated with AMP and traces of ADP. ADP also contains AMP. The only one that appears chromatographically pure is AMP.

Bidimensional chromatograms were run on square plates, 80 × 80 mm, the nucleotides were applied one after the other as one single spot near a corner, run in the first solvent in one direction, photographed and then run in the other direction with the second solvent. The results obtained for ATP, ADP and AMP are shown in Figs. 3 and 4.

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The use of polyamide in analyses of water soluble food dyes

IV. Thin layer chromatographic separation of water soluble food dyes

In a previous paper, a quantitative method for the isolation of food dyes directly from food materials using polyamide powder has been described[1]. After isolation food dyes or mixtures of synthetic dyes are generally separated on cellulose or inorganic adsorbents such as silica gel[2–5]. Although these methods give good separation, they are not much used for the separation of very small amounts of food dyes. The present paper describes a thin-layer chromatographic method using polyamide powder for the separation and estimation of small amounts of food dyes.

Materials and methods

Reagents. (1) Synthetic food dye solutions (0.005 % solution). (2) Polyamide powder (Chemical Fabrics Lovosice Workshop Rudnik, Czechoslovakia).

Procedure. For the preparation of thin layers, polyamide powder (12 g) was homogenized with methanol (40 ml), applied to the plates (thickness 0.2 mm) and the coated plates were dried at 40° for 30 min. Developing time for the chromatogram depended on the composition of the solvent system used, but it was less than 45 min in all cases.

For the isolation and separation of synthetic dyes in various food materials, the method used was the same as that described earlier[6]. To a weak acid extract of food dyes or to an acidified liquid sample of food, about 10 g of polyamide powder was added, thoroughly mixed and filtered. The polyamide powder together with the adsorbed dyes was washed with acetic acid solution (50 %, v/v) to remove natural dyes till the filtrate was colorless. The synthetic dyes were then eluted from the polyamide powder with a 5 % solution of ammonia in methanol (v/v). The eluate was

evaporated almost to dryness, dissolved in a known volume of water and used for thin layer chromatography.

TABLE I

INFLUENCE OF AMMONIA ON R_F VALUES

Solvents: Ammonia–methanol–water in the following ratios: (1) 1:15:84; (2) 2.5:15:82.5; (3) 5:15:80; (4) 10:15:75; (5) 15:15:70.

Colour	*Chromatographic solvent*				
	1	*2*	*3*	*4*	*5*
Amaranth	0.47	0.72	0.77	0.83	0.82
Azorubin	0.55	0.69	0.78	0.82	0.84
Echrot	0.19	0.27	0.42	0.65	0.59
Cochenillerot	0.15	0.26	0.34	0.58	0.56
Erythrosin	0.10	0.19	0.21	0.28	0.28
Sunset Yellow	0.47	0.66	0.72	0.78	0.74
Tartazin	0.76	0.85	0.88	0.90	0.90
Naftolgelb	0.32	0.45	0.62	0.64	0.63
Indigotin	0.53	0.65	0.70	0.78	0.74
Brillantschwarz	0.18	0.36	0.60	0.72	0.72

TABLE II

INFLUENCE OF METHANOL ON R_F VALUES

Solvents: Ammonia–methanol–water in the following ratios: (1) 2.5:5:92.5; (2) 2.5:10:87.5; (3) 2.5:15:82.5; (4) 2.5:20:77.5; (5) 2.5:25:72.5; (6) 2.5:30:67.5.

Colour	*Chromatographic solvent*					
	1	*2*	*3*	*4*	*5*	*6*
Amaranth	0.69	0.70	0.72	0.69	0.70	0.72
Azorubin	0.72	0.72	0.69	0.73	0.76	0.75
Echrot	0.26	0.28	0.27	0.34	0.35	0.39
Cochenillerot	0.25	0.26	0.26	0.26	0.36	0.36
Erythrosin	0.14	0.14	0.15	0.16	0.22	0.24
Sunset Yellow	0.63	0.63	0.64	0.63	0.67	0.69
Tartazin	0.85	0.85	0.85	0.82	0.84	0.82
Naftolgelb	0.41	0.47	0.45	0.49	0.50	0.53
Indigotin	0.56	0.60	0.65	0.50	0.56	0.55
Brillantschwarz	0.31	0.35	0.36	0.33	0.40	0.47

Results

For the separation of synthetic food dyes, ammonia–methanol–water mixtures containing varying amounts of ammonia and methanol were used as solvent systems (Tables I and II). Since our earlier results on the isolation of synthetic dyes from food materials have indicated that, in acidic medium, these dyes are firmly bound to the polyamide powder[1,6], in these tests only basic solvent systems were used. The results indicate that the mobilities of synthetic dyes are greatly affected by the pH of the solvent system. Concentration of methanol, on the other hand, had little affect on the mobilities of these dyes, and it was also possible to replace methanol by ethanol

without any adverse effect on the separation. The best separation was achieved with a solvent system containing ammonia–methanol–water (5 : 15 : 80). This solvent system also gave good separation of synthetic dyes from a number of samples of fruit jams, fruit jellies, food preserves, canned fruits, beverages and sweet candies.

Discussion

The results indicated that polyamide powder is an excellent material for thin-layer chromatographic separation of synthetic food dyes. Earlier work in our laboratories has also shown the efficiency of polyamide powder for quantitative isolation of synthetic dyes from food materials[1]. Probably the separation is achieved by binding hydrogen bridges between the dyes and the polyamide powder. The natural food dyes usually contain hydroxyl groups (–OH) which form weaker hydrogen bridges, while the synthetic food dyes contain sulfonic acid groups ($-SO_3H$) which form stronger hydrogen bridges, therefore it appears that the elution of natural dyes from the polyamide powder is possible in acidic medium without affecting the binding between the synthetic dyes and the polyamide powder. Since the separation of synthetic dyes depended only on the concentration of the ammonia in the solvent system, the separation of the synthetic dyes may be partly achieved as a result of the difference in the nature and number of functional groups forming hydrogen bridges.

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Bioautography of antibiotics on thin layer chromatograms

In a previous note[1], we described techniques for the bioautographic detection of antibiotics on thin layer chromatograms. We recommended *Streptococcus lactis* as an assay organism for antibiotics active against Gram positive microorganisms. This bacterium can grow underneath a glass chromatographic plate where oxygen is growth limiting for the more commonly used assay organism, *Staphylococcus aureus* 209P. In subsequent studies however, we found some antibiotics to be non-inhibitory for this bacterium, and hence we were required to use *Staphylococcus aureus* 209P.