Thin-layer chromatography of nucleotides with U.V. transparent plates

In the present work a modification of the thin-layer chromatographic technique is described which consists in substituting the ordinary glass for supporting the adsorbent by plates of U.V. transparent glass. This modification facilitates the study and the separation of compounds with absorption in the ultraviolet.

The present study is concerned with the application of this method to the study and resolution of mixtures of nucleotides.

Experimental

Glass plates (filters manufactured by Corning Glass Works No. 7910, CS No. 9.54) about 2 mm thick and 80 mm square, or rectangular (like microscope slides), 16.7 mm width by 80 mm length, were used. This material has a good light transmission between 250 and 400 mμ.

The adsorbent used was Cellex D Bio-Rad Laboratories (DEAE cellulose), an anion exchange material, which was purified by washing thoroughly with distilled water, then methanol and finally acetone. Sufficient dry Cellex D to give a coating of 4 to 5 mg/cm^2 was made into an aqueous suspension, poured on the plate placed on a horizontal surface, and spread with a spatula.

The following nucleotides were examined: adenosine mono-, di- and triphosphates (AMP, ADP and ATP) and diphosphopyridine and triphosphopyridine nucleotides (NAD and NADP).

The solvents employed were: (a) distilled acetone–water–HCl (65:35:0.1, v/v); (b) distilled water–methanol–HCl (60:40:0.1, v/v); and (c) a solution of 100 mg of potassium borate in 100 ml of water and distilled methanol mixed in the following

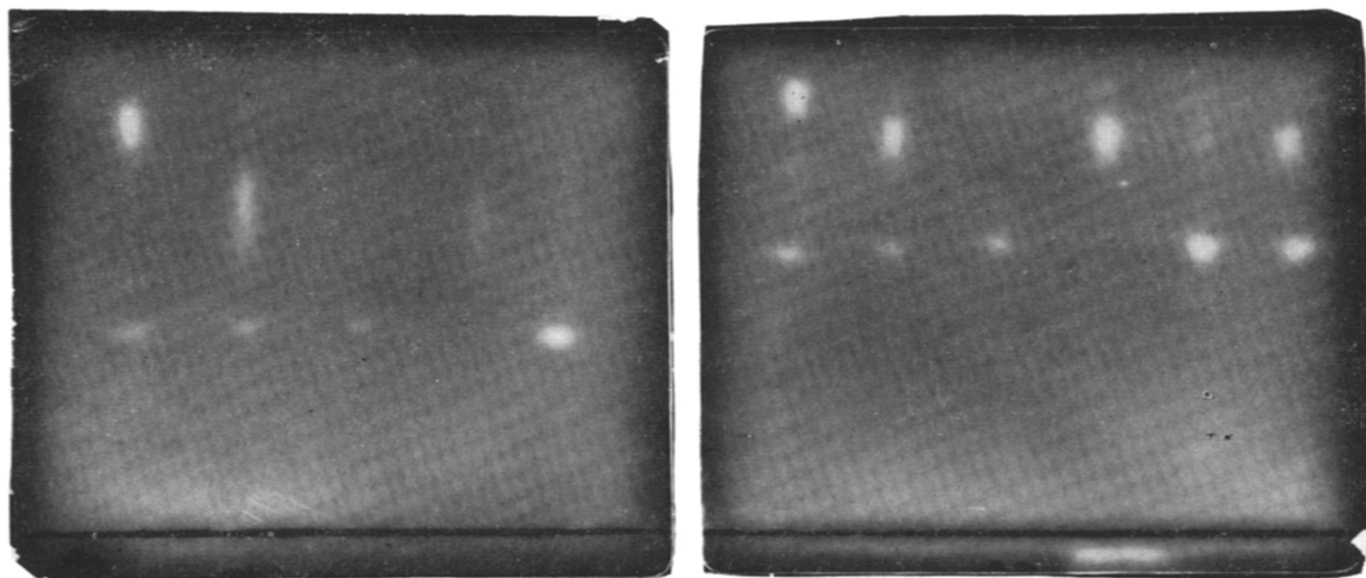

Fig. 1. Thin-layer chromatography of ATP, ADP, AMP, NAD and NADP applied as individual spots in the order listed. Solvent: acetone–water–hydrochloric acid (65:35:0.1).

Fig. 2. Thin-layer chromatography of ATP, ADP, AMP, NAD, NADP and NAD + NADP applied as individual spots in the order listed. Solvent: water–methanol–hydrochloric acid (60:40:0.1).

proportions: 70:30 and 0.1 part of HCl, v/v. In solutions (a) and (b) the pH was approximately 4.0.

After chromatography the plates were dried at room temperature and taken to the dark room and placed over photographic paper (Azo No. 4) and illuminated a few seconds with a source of U.V. light (mineral light lamp, short wave about 260 mμ), placed at a distance of 30 cm. In this manner visible and permanent records of the chromatograms are obtained.

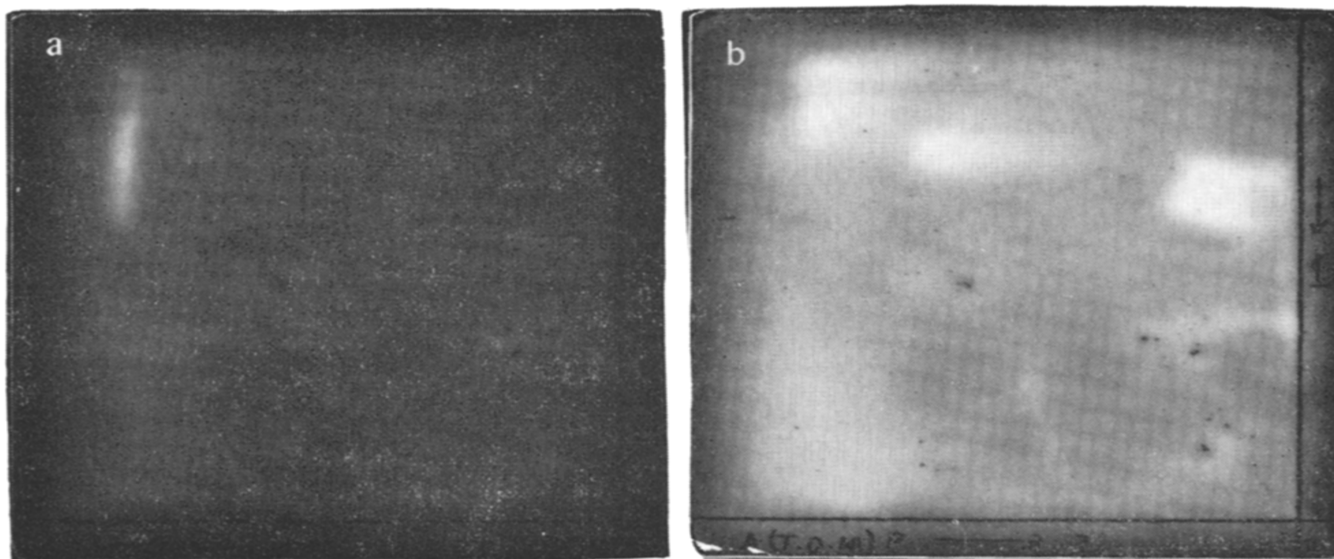

Fig. 3. (a) Chromatogram after first run (direction 1). Solvent: water–methanol–hydrochloric acid (60:40:0.1). (b) Same chromatogram after second run (direction 2). Solvent: potassium borate solution–methanol–hydrochloric acid (70:30:0.1).

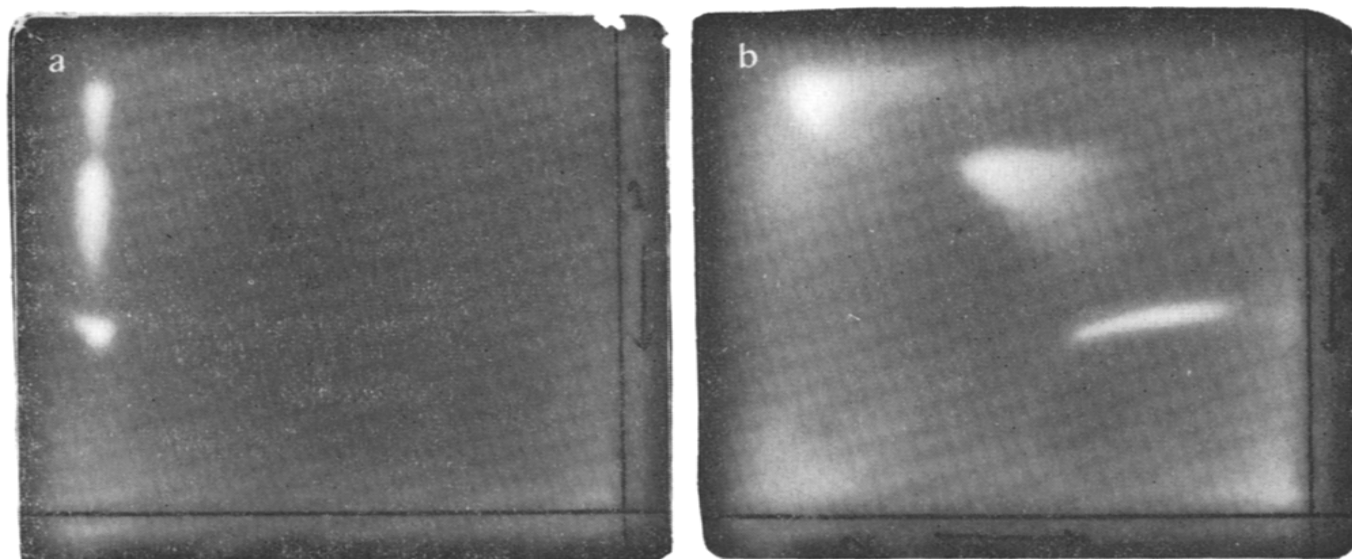

Fig. 4. (a) Chromatogram after first run (direction 1). Solvent: acetone–water–hydrochloric acid (65:35:0.1). (b) Same chromatogram after second run (direction 2). Solvent: potassium borate solution–methanol–hydrochloric acid (65:35:0.1).

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