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The Separation of Nucleotides by Ionophoresis and Chromatography on Paper

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The separation of a complex mixture of 5′-ribonucleotides by a combination of ionophoresis and chromatography on paper is described.

The development of ionophoresis on paper has greatly facilitated separations of substances which would be impossible by paper chromatography and time consuming by ion exchange methods. The combination of paper ionophoresis with chromatography, either carried out simultaneously or separately, sometimes permits separations to be effected that can not be carried out by either technique alone. Numerous workers have used this method for the separation of different substances but most of the methods concerned with the separation of nucleotides have been found to give satisfactory separations of only a few nucleotides. In the course of studies of the nucleotide content of various plant materials, a method has been developed for the separation of all the main components to be found in these materials [1]. The nucleotides are fractionated into groups by paper chromatography and then completely separated by ionophoresis. The present paper describes a two-dimensional fractionation of these substances on a single sheet of filter paper.

The mixture of nucleotides to be separated was applied as a narrow band about 15 mm in length and 6 cm from the edge of a large sheet of Whatman No. 1 filter paper (Fig. 1). The resolution in the first direction was obtained by ionophoresis using the long-paper apparatus described earlier [1]. The paper was impregnated with an acetate buffer of pH 4.15 and of ionic strength 0.1 by dipping as previously described. Then the paper was folded along the line CD and placed on a glass frame. The frame and paper were then immersed in a carbon tetrachloride vessel so that the ends FG and HI dipped into the electrode vessels. The frame was constructed as a three piece unit in order to facilitate placing the paper on it as illustrated in Fig. 2. The ionophoresis was carried out at 1 000 V for 10 h, at which time the nucleotides were distributed over the interval BE. The paper was then dried at room temperature and, after cutting away the tabs BCGF and EDHI, the separation in the second

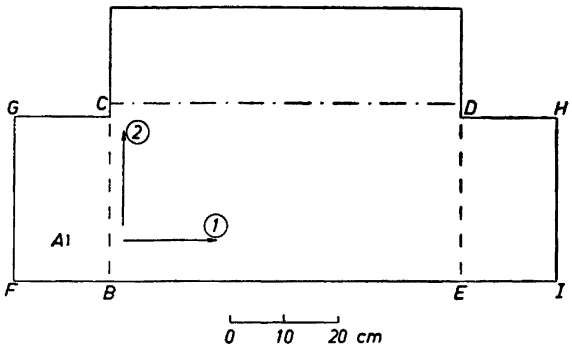

Fig. 1. The dimensions and shape of the filter paper sheet. The substances are applied at point A. The paper is folded along the line CD and the tabs FG and HI dip into the electrode vessels. The substances are fractionated by ionophoresis in direction 1 and then by paper chromatography in direction 2.

direction was achieved by chromatography. The previously described solvent system consisting of saturated ammonium sulphate solution — *iso*propanol — water (79:2:19) could be used for obtaining a complete separation of all the components, but better separation of the pyrimidine nucleotides was achieved using *n*-butanol instead of *iso*propanol. The paper sheet was impregnated with buffer salts from the ionophoretic development; but, when using the butanol mixture, these salts did not interfere with the separation of the various substances. The spots obtained by this method are as compact as those which result from other two-dimensional systems. All the 5′-mono-, di- and triphosphates of adenosine (AMP, ADP and ATP), guanosine (GMP, GDP and GTP), cytidine (CMP, CDP and CTP) und uridine (UMP, UDP and UTP) together with diphospho- and triphosphopyridine nucleotides (DPN and TPN) are separated as shown in Fig. 3. Uridine diphosphate glucose (UDPG) and

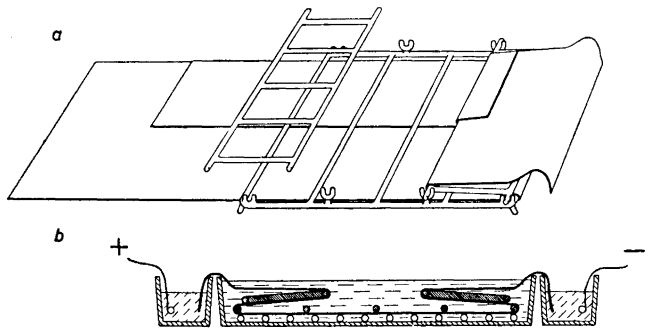

Fig. 2. a) The fitting of the paper to the glass frame. b) Cross section showing the paper and frame in place in the ionophoresis apparatus.

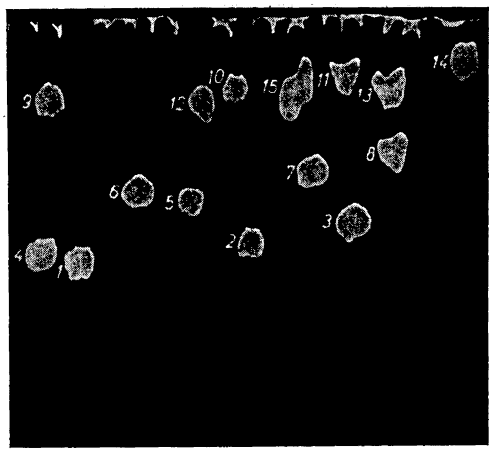

Fig. 3. The ultraviolet photograph of the two-dimensional ionophoretic — chromatographic separation of nucleotides.

1) AMP; 2) ADP; 3) ATP; 4) DPN; 5) TPN; 6) GMP; 7) GDP; 8) GTP; 9) CMP; 10) CDP; 11) CTP; 12) UMP; 13) UDP; 14) UTP; 15) UDPG and UDPAG.

uridine diphosphate acetylglucosamine (UDPAG) are not completely separated from each other, but are clearly separated from the other nucleotides.

The method described gives a rapid separation of the nucleotides. It is usable for preliminary analyses of mixtures and especially when only small amounts of material are available. An approximate estimation of the amounts of the different substances can be obtained by eluting the ultraviolet absorbing spots with 0.1 N hydrochloric acid and estimating the ultraviolet absorption of these eluents.

EXPERIMENTAL

The ionophoretic fractionation was performed using the apparatus and technique previously described [1]. The two-dimensional separation was carried out on Whatman No. 1 paper purified by washing with 1 N hydrochloric acid and water. The mixture to be separated was applied as a line to the dry paper and then the paper was impregnated with sodium acetate buffer of pH 4.15 and of ionic strength 0.1. The nucleotides were distributed over a maximum usable interval after the paper had been exposed to a voltage of 1 000 V for 10 h. The paper was dried at room temperature and then the initial fractionation of the mixture was observed by photographic printing in ultraviolet light of 254 mμ.

The chromatographic separation in the second direction was made after cutting away the unnecessary parts of the paper sheet. The best separations were obtained by the descending technique with a solvent system of saturated ammonium sulphate solution — *n*-butanol — water (79:0.5:19). The chromatogram was run for 10 h at 20—22°C. The resulting separations of the nucleotides were established by making a contact print in ultraviolet light of the whole sheet.

Materials. The nucleotides used as standard substances were obtained from the Sigma Chemical Company.

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