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## Note

# Extremely low electrophoretic mobility of picric acid in acetyl cellulose paper

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The electrophoretic mobility on a support depends on several factors which will vary with the experimental conditions[1]. Thus, in order to correlate the results of each run, it is necessary to include a standard compound in the sample. Picric acid is a useful standard because it is a strong acid and is coloured. I have used picric acid as a standard and never met any inconsistency in electrophoresis on pure cellulose paper. On the other hand, electrophoresis using modified cellulose paper is becoming very popular[2]. Acetyl cellulose (AC) paper is widely used because of its lower adsorption. However, when I used AC paper instead of a pure cellulose paper as a support, the mobility of picric acid was extremely low. Thus, in order to determine the interaction between AC paper and picric acid, the electrophoresis of picric acid and dinitrophenol compounds on AC paper and ion exchange cellulose papers has been studied.

## EXPERIMENTAL

The procedures and apparatus used were similar to those described previously[3]. All the reagents were of guaranteed grade (Wako, Osaka, Japan) and were used without further purification. Four different kinds of papers were used as supports: Toyoroshi No. 51A (pure cellulose paper), Sartorius Membranfilter (acetyl cellulose paper), Whatman DEAE-cellulose paper (DE-81, anion exchange cellulose paper) and Whatman phosphate paper (P-81, cation exchange cellulose paper). All the papers were cut to a size of 1 × 40 cm before use. The supporting buffer solutions were prepared according to Sørensen's method[4]. A 5-$\mu$l volume of a sample solution ($10^{-2}$ *M*) was spotted at a marked position on a support wetted with buffer solution. The support was then immersed in hexane and a constant voltage applied to it for a definite time, keeping the temperature constant.

## RESULTS AND DISCUSSION

The observed migration distances of adenosine nucleoctides along with that of picric acid are shown in Fig. 1, for AC and 51A paper. In previous experiments[5] using ion exchange cellulose papers the migration distances of adenosine nucleotides were shorter on both the anion and cation exchange papers than on pure cellulose paper. However, in the present experiments, adenosine nucleotides and adenine migrate

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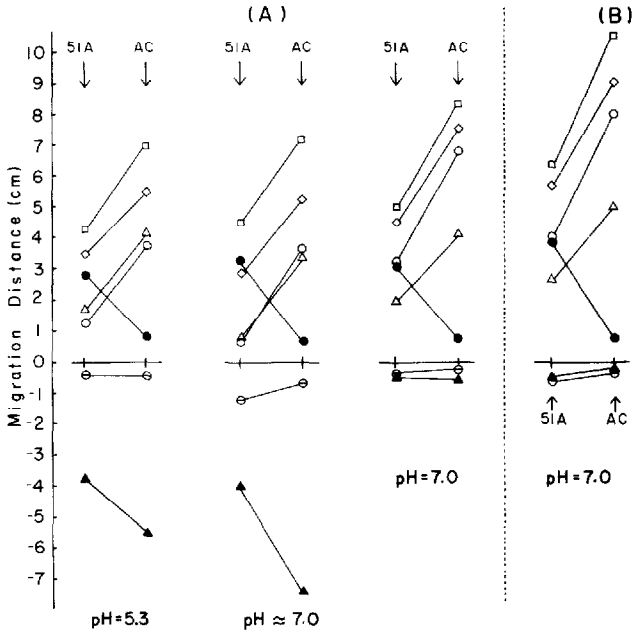


Fig. 1. Observed migration distances of adenine, adenosine and adenosine nucleotides on acetyl cellulose (AC) and pure cellulose (51A) papers. Sample solutions: □, ATP; ◇, ADP; ○, AMP; △, cyclic 3′,5′-AMP; ⊖, adenosine; ▲, adenine; ●, picric acid. Electrophoresis conditions: 1000 V per 30 cm, 30 min; migration temperature, 10°C (A) and 20°C (B), supporting solutions, pH ≈ 7.0, 0.1 *M* NaCl; pH = 5.3 and 7.0, phosphate buffers; spotting position, the centre of the support. Positive movement is towards the anode, negative movement towards the cathode.

much further on AC paper than on 51A paper. This seems to be the main reason why AC paper is now preferred. Surprisingly, the migration distances of picric acid in 0.1 *M* NaCl and phosphate buffer were much shorter on AC paper than on 51A paper. The above trends were found also at 20°C, (see Fig. 1B). Although the migration distances of adenosines on both the AC and 51A papers increased with increasing temperature, that of picric acid did not increase.

The effect of an electroosmotic flow on the migration of picric acid is not considered to differ greatly from AC paper to 51A paper because the movement of glucose on AC paper was similar to that on 51A paper[5]. Additionally, the effect of capillary action will be negligibly small because the sample was spotted at the centre of the support. Thus, it is assumed that the cause of the retardation of picric acid was a chemically specific interaction. As picric acid migrates to the anodic side of the support, it is clear that it behaves as an anion, not as a neutral molecule.

In order to determine the effect of nitro groups, three isomers of dinitrophenol and picric acid were allowed to migrate from different spotting positions and on different supports (Fig. 2). The differences between the migration distances of a given compound from the different spotting positions are due mainly to capillary action, which is towards the centre of a support from both the sides. The movements due to this capillary action were measured by allowing the sample to stand under conditions similar to electrophoresis but in the absence of an applied voltage. The dotted lines

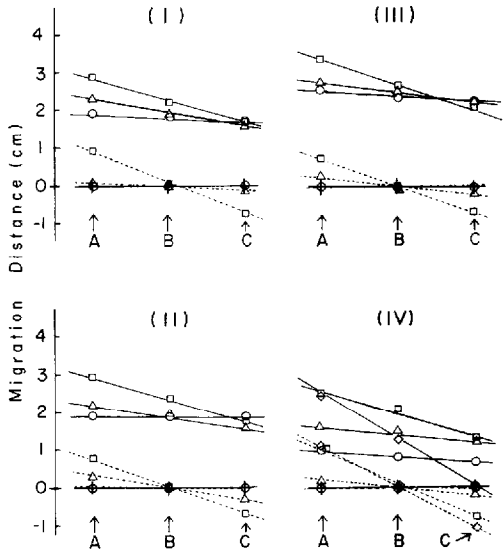


Fig. 2. Observed migration distances of dinitrophenols and picric acid from different spotting positions on various supports. Samples: I, 2,4-dinitrophenol; II, 2,5-dinitrophenol; III, 2,6-dinitrophenol; IV, 2,4,6-trinitrophenol (picric acid). Supports: ○, AC; □, 51A; △, DE-81; ◇, P-81. Spotting positions: A, 5 cm to the cathodic side from the centre of the support; B, the centre of the support; C, 5 cm to the anodic side from the centre. Electrophoresis conditions: 500 V per 30 cm, 30 min, at 25°C.

show the movements due to the capillary action, the slopes being proportional to the strength of the capillary action. The capillary action was found to increase in the order AC < DE-81 < 51A < P-81. In the case of AC paper, the migration distance of a given compound from three different positions did not vary greatly, showing that the effect of the capillary action is small. The effect of electroosmotic flow under these conditions was estimated from previous experiments[6] to be +0.25, −0.5, −0.5 and −1.2 cm for DE-81, AC, 51A and P-81, respectively. Therefore, the effect is small, except for P-81. The signes (+ or −) before the above values indicate the direction of the flow, the positive sign representing movement towards the anode and the negative

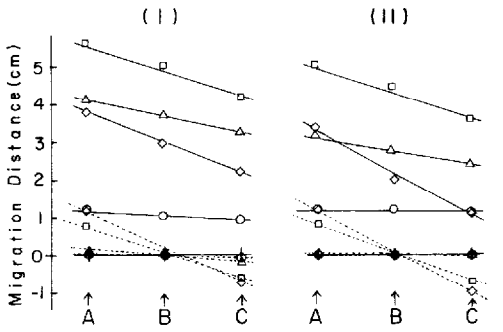


Fig. 3. Observed migration distances of picric acid on acetyl cellulose paper at a higher voltage gradient. Electrophoresis conditions: 1000 V per 30 cm, 30 min, at 25°C. Supporting solutions: I, pH ≈ 7.0, 0.1 *M* NaCl; II, pH = 7.0, phosphate buffer. Supports and spotting positions as in Fig. 2.

sign that towards the cathode. The observed migration distances of dinitrophenols on the different supports exhibited less variation than those of picric acid. In other words, the migration of dinitrophenol is affected by the chemical nature of the support to a lesser extent than that of picric acid, provded that the buffering capacity of the supporting solution is large enough[5]. In the separation of nucleotides, AC paper was superior to other papers, but this is not the case in the separation of nitrophenols.

The interaction between the acetyl groups of AC paper and picric acid was studied by carrying out the electrophoresis at higher voltage gradients and with different supporting solutions. The extremely low mobility of picric acid in AC paper was also observed in this case (see Fig. 3).

It is concluded that the low mobility of picric acid in AC paper is due to a chemically specific but weak interaction between the acetyl groups of AC paper and picric acid.

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