Journal of Chromatography, 355 (1986) 407–411
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 385

Note

Effect of surfactants on electrophoretic zone mobility and its application to the separation of adenosine nucleotides

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(Received December 3rd, 1985)

The migration distance in electrophoresis using a support is affected by the physicochemical nature of the support. One of the main factors is the capillary action from both the electrode cells to the centre of the support[1–3]. This results in different migration distances depending on the spotting position on the support.

In the course of a study on the component of the supporting solution in zone electrophoresis, we found that the migration distances from different positions on a support showed the same values by the use of a supporting solution containing a surfactant. This means that it is not necessary to take care over the spotting position and that it becomes easier to evaluate the zone mobility theoretically. We now describe the effect of the surfactant on the zone mobility of picric acid on various supports and its application to the separation of adenosine nucleotides.

MATERIAL AND METHODS[3]

Sodium dodecyl benzenesulphonate (SDBS), laurylpyridinium chloride (LPC) and sodium dodecyl sulphate (SDS) of guaranteed grade were purchased from Wako Chemicals (Osaka, Japan), adenosine nucleotides from Sigma (St. Louis, MO, U.S.A.). Four different kinds of papers were used as supports: Toyoroshi No. 51A (51A, pure cellulose paper), Sartorius Membranfilter (AC, acetyl-cellulose paper), Whatman DEAE-cellulose paper (DE81, anion-exchange cellulose paper) and Whatman phosphate paper (P81, cation-exchange cellulose paper). All the papers were cut to a size of 40 × 1 cm before use.

A known weight of surfactant was dissolved in 0.1 *M* sodium chloride solution or in a phosphate buffer solution (pH = 7.0, ionic strength = 0.1), prepared by mixing equimolar amounts of disodium hydrogen phosphate (0.2 *M*) and sodium dihydrogen phosphate (0.2 *M*). Solutions with the surfactant or without were used as the supporting solutions. A strip (40 × 1 cm) of a filter-paper was dipped in a supporting solution and the excess of solution on the paper was removed by another paper. A 5-μl volume of a sample solution was spotted and its position marked by a lead pencil. The strips were then dipped in hexane in a migration chamber. A

0021-9673/86/$03.50

constant voltage (1000 V per 30 cm) was applied for 30 min at *ca.* 20°C. The electric current varied with the chemical nature of the support and supporting solution. The position of adenosine nucleotides on the strip was detected by irradiation with UV light (253 nm).

RESULTS AND DISCUSSION

The electrophoretic zone mobility is affected by many factors[4]. In order to evaluate the observed migration distances, it is necessary to know the chemical nature of a supporting solution and the physicochemical nature of the support. We spotted a sample solution at three different positions on various supports and then carried out the electrophoresis.

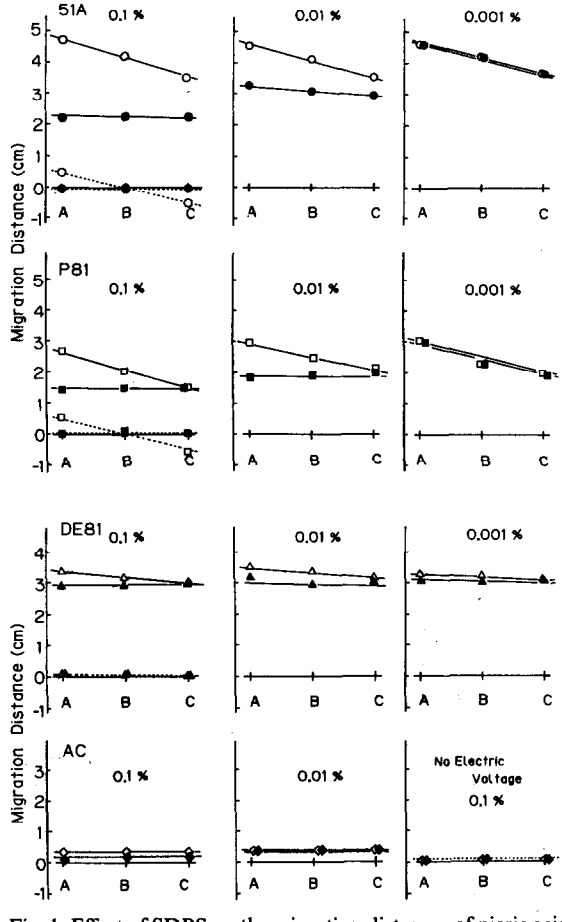

Fig. 1. Effect of SDBS on the migration distances of picric acid from different spotting positions on various supports. Sample: 10^{-3} *M* picric acid. Supporting solutions: 0.1 *M* sodium chloride solution (○, □, △, ◇) and 0.1 *M* sodium chloride containing SDBS (●, ■, ▲, ◆); the percentages of SDBS is shown. Supports: 51A, P81, DE81 and AC. 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: 1000 V per 30 cm, 30 min, 20°C. The dotted lines show the movements due to capillary action.

The observed migration distances of picric acid from three positions on various supports with or without the surfactant (SDBS) are shown in Fig. 1. The dotted lines show the movements observed when the sample was allowed to stand under conditions similar to those of the electrophoresis but in the absence of an applied voltage. When 0.1% (w/w) of SDBS was added to the supporting solution, the movements due to capillary action were almost zero on all supports. The effects of SDBS depended on its concentration and varied with the nature of the support.

Taking 51A paper as an example, when electrophoresis was carried out in neat 0.1 *M* sodium chloride solution as a supporting solution, different migration distances were obtained from different positions where the capillary action is different. The slope of the plots of migration distance *vs.* spotting position is proportional to the strength of the capillary action. Generally, the direction of the capillary action is towards the centre of the support from both sides and usually disappears in 1 h, depending on the physicochemical natures of the supporting solution and the support[5]. Upon addition of 0.1% (w/w) surfactant to the supporting solution, we were surprised to see that the migration distances from three different positions showed the same values irrespective of the spotting position, as shown in Fig. 1. The effect of the surfactant decreased with decreasing concentration. When 0.1% (w/w) surfactant was added to the supporting solution and the support allowed to stand in the migration chamber for 30 min without an electric voltage, no migration of picric acid was observed. It was assumed that there was little capillary action because of the lowering of the surface tension upon adding the surfactant[6].

In a separate experiment[7], when electrophoresis was carried out without dipping the support into hexane, the supporting solution containing 0.1% (w/w) SDBS evaporated more rapidly than usual, again because of the lowering of the surface tension.

In addition to the effect on the capillary action, the fact that all the migration distances of picric acid in 0.1% (w/w) SDBS were less than in the absence of SDBS

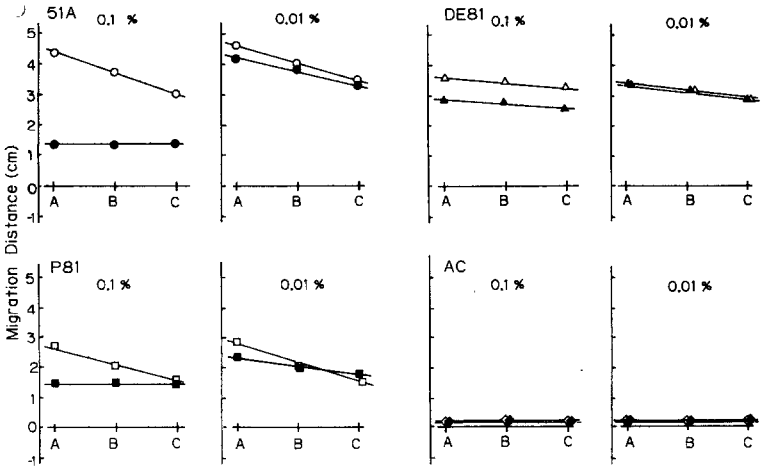

Fig. 2. Effect of SDS on the migration distances of picric acid from different spotting positions on various supports. Electrophoresis conditions and symbols as in Fig. 1. SDS was substituted for SDBS.

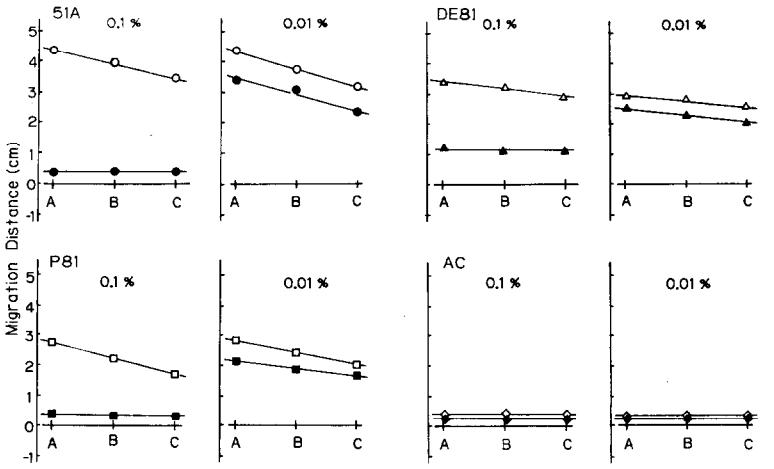

Fig. 3. Effect of LPC on the migration distances of picric acid from different spotting positions on various supports. Electrophoresis conditions and symbols as in Fig. 1. LPC was substituted for SDBS.

means that there is an interaction between picric acid and SDBS. Thus, adding an excess of SDBS to the supporting solution is not profitable.

Previously, we found[8] that the capillary action increases in the order AC $\approx$ DE81 < 51A < P81. This order is also true of the effect of SDBS; the effect of SDBS was large on P81 and 51A papers, but slight on AC and DE papers.

The effects of SDS and LPC were similar, as shown in Figs. 2 and 3, respectively; at 0.1% (w/w) concentration, LPC is the most effective and SDBS and SDS

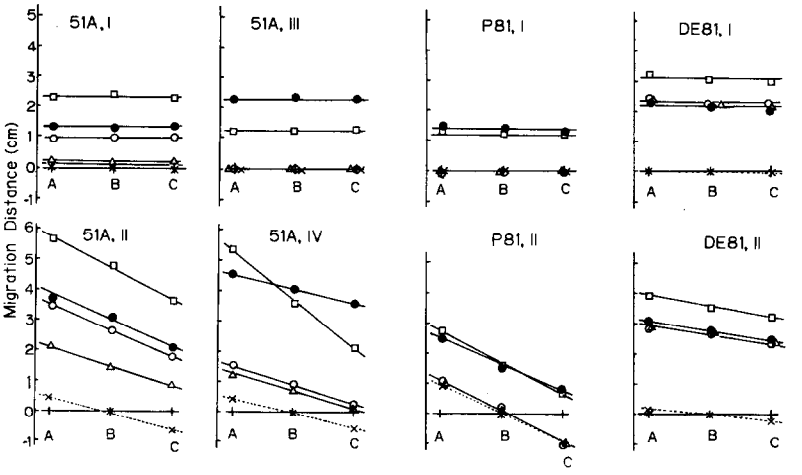

Fig. 4. Separation of adenosine nucleotides in a supporting solution containing SDBS on various supports. Sample: 5 μl of $5 \cdot 10^{-3}$ *M* solution; O—O, AMP; △—△, cAMP; □—□, ATP; ●—●, picric acid; × · · · ×, the movement of picric acid due to capillary action. Supports: 51A, P81, DE81, AC. Supporting solutions: phosphate buffer (pH = 7.0, ionic strength = 0.1) + 0.1% SDBS (I); phosphate buffer (II); 0.1 *M* sodium chloride + 0.1% SDBS (III); 0.1 *M* sodium chloride (IV). Electrophoresis conditions: 1000 V per 30 cm, 30 min, 20°C.

are equivalent. The effects of the chemical structure or charge of the surfactants remain unknown. From these results, we recommend the addition of *ca.* 0.1% (w/w) surfactant to the supporting solution.

A supporting solution containing a surfactant was then applied to the separation of adenosine nucleotides. Fig. 4 shows the observed migration distances of adenosine nucleotides and picric acid in 0.1% (w/w) SDBS–phosphate buffer together with those in phosphate buffers. In the experiments described in the previous section, we used 0.1 *M* sodium chloride solution as the supporting solution. However, for greater reproducibility of experimental results, it is desirable to use a solution of high buffering capacity as a supporting solution. In the supporting solution containing SDBS, the migration distances of the nucleotides showed the same values irrespective of the spotting positions, as expected. A good separation of adenosine 5′-monophosphate (AMP) from adenosine cyclic-3′,5′-monophosphate (cAMP) was achieved only on 51A paper in phosphate buffer, but not in 0.1 *M* sodium chloride solution. In the case of P81 or DE81, AMP and cAMP were not separated. Probably, the ionic moieties of the support affected the movement of the anions of the nucleotides. The fact that all the migration distances of the nucleotides in SDBS solutions were shorter than those solutions without surfactant suggests the occurrence of an interaction between the nucleotides and SDBS.

It is concluded that the presence of a surfactant in a supporting solution markedly affects the capillary action of a support and interacts with the sample to an extent dependent on the chemical nature of the latter.

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