H₃O⁺ Used as Terminating Ion in Isotachophoresis

ANDERS VESTERMARK

Department of Biochemistry, University of Stockholm, S-113 82 Stockholm, Sweden

In 1908 W. Lash Miller discussed the moving boundary method for determination of transport numbers.[1] Miller stressed that to ensure a sharp meniscus in such experiments the direction of the field had to be chosen so that the "specifically slower" ion species moved behind the "specifically faster species". A stable meniscus between water solutions of acetic acid and sodium acetate could according to Miller be expected if the positive current was arranged to move from the acetic acid to the sodium acetate. This idea was confirmed by experiments carried out by Dawson and Graham in Miller's laboratory. The net mobility [2] of the hydrogen ion was evidently under the conditions used lower than that of the sodium ion.

The aim of the work presented here has been to demonstrate that the hydrogen ion can be used as terminating ion in isotachophoretic experiments and that the net mobility of the hydrogen ion can [be changed by employing different counterions or mixtures of counterions.

Experimental. One series of experiments was performed in sucrose gradients (from 10 to 50 % sucrose) in vertical U-tubes, 0.9 cm i.d.[3] 0.1 M solutions of acetic acid, formic acid, monochloroacetic acid or trichloroacetic acid were used as terminating electrolytes. 0.1 M solutions (pH about 7) of the potassium salts of the acids were used as leading electrolytes. In some experiments, mixtures of acetic acid and trichloroacetic acid were used as terminating electrolytes and mixtures of the corresponding potassium salts as leading electrolytes. One ml of a 0.5 M pyridinium salt solution of pH 5.2 was used as sample. The counterion in the pyridinium salt solution was the same as that in the leading electrolyte.

The voltage was adjusted to give a downward movement of the pyridinium zone of about 15 cm in 15 to 20 h. The pH and UV-absorption (256 nm) in the eluate of the contents of the anodic part of the U-tube was determined. The presence of potassium was determined by the flame reaction.

For another series of experiments a cooled glass plate apparatus of the Crestfield and Allen type was employed.[4] Cellulose acetate strips 1 × 20 cm (Machery-Nagel & Co., Düren, Germany) were used as anticonvectional medium. The strips moistened with the leading electrolyte were placed with one end in contact with the wick of the leading electrolyte. A piece of filterpaper moistened with the sample was placed in contact with the other end of the strip and the wick of the terminating electrolyte. In some experiments 0.5 M solutions of copper, cobalt or ferric sulphate were employed as samples. In other experiments histidine or glycine acted as samples together with small amounts of a mixture of malachite green and toluyleneblue. The two dyes contained impurities which indicated the limits of the amino acid zones developed during the experiments.

The isotachophoretic experiments on strips were run at a tension of 600 to 1000 V and lasted for 1 h.

Results. In the density gradient experiments with acetic, formic and monochloroacetic acids a pH shelf developed between pH 4 and 5 (Fig. 1). The position of a UV
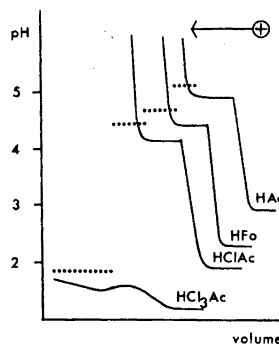

Fig. 1. pH determinations in experiments with acids as terminating and their corresponding potassium salts as leading electrolytes. The arrow indicates the direction of the positive current. The dotted line denotes presence of potassium. Well collected UV-absorbing zones, coinciding with the pH shelves were found in the experiments with acetic (HAc), formic (HFo) and monochloroacetic (HClAc) acids. To make the figure simpler the UV-zones have been left out. They were all of the same shape as the one shown in the experiment with 100 % HAc in Fig. 2. In the experiment with trichloroacetic acid (HCl₃Ac) no pH shelf developed.

absorbing zone coincided with the shelf. In front of this zone (to the left in Fig. 1) potassium ions were found. In the main part of the potassium-region (outside Fig. 2) pH reached a level of 7 or higher. Behind the shelf pH was low. These findings indicate that the order of the net mobilities of the positive ions in the experiments was potassium > pyridinium > hydrogen. When trichloroacetic acid was employed, no pH shelf developed (Fig. 1). During the experiment, H_3O^+ ions moved from the terminating solution through the pyridinium zone into the leading electrolyte. The resulting pH in the now zone-electrophoretically moving pyridinium zone was $3-4$ units below the pK_a value, 5.2, of the pyridinium ion. No pH shelf could be expected. The order of the net mobilities of the positive ions was obviously in this experiment hydrogen > potassium > pyridinium.

In all the experiments with mixtures of acetic and trichloroacetic acids the front boundary was sharp (Fig. 2). Also the rear boundary was sharp in the experiments with mixtures containing 70 % acetic acid or more. At lower concentrations of acetic acid a sharp boundary did not develop on the terminating side of the pyridinium zone.

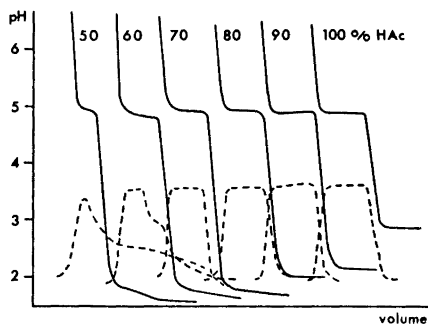The net mobility of the hydrogen ion and the pyridinium ion increases when the

Fig. 2. pH determinations in experiments with mixtures of acetic and trichloroacetic acids as terminating and mixtures of the corresponding potassium salts as leading electrolytes. The broken line indicates the absorption at 256 nm (arbitrary units). The leading boundary of the pyridinium zone was sharp in all experiments. Potassium ions were present in about the same region as in the corresponding experiment in Fig. 1. In the experiments with 50 and 60 % acetic acid a sharp rear boundary did not develop.

content of the stronger acid in the terminating acid mixture increases. The difference in net mobility between the pyridinium ion and the hydrogen ion decreases. This difference has evidently in the experiments with 50 or 60 % acetic acid been too small to produce a sharp boundary between the pyridinium and hydrogen ions. For the development of such a boundary a longer migration distance would have been necessary.

The experiments in Fig. 1 illustrate that the net mobility of the hydrogen ion can be changed stepwise by changing the counter ion species. The experiments in Fig. 2 indicate that the net mobility of the hydrogen ion can be modified in a continuous manner by changing the ratio of the components in the counter ion mixture.

In the experiments on cellulose acetate strips cobalt, copper, and iron formed nice zones when acetic acid was used as terminating solution. In one experiment a mixture of the three metal ions produced three consecutive zones, each one centimeter long, with no observable overlapping. Histidine also formed a zone in the acetic acid system. Glycine could not be shown to move isotachophoretically under these conditions. The ferric ions moved immediately behind the hydrogen ion front in the formic acid system in a non-isotachophoretic manner. The other four ions developed isotachophoretically moving zones in this system as well as in the monochloroacetic acid system. The two amino acids gave when run together two distinct zones, the glycine zone moving behind that of histidine. None of the five ions tested developed distinct zones when trichloroacetic acid was used as terminator.

By the experiments on cellulose acetate it has been demonstrated that the hydrogen ion can be used as terminating ion in isotachophoretic systems for the separation of metal ions. The fact that the pH is low in the terminating electrolyte is of special interest when the isotachophoretic procedure is used for extraction [5] of say metal ions from biological materials. The low pH counteracts binding of metals to carboxyl groups in the biological material.

H_3O^+ acts not only as a terminating ion, the mobility of which can be easily controlled, but also as a source of positive charges for amino acids and other amines. Protons seem to be ideal terminating ions for the isotachophoretic separation of amino acids.