Some Physico-chemical Characteristics that make D_2O-based Buffer Solutions Useful Media for Capillary Electrophoresis

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Capillary electrophoresis (CE) carried out in deuterium oxide based buffers can give much improvement and flexibility in analysis owing to a lowering in Joule heating and an increase in the viscosity within the capillary; moreover, as most acids are weaker in deuterium oxide than in water, pK_a differences can result in enhanced resolution.

Capillary electrophoresis (CE) is rapidly becoming a valuable technique for the analysis of a variety of polar molecules.[1–3] To date, CE separations have been largely carried out in H_2O-based buffer solutions covering a wide pH range. We recently introduced[4,5] D_2O-based buffers as a useful alternative or complement to H_2O-based measurements. In many cases, a marked improvement in analysis was found and this was thought to be due to a lowering of electroosmotic flow in heavy-water based buffers. In the present study we have carried out a detailed physico-chemical analysis of this isotope effect and report differences in current, viscosity and dissociation of substrates that have been observed in the two buffer media and that can lead to enhanced resolution.

Capillary electrophoresis measurements were performed in a fused silica tubing (50 μm i.d. and 570 mm in total length) using a Beckman P/ACE system. Phosphate buffers were used over the whole pH or pD range. Acidity in heavy water was measured by a conventional pH meter and pD values were calculated by adding 0.4 units to the meter reading.[6]

To compare the flow of current in H_2O and D_2O media, solutions containing two different amounts of total buffer (50 and 150 mmol dm^{-3}) and adjusted to an appropriate pH or pD value were prepared. For all the measurements of the variation of current with acidity, the voltage was kept at 20 kV. The dependence of current on pH and pD is shown in Fig. 1. At low pH the increase in current is proportional to the proton concentration in solution, whereas at pH or pD values above 6 the increase in current is related to the increase in the free base of the phosphate buffer. Although the shapes of the current/pH or pD curves are similar, the current in D_2O solution is significantly lower than that in H_2O. This means that Joule heating is lower in heavy-water-based solutions,

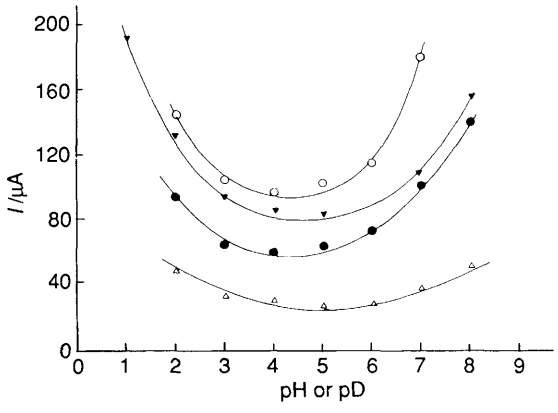

Fig. 1 Measurement of the variation of current with pH or pD in H_2O- and D_2O-based phosphate buffers, ○, 150 mmol dm^{-3}, 20 kV, H_2O; ▼ D_2O; ● 50 mmol dm^{-3}, 20 kV, H_2O; △ D_2O

resulting in a lower temperature difference between the centre and the wall of the capillary. Lower Joule heating is desirable for CE separations because if radial diffusion of solutes is slowed down, band broadening is diminished. These measurements have shown that the use of D_2O in CE provides the ability to carry out studies over a wide acidity range and more flexibility in voltages and total buffer concentration.

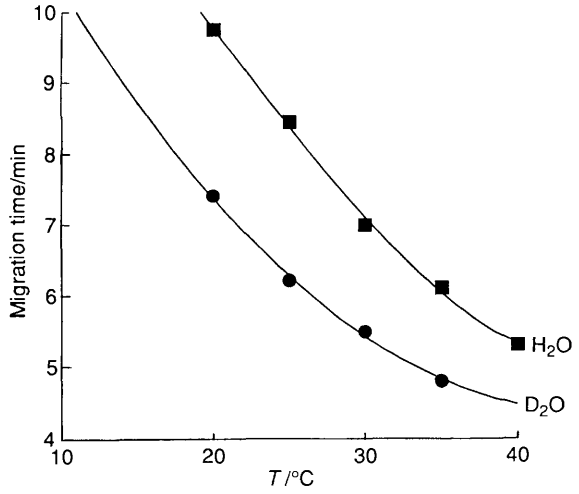

Fig. 2 Variation of migration time of mesityl oxide with temperature at a pH = pD = 5.98. (CE conditions: buffer, 150 mmol dm^{-3} sodium phosphate; separation voltage, 20 kV; separation length, 50 mm; total length of capillary, 570 mm; i.d. of capillary, 50 μm; absorbance at 230 nm).

A lower current in D_2O is also related to its viscosity being *ca.* 25% higher than that of H_2O. An increase in viscosity results in a lowering of electroosmotic flow, desirable in CE separations. To monitor the variation of viscosity with temperature we followed the migration of an uncharged species, mesityl oxide, in the range 20 to 35 °C. Using viscosity data from the literature[7] for H_2O and D_2O we found that viscosity was proportional to the migration time of mesityl oxide, which travels only under the influence of electroosmosis. These measurements make the assumption that the presence of phosphate buffer (150 mmol dm^{-3}) does not produce a significant change in the viscosity of either light or heavy water alone. The relationship of migration time and temperature is shown in Fig. 2. The shape of the curves in H_2O and D_2O is similar to those reported by Korson[8] and by Powell,[9] respectively, for the variation of viscosity with temperature for the two solvents. The bigger viscosity difference at a lower temperature is consistent with the increased structure of D_2O. The control of electroosmotic flow in heavy water is beneficial as this can lead to superior separations without the use of additives.[10]

The pK_a values of the silanol groups on the inside of the capillary, and of substrates are higher in D_2O than in H_2O solution. The changes in pK_a increase with an increase of the pK_a of substrates in water.[11] Assuming the pK_a of the silanol groups is of a similar magnitude as that of silicic acid[12] (H_4SiO_4) this will increase from *ca.* 9.8 in water to *ca.* 10.5 in D_2O. Such a decrease in ionisation is expected to lead to an increase in 'capping' of the silanol groups in D_2O-based buffer solutions (below a pH of *ca.* 10) as compared to H_2O solutions of the same acidity. This phenomenon is related to a decrease in electroosmotic flow observed in D_2O solution.

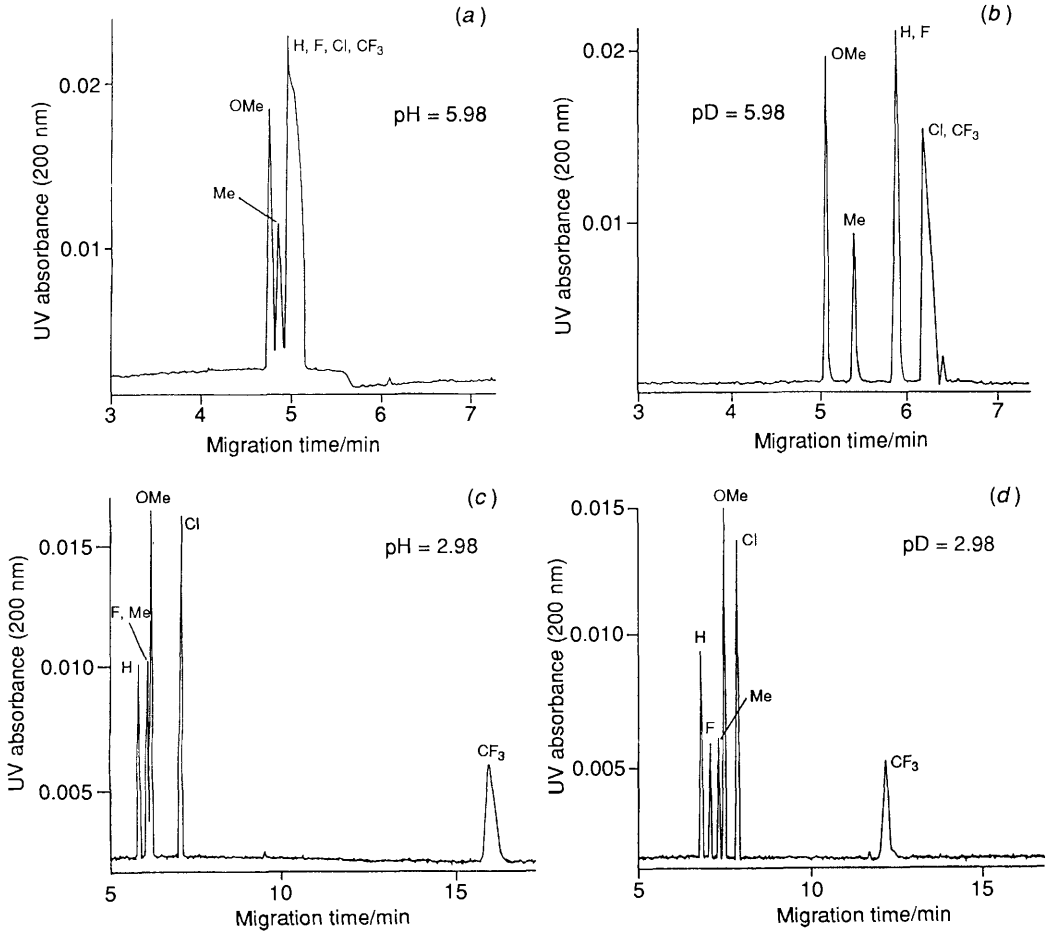

Fig. 3 CE separation of six aniline derivatives (p-$RC_6H_4NH_2$) at (*a*) pH = 5.98; (*b*) pD = 5.98; (*c*) pH = 2.98 and (*d*) pD = 2.98. (Separation conditions: buffer, 150 mmol dm^{-3} sodium phosphate; separation voltage, 20 kV; separation length, 50 mm; total length of capillary, 50 mm; i.d. of capillary 50 μm; temperature, 35 °C; absorbance at 200 nm).

Table 1 Dissociation constants and migration times for p-$RC_6H_4NH_2$ at pH = pD = 2.98

R	Dissociation constant in H_2O (pK_a)	Migration time/min (at pH = 2.98)	Migration time/min (at pD 2.98)
H	4.63	5.64	6.78
F	4.65	6.08	7.04
Me	5.08	6.08	7.28
OMe	5.34	6.18	7.45
Cl	3.99	7.08	7.83
CF_3	2.60	15.99	12.20

Differences in the pK_a values of substrates due to an isotope effect can also lead to enhanced efficiency and resolution. We have demonstrated this effect by analysing the CE separation of six *para*-substituted anilines (p-$RC_6H_4NH_2$) of pK_a values ranging from *ca.* 2.5 to 5.5 in water. CE measurements were carried out at a pH or pD equal to 2.98 and 5.98. Electropherograms are shown in Fig. 3(*a*) to (*d*). At a pH = 5.98, only anilines containing a methoxy- and methyl-substituent are partially ionised whereas the remainder are present in the neutral form. This is consistent with the separation pattern shown in Fig. 3(*a*). Raising the pK_a values by *ca.* 0.5 units in D_2O solution gives a markedly different picture at pD = 5.98. Four peaks are now observed. The chloro- and fluoro-substituted anilines have a low pK_a (<5.0) in H_2O so that increasing this by 0.5 units in D_2O does not lead to an appreciable increase in the concentration of the protonated species for these molecules at pH = 5.98. Thus, unlike the remaining four anilines, trifluoromethylaniline and chloroaniline have not been pulled away from the electroosmotic front [Fig. 3(*b*)].

The electropherograms shown in Fig. 3(*c*) and (*d*) compare the migration behaviour of the above anilines at a pH or pD = 2.98. At these acidic pHs a better resolution of the six anilines is obtained than at pH or pD = 5.98. In H_2O solution at this pH trifluoromethylaniline (pK_a = 2.57) exists predominantly as the free base (~72%). The rest of the aniline derivatives with (pK_a > 2.98) are largely protonated (Cl ≃ 88, F = H = 97, Me = OMe ~ 100%). The acid strength of the anilinium cations and the respective migration times in H_2O and D_2O at pH = pD = 2.98 are shown in Table 1. A plot of the data gives an inverted 'bell-shape' curve (Fig. not shown), which indicates that two mechanisms may be operative: separation and the order of migration of the methyl- and methoxy-anilines is related to the electron-donating ability of the substituents in these molecules and consequent slight changes in the positive nature of the respective anilinium cations. However, the percentage dissociation of the remaining anilinium cations plays a major role in their electrophoretic behaviour.

The six aniline derivatives were finally completely resolved at pH = 2.98. Remarkably, the trifluoromethyl derivative moves faster in D_2O-based buffer. This compound with a pK_a *ca.* 3 in D_2O solution is expected to be more ionised than in H_2O of the same acidity. The concentration of the anilinium cations for the other compounds in D_2O-based buffer is *ca.* 100% so that these compounds separate owing to slight differences in the positive nature of these ions. The delay in the mobility of these compounds in D_2O is caused by a 15.5% lowering of electroosmotic flow in this solvent.

In conclusion, we have shown that replacement of H_2O- by D_2O-based buffers can be beneficial in that it can provide more flexibility in the application of CE owing to a number of interrelated physico-chemical properties such as lowering in Joule heating and viscosity and slight changes in the separation mechanism of substrates.

Received, 4th April 1991; Com. 1/01588E

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