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Temperature dependence of acidity constants, a tool to affect separation selectivity in capillary electrophoresis

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Abstract

The mathematical models of migration and dispersion in capillary zone electrophoresis of small molecules form a sound basis for separation strategies of complex mixtures. It turned out that the key property is the effective mobility of the sample ions. To tune resolution parameters such as pH, complexation constants and ionic strength are widely used; temperature however is not although mobilities and pK_a values depend in a more or less degree on temperature. From the temperature dependences of pK_a values of a number of compounds listed in the literature a general rule can be derived: for carboxylic and inorganic acids d pK_a/dT values are very small and the pK_a values change less than ±0.05 units/10 K. Thermodynamically speaking, these compounds exhibit dissociation enthalpies close to zero. Phenols and amines, on the other hand, have systematically larger d pK_a/dT values of about −0.1 to −0.2 units per 10 K (the results of dissociation enthalpies of 20–70 kJ/mole). Based on this classification, a distinction can be made between different situations in capillary electrophoresis: (i) selectivity changes with temperature are largely due to the temperature dependence of the pK_a of the buffering compound in the background electrolyte, (ii) selectivity changes mainly result from the temperature dependence of the pK_a of the sample ions, and (iii) temperature effects on the pK_a values of both, sample and buffer play a role. This work demonstrates such effects on selectivity in capillary electrophoresis highlighting the fact that in some instances temperature can be used to fine-tune separations.

Keywords: Ionisation constant; pK_a value; Acidity; Effective mobility; Degree of ionisation; Temperature; Capillary electrophoresis; Dissociation enthalpy

1. Introduction

Temperature is an important working parameter in capillary electrophoresis (CE). It has an influence on both separation efficiency and selectivity (see e.g. recent reviews [1,2]). It affects the peak dispersion by the occurrence of a parabolic temperature profile in the capillary lumen. However, its contribution to the total plate number is most often overestimated under the normal experimental conditions applied in CE (low conductance of the background electrolyte, capillary inner diameter smaller than 100 μm) [3]. This does not mean that the temperature increase due to Joule heating can be neglected; it can in contrary even reach the boiling point of the liquid, especially when there is natural convection instead of enforced heat transport from the capillary. Although advanced instrumentation uses circulating fluids for temperature control, a part of the capillary always remains outside of the thermostated region and here temperature deviates.

In water the absolute ionic mobility – that at zero ionic strength – has a strong dependence on the temperature [2]. It changes roughly inversely proportional to the solvent viscosity, η, i.e. it increases by about 2–2.5% per degree (in organic solvents the influence is lower). This increase is approximately linear over a temperature range of several ten degrees and can be expressed over a wider temperature range by inclusion of a quadratic term [3]. The change in temperature can either be by intention (by selecting the according thermostating temperature of the instrument), or it takes place unintentionally by the above mentioned non-controlled temperature conditions in some sections of the capillary. It should be noted that the actual mobility, μ_{act} – the mobility of the fully charged ion at finite

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ionic strength of the solution during separation – depends on the temperature as well, on the one hand due to the change in absolute mobility, on the other hand due to the dependence of the Debye–Hückel parameters on $T^{-3/2}$ and $T^{-1/2}$, respectively, and implicitly on η^{-1}.

In case that the analytes are weak acids or bases the most commonly known parameter determining the effective mobility (the mobility of the partially charged ion) is certainly the pH of the background electrolyte (BGE), which is expressed for a certain buffer consisting of a weak acid HA and its salt A^- with activity, a, by the common relation

$$\mathrm{pH} = \mathrm{p}K_{\mathrm{a,HA}} + \log\left(\frac{a_{\mathrm{A}^-}}{a_{\mathrm{HA}}}\right) \quad (1)$$

For a monobasic analyte this dependence can be expressed by

$$\mu_{\mathrm{eff},i} = \alpha\mu_{\mathrm{act},i} = \frac{\mu_{\mathrm{act},i}}{1 + 10^{\mathrm{p}K_{\mathrm{a},i} - \mathrm{pH}}} \quad (2)$$

It can be seen that the effective mobility of the sample ion, i, is a function of its actual mobility, its $\mathrm{p}K_{\mathrm{a},i}$ value and the pH of the BGE, which in turn is determined by its own $\mathrm{p}K_{\mathrm{a,BGE}}$. The interpretation of Eqs. (1) and (2) leads to the conclusion that there might be an additional effect of the temperature on the effective mobility, beside that on the actual mobility: given that the $\mathrm{p}K_{\mathrm{a}}$ values of the samples and the BGE are temperature dependent, the change of this variable might influence the effective mobility and thus the separation selectivity. The clarification of this aspect is the topic of the present paper.

2. Experimental

CE was carried out with a Beckman P/ACE 5500 instrument with circulating liquid thermostating, equipped with a UV absorbance detector set at 254 nm. Electrophoresis was carried out in fused silica capillaries (203/269 mm length, 75 μm i.d.), coated to suppress the electroosmotic flow according to the procedure described by Chiari et al. [4]. Separation voltage was −20 kV. Chemicals for the BGE and samples were purchased from E. Merck (Darmstadt, Germany). The BGE solutions were prepared on a mass basis (NaH_2PO_4 and Na_2HPO_4 for phosphate buffer, histidine and histidine·HCl for histidine buffer), using literature data for $\mathrm{p}K_{\mathrm{a}}$ values at 25 °C, and subsequently measured at that temperature using a calibrated pH meter. The sample solution in water had a concentration of approximately 0.001 mole/L. Injection was by pressure at 35 mbar for 1 s.

3. Results and discussion

For many substances data on the temperature dependence of the $\mathrm{p}K_{\mathrm{a}}$ values are available in the literature [5–7]. It is remarkable that they mirror a chemical classification of the compounds. Whereas the dissociation of carboxylic and inorganic acidic groups acids have $\mathrm{d}\mathrm{p}K_{\mathrm{a}}/\mathrm{d}T$ values close to (and around) zero, these values are between −0.007 and −0.012 for phenols and secondary amines. An even stronger dependence is seen for primary amines with $\mathrm{d}\mathrm{p}K_{\mathrm{a}}/\mathrm{d}T$ values of −0.012 to −0.022. Note that the $\mathrm{p}K_{\mathrm{a}}$ values decrease with increasing temperature for these compounds, e.g. by nearly one logarithmic unit for a temperature difference of 40 °C. It has to be pointed out that the substances can either be BGE constituents, or analytes.

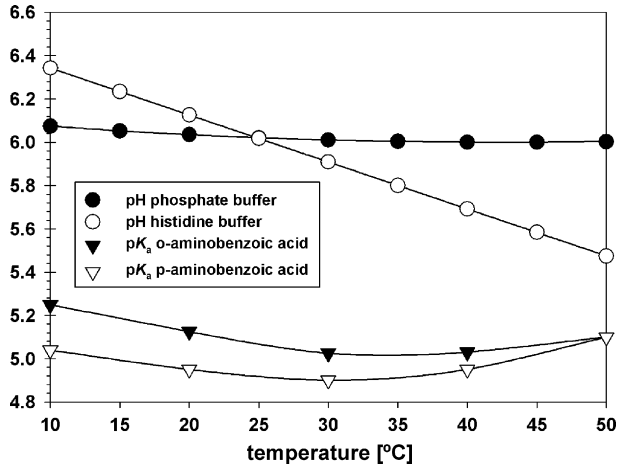

Fig. 1. Temperature dependence of the pH of histidine buffer, the pH of phosphate buffer, the $\mathrm{p}K_{\mathrm{a}}$ of *o*-aminobenzoic acid and the $\mathrm{p}K_{\mathrm{a}}$ of *p*-aminobenzoic acid.

If the temperature of a BGE changes, and its $\mathrm{p}K_{\mathrm{a}}$ is temperature dependent, then the pH (Eq. (1)) becomes also a function of the temperature: for solutions in the "safe" pH range it follows that $\mathrm{d}\mathrm{pH}/\mathrm{d}T = \mathrm{d}\mathrm{p}K_{\mathrm{a}}/\mathrm{d}T$. This means that the temperature change might influence the effective mobility of the sample via its degree of ionisation (Eq. (2)). If, on the other hand, the $\mathrm{p}K_{\mathrm{a}}$ of the sample compound is dependent on the temperature, even at constant pH of the BGE a change in T can influence the effective mobility of the analyte. Finally, if both $\mathrm{d}\mathrm{p}K_{\mathrm{a}}/\mathrm{d}T$ values, that of the analyte and of the BGE are similar, no significant change of the degree of ionisation should take place. It is clear that an effect can be expected only when the $\mathrm{p}K_{\mathrm{a}}$ value of the analyte and the pH of the BGE are similar. As the BGE buffers only in the region of its $\mathrm{p}K_{\mathrm{a}}$, those cases are of interest in which the $\mathrm{p}K_{\mathrm{a}}$ of the BGE is sufficiently close to that of the analyte. We consider in the following two combinations of BGE and analyte according to the properties of their $\mathrm{p}K_{\mathrm{a}}$ values as function of T, and illustrate these cases by typical examples.

As BGEs either phosphate or histidine buffers were selected, both with the same pH (6.02) and ionic strength (20 mmol/L). As samples *o*-aminobenzoic acid ($\mathrm{p}K_{\mathrm{a}} = 5.08$) and *p*-aminobenzoic acid ($\mathrm{p}K_{\mathrm{a}} = 4.93$) were chosen. These $\mathrm{p}K_{\mathrm{a}}$ values correspond to the dissociation of the COOH groups, being both about one unit below the pH of the BGE (data are at 25 °C). They are thus ionised by about 90% at 25 °C. Phosphate has a very small $\mathrm{d}\mathrm{p}K_{\mathrm{a}}/\mathrm{d}T$ value, that of histidine is −0.0217. Their $\mathrm{p}K_{\mathrm{a}}$ versus T plots are shown in Fig. 1. It can be seen that the $\mathrm{p}K_{\mathrm{a}}$ of phosphate decreases only marginally between 10 and 50 °C, whereas the $\mathrm{p}K_{\mathrm{a}}$ of histidine decreases from 6.0 to 5.5 in this T-range, with the according change of the pH of the solution when these compounds are used as buffering BGEs. The literature data of the $\mathrm{p}K_{\mathrm{a}}$ of the analytes are shown in the same Fig. 1 as function of T.

Based on these data we can predict the following effect of the temperature on the electrophoretic behaviour of the analytes. Phosphate as BGE will keep an about constant pH, and

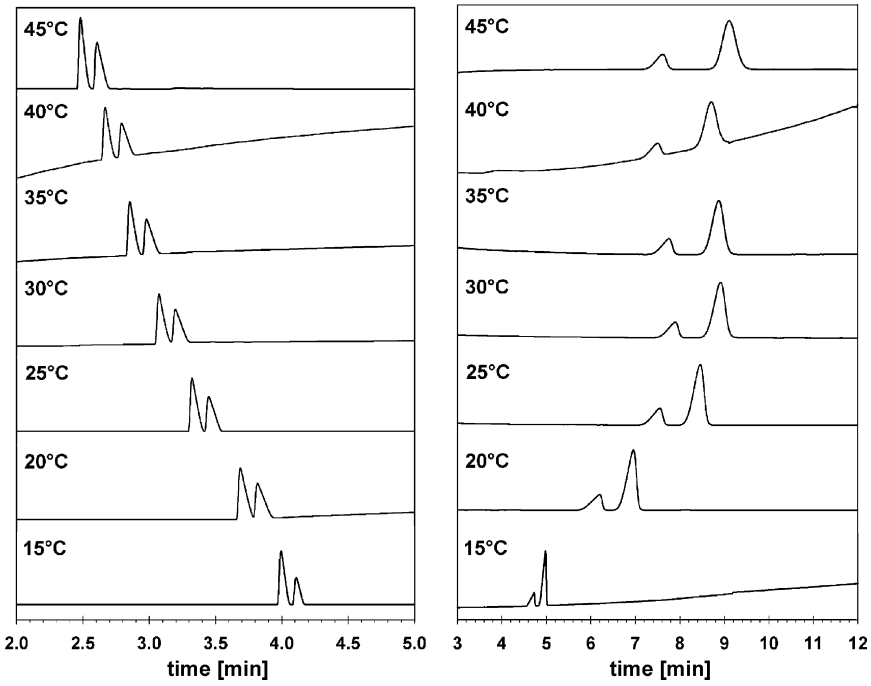

Fig. 2. Separation of *p*-aminobenzoic (left peak) and *o*-aminobenzoic acid in phosphate BGE (left figure) and histidine BGE (right figure) at different temperatures. pH of both BGEs at 25 °C: 6.02, ionic strength 20 mmol/L. For experimental details, see text.

because of the only slight change of the analytes, pK_a values their separation will not be significantly influenced. Agreement with the forecasted result can indeed be observed from the measured electropherograms obtained between 15 and 45 °C shown in Fig. 2(left). Obviously the migration is faster at higher temperature due to the increase in the actual mobilities, but the analytes are just baseline separated at all temperatures.

Histidine buffer decreases its pK_a when the temperature rises, and thus the pH of the BGE is shifted closer to the pK_a of the analytes. The degree of ionisation is thus reduced, and the effective mobilities become smaller. As the *o*-aminobenzoic acid has a 0.15 units higher pK_a value than the *p*-isomer, it is stronger affected by this shift, and its effective mobility should decrease more markedly. This is what can be seen from the measured electropherograms (Fig. 2(right)). All effective mobilities decrease (although the solvent viscosity decreases), and the *o*-isomer is progressively more separated from the *p*-isomer.

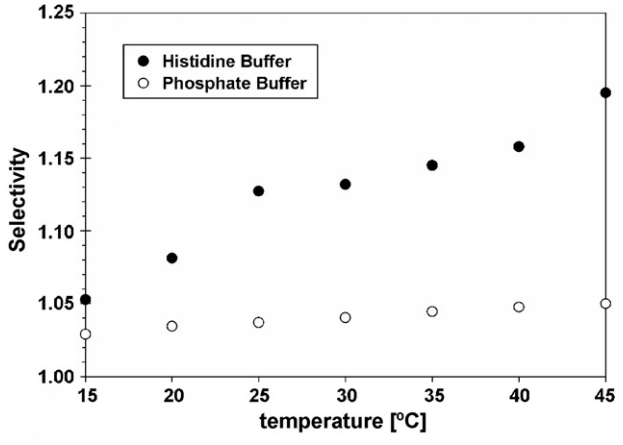

Fig. 3. Selectivity (expressed as ratio of effective mobility) as a function of operating temperature in phosphate BGE (squares) and histidine BGE (circles). Data calculated from the electropherograms depicted in Fig. 2.

This change in migration behaviour can be depicted even more pronouncedly by regarding the separation selectivity of the analyte pair, defined here as the ratio of the mobilities. It is shown for the two BGEs in Fig. 3. Whereas in the phosphate buffer it changes between 15 and 45 °C only from 1.03 to 1.05, a remarkable increase from 1.05 to 1.20 is observed in the BGE consisting of histidine. This variation is clearly related to the different change of the pH with the temperature of the BGE.

4. Conclusions

pK_a data as function of the temperature are available in the literature for many weak acids. Note that for a base, B, the pK_a corresponds to the dissociation of the conjugated cation acid, HB^+. It is observed that different classes of these acids exhibit a different dependence of the pK_a values on temperature. The dpK_a/dT values are larger for amines (better said for their corresponding ammonium compounds) and phenols than for carboxylic acids; the dpK_a/dT values of the latter class tend to zero. Primary amines exhibit a larger dpK_a/dT than secondary amines. Of practical interest is that phosphate (at pK_a = 7.23) does not shows a significant T-dependence, in contrast to, e.g. boric acid, which has a dpK_a/dT of −0.01.

Temperature changes could result in a different migration behaviour of the analytes when the dpK_a/dT values of the buffer and analyte differ significantly. On the other hand, in case that the dpK_a/dT values of both, analytes and buffer are similar, selectivity might remain uninfluenced by the temperature. It is a trivial prerequisite that the pH of the BGE and the pK_a values of the sample have to be close enough.

It is clear that the most common tool to adjust the effective mobilities by the pH is the composition of the BGE. However, the role of the temperature and its influence on the acid–base equilibria should not be overlooked. This means that it is important in practice to be attentive (i) to a well defined and documented operating temperature, (ii) to the adjustment of the pH of the BGE just at the operating temperature (especially when amines or boric acid are used as buffers), (iii) to consider the possible influence of the non-thermostated parts of the capillary on the separation and its reproducibility, and finally, (iv) to take into consideration the use of potential $pK_a(T)$ effects for difficult separations, to name a few.

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