

Three different procedures are used to measure the pH of hydroorganic mobile phases in HPLC. A typical one consists on calibrating the electrode systems with commercial aqueous standard buffers, and then measuring the pH of the aqueous buffer before mixing it with the organic modifier. This way the pH value is obtained in the  ${}^w\text{pH}$  scale (19). In our opinion this is not the best option because the pH of the solution changes after dilution of the aqueous buffer with the organic modifier. If the electrode system is calibrated with standard buffers prepared in the same solvent composition used as mobile phase and the pH is measured in this particular mobile phase composition, the  ${}^s\text{pH}$  value is obtained. Working in the  ${}^s\text{pH}$  scale requires a careful preparation and maintenance of the standard buffers and electrodes, and often these standards are not commercially available. Finally, when pH is measured in the hydroorganic mixture, but the electrode system is calibrated with aqueous buffers, the  ${}^s\text{pH}$  values are obtained. Notice that here the IUPAC nomenclature has been used: the left hand superscript indicates the medium where the quantity is measured ( $w$  for water and  $s$  for hydroorganic mixture), and the subscript indicates the standard state medium (i.e. the solvent where activity coefficients are taken as equal to unity at infinite dilution), which means in practice, the solvent ( $w$  or  $s$ ) in which electrode systems are calibrated. It has been widely reported that better results are obtained when the pH in the mobile phase is considered instead of the aqueous pH of the buffer.  ${}^s\text{pH}$  can be easily converted to  ${}^w\text{pH}$  by means of  $\delta$  parameter:

$${}^w\text{pH} = {}^s\text{pH} + \delta$$

The  $\delta$  term is a constant value for each mobile phase composition. It includes the primary medium effect and the difference between the liquid junction potential of the electrode system in the hydroorganic mobile phase and in water. The primary medium effect (related to the standard Gibbs energy change for the transfer of the  $\text{H}^+$  ion from water to the non-aqueous or hydroorganic solvent at infinite dilution) depends only on the mobile phase solvent composition, but the liquid junction potential depends also on the particular electrode system, pH standards, and sample composition. Therefore, general interlaboratory conversion between both pH scales is only possible if the different electrode systems are designed to have a negligible residual liquid junction

potential. In practice, this requirement is fulfilled using a combination electrode containing a reference electrode with a concentrated KCl solution in water as a salt bridge. These  $\delta$  values for methanol-water mixtures were studied by various authors and they can be estimated from the solvent composition through the empirical equation:

$$\delta = \frac{0.09\phi_{\text{MeOH}} - 0.11\phi_{\text{MeOH}}^2}{1 - 3.15\phi_{\text{MeOH}} + 3.51\phi_{\text{MeOH}}^2 - 1.35\phi_{\text{MeOH}}^3}$$

where  $\phi_{\text{MeOH}}$  is the volume fraction of methanol in the hydroorganic mixture.  $\delta$  values for acetonitrile-water mixtures up to 60% (v/v) of organic modifier can be also estimated from the solvent composition through the equation:

$$\delta = \frac{-0.446\phi_{\text{MeCN}}^2}{1 - 1.316\phi_{\text{MeCN}} + 0.433\phi_{\text{MeCN}}^2}$$

The relationship between  ${}^s\text{pH}$  and  ${}^w\text{pH}$  depends on the organic solvent fraction in the mixture, whereas the difference between  ${}^w\text{pH}$  and  ${}^s\text{pH}$  (or  ${}^w\text{pH}$ ) depends not only on the mobile phase composition but also on the particular buffering solution employed.  $\delta$  values are also useful to convert  ${}^w\text{p}K_a$  values to  ${}^s\text{p}K_a$ , and  ${}^w\text{p}K_{\text{ap}}$  to  ${}^s\text{p}K_{\text{ap}}$ , where  $\text{p}K_a$  refers to the analyte acid-base constant and  $\text{p}K_{\text{ap}}$  to the autoprotolysis constant of the solvent (organic solvent-water mixture).