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Determination of acidity constants by the capillary electrophoresis internal standard method. IV. Polyprotic compounds

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ABSTRACT

The IS-CE method is developed for pK_a determination of polyprotic compounds. In this method, the internal standard (IS) and the polyprotic test compound are injected into the capillary electrophoresis (CE) system in buffers with appropriate pH. The pH of the buffers is not externally measured, but determined inside the capillary from the mobilities of the internal standards. Then the pK_a values of the polyprotic compounds are obtained by fitting its mobilities to the in situ pH values. The method is faster than the classical CE method (a diprotic compound can be done in less than 15 min), and also than other methods like potentiometric and spectrophotometric titrations. The method has been successfully applied to 20 polyprotic test compounds of different chemical nature, including compounds with extreme or very close pK_a values.

1. Introduction

Acidity determination is important in many fields of research, specifically in drug development because the ionization state of a compound governs its physicochemical properties such as solubility, lipophilicity and protein binding. Many drug compounds contain at least one acidic and/or basic functional group and it has been estimated that 95% of the commercial drugs are ionizable [1,2].

A need for rapid filing of patents for promising drugs compels pharmaceutical companies to characterize a great number of potential drugs and chemical precursors in a relatively short time to select the ones which are more suitable for their further test and development. This fact justifies the increasing demand of fast and reliable physicochemical characterization techniques [1,2]. In addition, there is a significant need of measuring the dissociation constants of environmentally relevant drugs and pollutants to determine their occurrence, fate and effects [3].

Our research group has developed the internal standard (IS) method, based on capillary electrophoresis (CE) for the determination of acidity constants [4–7]. The IS-CE method is fast and efficient, and offers an attractive alternative to the classical method [8–14] and to other common methods, such as potentiometric [15–17] and spectroscopic titrations [15,18]. It is easily automatable and requires very small amounts of compounds that may only be available in very limited quantities (e.g. from combinatorial syntheses). Using this method, the acidity constant of a monoprotic compound can be easily determined in few minutes in an standard CE instrument.

The method is based on the use of an internal standard with pK_a similar to that of the analyte. The analyte and the internal standard are injected through sequential injection into the capillary and run sequentially in two different buffers: a buffer in which the analyte and the IS are partially ionized (pH in the range p$K_a \pm 1$), and a second buffer in which both are completely ionized (pH $\gg$ pK_a for a neutral acid or pH $\ll$ pK_a for a neutral base). The difference between the pK_a of the analyte and the one of the IS can be determined from the mobility ratio of the analyte and the IS. Consequently, when the pK_a of the IS is well known, the pK_a of the analyte can be accurately determined [4–7]. This procedure provides a measure of the pK_a of the test compound as accurate as the pK_a of the IS. In any case, the precision can be improved by using more internal standards and/or buffer solutions.

At the moment, the IS-CE method has been only developed for the pK_a determination of monoprotic weak acids and bases [4–7]. However, many drugs contain more than one acidic and/or basic group and thus the method needs to be extended to polyprotic compounds. The present paper extends the application of the method to compounds with several acidity constants, and emphasizes those

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particularly troublesome cases, like compounds with extreme or very close pK_a values.

2. Theory

The general acid–base equilibria for a fully protonated species, H_nX^z, can be expressed by:

$$\begin{array}{ll} H_nX^z \rightleftarrows H_{n-1}X^{z-1} + H^+ & \mathrm{p}K'_{a1} = \mathrm{pH} - \log\dfrac{[H_{n-1}X^{z-1}]}{[H_nX^z]} \\ \vdots & \vdots \\ H_{n-i+1}X^{z-i+1} \rightleftarrows H_{n-i}X^{z-i} + H^+ & \mathrm{p}K'_{ai} = \mathrm{pH} - \log\dfrac{[H_{n-i}X^{z-i}]}{[H_{n-i+1}X^{z-i+1}]} \\ \vdots & \vdots \\ HX^{z-n+1} \rightleftarrows X^{z-n} + H^+ & \mathrm{p}K'_{an} = \mathrm{pH} - \log\dfrac{[X^{z-n}]}{[HX^{z-n+1}]} \end{array} \quad (1)$$

where n is the total number of ionizable groups, z the charge of the fully protonated species, and pK'_{ai} the dissociation equilibrium constant of the ith dissociation step (at constant ionic strength).

As the ionization degree is related to the acidity constants, the effective electrophoretic mobility (μ_{eff}) of a polyprotic compound can be expressed as a function of the pK_a values of each species and the pH of the background electrolyte through the following general equation [9,11,19–22]:

$$\mu_{eff} = \frac{\mu_{H_nX^z} + \sum_{i=1}^{n} 10^{ipH - \sum_{j=1}^{i} \mathrm{p}K'_{aj}} \mu_{H_{n-i}X^{z-i}}}{1 + \sum_{i=1}^{n} 10^{ipH - \sum_{j=1}^{i} \mathrm{p}K'_{aj}}} \quad (2)$$

where $\mu_{H_{n-i}X^{z-i}}$ is the limiting mobility of the subscript species. The term corresponding to the uncharged species has $\mu = 0$ and it is removed from expression (2).

Such mobilities can be directly calculated from the migration time of the test solute t_m and the electroosmotic flow marker t_0 (min) by means of the expression:

$$\mu = \frac{L_T L_D}{V}\left(\frac{1}{t_m} - \frac{1}{t_0}\right) \quad (3)$$

where L_T and L_D are the total length and the effective length of the capillary, respectively (cm), and V is the applied voltage (V).

In order to obtain each pK'_{ai} of a studied compound, data pairs pH − $\mu_{eff(AN)}$ can be fitted to Eq. (2) using nonlinear regression analysis. Later, the thermodynamic pK_{ai} values can be easily calculated from the working pK'_{ai} values by using Eq. (4):

$$\mathrm{p}K_{ai} = \mathrm{p}K'_{ai} - \log\frac{\gamma_{H_{n-i}X^{z-i}}}{\gamma_{H_{n-i+1}X^{z-i+1}}} \quad (4)$$

where $\gamma_{H_{n-i}X^{z-i}}$ and $\gamma_{H_{n-i+1}X^{z-i+1}}$ are the activity coefficients of the involved species. Activity coefficients are usually estimated by Debye–Hückel equation, which depends on the ionic strength (I) of the solution:

$$-\log\gamma = \frac{Az^2\sqrt{I}}{1 + Ba\sqrt{I}} \quad (5)$$

where A and B depend on the solvent dielectric constant and temperature (their values are 0.509 and 0.33, respectively, in water at 25 °C), z is the charge of the ion, and a is the hydrated radius of the ion. The value of a depends on the hydrated ion, although a value of 4.5 Å (value for hydrogen ion) is commonly taken for most ions. This equation is valid for ionic strength values lower than 0.2 M. As usual, activity coefficients of neutral species ($z = 0$) are assumed to be unity.

In the classical CE method, the pH of the buffer solution is measured by potentiometry and the mobility of the analyte in this buffered solution by capillary electrophoresis. In the IS-CE method, both parameters can be obtained at the same time in the same CE experiment. The test compound and the IS are injected together into the capillary. Since the pK_a of the IS is known, once μ_{eff} and the limiting mobility of the IS have been measured in an appropriate buffer, the pH of the buffer solution inside the capillary can be easily calculated from Eq. (2) avoiding pH variations between potentiometric pH and electrophoretic mobility measurements.

The calculation of the pH inside the capillary is particularly simple if we chose monoprotic internal standards, such as the ones we proposed in earlier work [6,7]. In this instance, Eq. (2) can be transformed into Eq. (6) when the IS is a monoprotic neutral acid ($n = 1$, $z = 0$), or Eq. (7) when it is a monoprotic neutral base ($n = 1$, $z = 1$) [4].

$$\mathrm{pH} = \mathrm{p}K'_{a(IS)} - \log\frac{\mu_{IS^-} - \mu_{eff(IS)}}{\mu_{eff(IS)}} \quad (6)$$

$$\mathrm{pH} = \mathrm{p}K'_{a(IS)} + \log\frac{\mu_{IS^+} - \mu_{eff(IS)}}{\mu_{eff(IS)}} \quad (7)$$

In these equations the pK'_a is related to the thermodynamic pK_a and the activity coefficient using Eq. (4), μ_{IS^-} is the mobility of the fully deprotonated neutral acid IS (measured at pH ≫ p$K'_{a(IS)}$) and μ_{IS^+} is the mobility of the fully protonated neutral base IS (measured at pH ≪ p$K'_{a(IS)}$).

μ_{eff} of the analyte is also measured together with μ_{eff} of the IS. Fitting all pairs pH − $\mu_{eff(AN)}$ to Eq. (2) and using nonlinear regression analysis, all pK'_{ai} and limiting mobilities of the test compound not directly measured can be calculated.

3. Experimental

3.1. Apparatus

Experiments have been performed using an Agilent Technologies (Santa Clara, CA, USA) Capillary Electrophoresis system equipped with a diode-array spectrophotometric detector. Capillary was of fused silica 50 μm I.D., 375 μm O.D., and 33.5 cm of total length (25 cm to the detector). The temperature of the capillary was 25.0 °C ± 0.1 °C. Samples were injected hydrodynamically at a pressure of 50 mbar for 2 s and the applied voltage was 20 kV. Sequential injection was performed. A pressure of 25 mbar is applied during the electrophoretic runs. With these conditions each run took around 1.5 min. The UV detector was set at 214, 254, and 280 nm.

The capillary conditioning previously reported [6,7,22] was time optimized. The very first time the capillary was conditioned by rinsing successively with 1.0 M NaOH (20 min), H_2O (10 min), and the buffer solution (10 min). Before every other use, the capillary was conditioned with 1.0 M NaOH (2 min), H_2O (0.5 min), and finally the buffer to be used in the experiment (2 min). When the pH of the buffer used was changed, the capillary was cleaned with the new buffer solution (10 s). At the end of each session, the capillary was washed with 0.1 M NaOH (2 min) and water (2 min).

3.2. Reagents

Dimethyl sulfoxide (DMSO, >99.9%), 0.5 M sodium hydroxide, 0.5 M hydrochloric acid, and sodium dihydrogenphosphate monohydrate (>99%) were from Merck (Darmstadt, Germany). Anhydrous sodium acetate (>99.6%) was purchased from J.T. Baker (Deventer, The Netherlands). 2-(Cyclohexylamino)ethanesulfonic acid (CHES, >99%), and 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS, >98%) were from Sigma (St. Louis, MO, USA). 2,2-Bis(hydroxymethyl)-2,2′,2″-nitrilotriethanol (BisTris, >99.9%), and sodium formate were from Fluka (Buchs, Switzerland). Tris(hydroxylmethyl)amino-methane (Tris, >99.9%) was purchased