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Fast high-throughput method for the determination of acidity constants by capillary electrophoresis I. Monoprotic weak acids and bases

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ABSTRACT

A new and fast method to determine acidity constants of monoprotic weak acids and bases by capillary zone electrophoresis based on the use of an internal standard (compound of similar nature and acidity constant as the analyte) has been developed. This method requires only two electrophoretic runs for the determination of an acidity constant: a first one at a pH where both analyte and internal standard are totally ionized, and a second one at another pH where both are partially ionized. Furthermore, the method is not pH dependent, so an accurate measure of the pH of the buffer solutions is not needed. The acidity constants of several phenols and amines have been measured using internal standards of known pK_a, obtaining a mean deviation of 0.05 pH units compared to the literature values.

1. Introduction

The knowledge of the acidity constant for substances with acid–base properties is of main importance in many fields. In fact, the neutral and ionic forms of a compound may exhibit very different physicochemical and biological properties, being the acidity constant, sometimes, decisive for a given application. The determination of this parameter is of particular interest in the pharmaceutical industry [1,2]. Many potential drugs are weak acids or bases, and their use in further studies depend on the value of certain physicochemical properties such as acidity, hydrophobicity, solubility, etc. [3].

There are several methodologies for the determination of acidity constants. The ones most used are based on potentiometric [4–6], spectrophotometric [4,7] and electrophoretic measurements [8–14], and all of them have some advantages and some drawbacks when applied. For example, potentiometric titrations have the advantage of providing very accurate results, but the sample must be of high purity and completely soluble in the whole pH range, it requires relatively high amounts of sample, and it is not a fast technique. Spectrophotometric titrations are more sensitive, but they are also slow and only applicable when the compound of interest has a chromophore in its structure with different spectral properties for the ionic and the neutral form. In addition, also high purity of the sample is required. Capillary electrophoresis has also been widely used for the determination of acidity constants. It is a highly automated technique and low amounts of sample and reagents are required. Purity is not always a problem because impurities are separated from the analyte in the electrophoretic process, but it is also a slow method. In fact, electrophoretic mobilities must be determined in a wide range of pH values, and several considerations must be taken into account such as keeping the temperature and the ionic strength constant for all the measurements, and be aware of specific interactions between the buffers and the analytes [12–16].

The proposed method is also based on capillary zone electrophoresis (CZE) measurements, so it takes profit of the advantages of the technique, but it is focused on the use of a reference compound (or internal standard, I.S.) of similar nature and also acidity constant as the analyte. The reference compound and the analyte are supposed to behave in the same way under the analysis conditions, therefore the differences between the relative mobility values of both compounds can be directly related to differences in their acidity values. Among other advantages, this method requires only two electrophoretic runs for the determination of an acidity constant, and the exact measure of the pH of the buffer solutions is not needed. In order to test the proposed method, several phenols and amines with known acidity constants have been selected. Some of them have been used as internal standards and some others as test compounds, so that the accuracy of the method can be checked.

2. Theory

The effective electrophoretic mobility, μ_{eff}, of a monoprotic neutral acid (HA) can be expressed as a function of its acidity constant (or its logarithmic form, pK_a) and the pH of the BGE by the following

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equation [15]:

$$\mu_{\text{eff}} = \frac{\mu_{A^-}}{1 + 10^{pK'_a - pH}} \quad (1)$$

where μ_{A^-} is the mobility of the deprotonated species, and pK'_a is related to the thermodynamic pK_a through the following equation:

$$pK_a = pK'_a - \log \gamma_{A^-} \quad (2)$$

provided that pK'_a involves the activity of the proton and the concentration of acidic and basic species.

In this equation, γ is the activity coefficient of the subscript species and corrects the effect of the ionic strength on solute ionization.

If Eq. (1) is rearranged, Eq. (3) is obtained:

$$pK'_a = pH + \log \frac{\mu_{A^-} - \mu_{\text{eff}}}{\mu_{\text{eff}}} = pH + \log Q \quad (3)$$

For a given acid, Q is a quotient that involves its limiting mobility (i.e. of the fully deprotonated form, μ_{A^-}) and its effective mobility (i.e. the mobility at a pH where the ionizated and the neutral form of the acid coexist, μ_{eff}). These mobility values ($cm^2\ V^{-1}\ min^{-1}$) are directly calculated from the migration times of the analyte (t_m) and the electroosmotic flow marker (t_0) through the following equation:

$$\mu = \frac{L_T L_D}{V}\left(\frac{1}{t_m} - \frac{1}{t_0}\right) \quad (4)$$

where L_T and L_D are the total and effective capillary length, respectively (cm), V is the applied voltage (V) and migration times are usually expressed in minutes.

When an I.S. is used, two different equations 3 are defined, the first one for the analyte (AN), and the second one for the I.S. If both equations are subtracted, the following expression is obtained:

$$pK'_{a,AN} - pK'_{a,IS} = \log Q_{AN} - \log Q_{IS} \quad (5)$$

This equation is not pH dependent. The limiting and effective mobilities of the analyte and the internal standard are the only parameters to be measured. It means that both compounds have to be injected together in a buffer at a pH where they are totally ionized in order to determine μ_{A^-}, and in a buffer at a pH where they are only partially ionized to obtain μ_{eff}. The pH of both buffers does not need to be measured, nor exactly known. With the use of a reference compound with known pK'_a value, the acidity constant of the analyte is easily calculated through the following equation:

$$pK'_{a,AN} = pK'_{a,IS} + \log Q_{AN} - \log Q_{IS} \quad (6)$$

In an analogous way, the μ_{eff} of a neutral monoprotic base (B) is related to its pK'_a value and the pH of the background electrolyte through the following equation:

$$\mu_{\text{eff}} = \frac{\mu_{BH^+}}{1 + 10^{pH - pK'_a}} \quad (7)$$

and the pK'_a is related to the thermodynamic one through the following equation:

$$pK_a = pK'_a + \log \gamma_{BH^+} \quad (8)$$

Rearrangement of Eq. (7) gives the following equation for a monoprotic base:

$$pK'_a = pH - \log \frac{\mu_{BH^+} - \mu_{\text{eff}}}{\mu_{\text{eff}}} = pH - \log Q \quad (9)$$

Now Q depends on the mobility of the protonated form of the base (μ_{BH^+}) and again on the effective mobility. As for Eq. (3), when Eq. (9) is defined for the analyte and the I.S. and they are subtracted and rearranged, Eq. (10) is obtained:

$$pK'_{a,AN} = pK'_{a,IS} - \log Q_{AN} + \log Q_{IS} \quad (10)$$

3. Experimental

3.1. Apparatus

Experiments were performed using two different capillary electrophoresis systems: a Beckman (Palo Alto, CA, USA) P/ACE 5500, equipped with a diode-array spectrophotometric detector, and an Agilent (Santa Clara, CA, USA), also equipped with a diode-array spectrophotometric detector. A fused-silica capillary of 50 μm I.D., 375 μm O.D. and 47 cm of total length (40 cm to the detector) for the Beckman system and 48.5 cm total length (40 cm to the detector) for the Agilent system obtained from Composite Metal Services (Ilkley, UK) were used to carry out the experimental mobility determinations. The temperature of the capillary was kept at 25.0 °C (±0.1 °C). Samples were injected hydrodynamically, and the applied voltage was 20 kV. UV detection was carried out at 214 nm.

3.2. Chemicals

Benzyl alcohol (analytical-reagent grade), 0.5 M sodium hydroxide, 0.5 M hydrochloric acid, and potassium chloride (>99.5%) were from Merck (Darmstadt, Germany). Sodium acetate anhydrous (>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), and sodium formate were from Fluka (Buchs, Switzerland). Tris(hydroxymethyl)aminomethane (Tris, >99.9%) was purchased from Aldrich (Milwaukee, WI, USA). Water was purified by a Milli-Q plus system from Millipore (Bedford, MA, USA), with a resistivity of 18.2 MΩ cm.

The test solutes employed were: 2-nitrophenol, 4-nitrophenol, 3-nitrophenol, 2-chlorophenol, 2-fluorophenol, 3-bromophenol, vanillin, 3,5-dichlorophenol, 7-hydroxyflavone, aniline, quinine, diphenhydramine, propranolol, ephedrine, pyridine, lidocaine, procainamide, *N*,*N*-dimethyl-*N*-benzylamine, 1-aminoethylbenzene, nortriptyline, and imipramine. All the compounds were reagent grade or better and were obtained from Sigma, Fluka, or Carlo Erba (Milan, Italy).

3.3. Procedure

Capillary conditioning methodology was reported previously [16]. Briefly, before the first use it was conditioned with 1 M NaOH, water and the running buffer; when the buffer was changed it was rinsed with water and the new buffer; and between runs it was rinsed for 3 min with the running buffer. At the end of the working session the capillary was flushed with water.

Several running buffers covering the pH range between 3 and 12 were prepared. Table 1 shows the employed buffers, its pK_a value, the covered pH range, and the stock solutions used in their preparation. In order to obtain a desired pH value, 50 mL of the stock solutions (0.1 M) were mixed with an appropriate amount of 0.5 M HCl, 0.5 M NaOH or 0.5 M KCl and diluted up to 100 mL, to have an ionic strength of 0.05 M.

Table 1
Buffer solutions used in the acidity constant determination.

Buffer constituents	pK_a	Covered pH range	Stock solutions
$HCOOH/HCOO^-$	3.75	2.60–4.80	0.1 M HCOONa + 0.5 M HCl
CH_3COOH/CH_3COO^-	4.76	3.70–5.80	0.1 M CH_3COONa + 0.5 M HCl
$BisTrisH^+$/BisTris	6.48	5.50–7.50	0.1 M BisTrisHCl + 0.5 M NaOH
$TrisH^+$/Tris	8.08	7.00–9.00	0.1 M TrisHCl + 0.5 M NaOH
$CHES/CHES^-$	9.50	8.40–10.10	0.1 M CHESNa + 0.5 M HCl
$CAPS/CAPS^-$	10.40	9.40–11.60	0.1 M CAPSNa + 0.5 M HCl
NaOH		11.8–13.00	0.5 M NaOH + 0.5 M KCl