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Review

Application of capillary electrophoresis in determination of acid dissociation constant values

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ABSTRACT

The chemical groups undergoing protonation or deprotonation in solution are described by the acid dissociation constant value, the key parameter for physicochemical characterization of biologically- and pharmacologically-important compounds. Capillary electrophoresis (CE) proved to be suitable technique for its determination: it enables automated and accurate measurements even for minute amount of sample, does not require the information about concentration, and handle both the impure and complex samples. In this review, a number of contributions reporting on the application of CE in pK_a prediction has been summarized and critically discussed. The reader will find herein the brief introduction of theory, summary of all works published in the last decade, considerations on the most important innovations and achievements, and the discussion of pK_a-related issues as e.g. the role of pK_a-shifts in the chiral separation mechanism or the elucidation of migration order reversals observed during CE-mediated separations.

Contents

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1. Introduction

The acid dissociation constant (K_a) value, usually expressed as its pK_a, is a fundamental parameter of chemical compounds characterizing the respective ionization equilibria corresponding to their acid–base properties [1–3]. For compounds possessing only a single ionizable group, pK_a indicates the pH at which the ionization level amounts 50%. Neutral and ionized forms of compounds usually differ in physicochemical properties, e.g. water solubility and membrane permeability, which are particularly important features in the case of pharmaceuticals and environmental agents. In general the extent of ionization is strictly related to the stabilization energy of solvated ions, which varies in relation to different chemical structures and other groups present in the vicinity of group of interest. The ionization equilibrium is also very sensitive on the presence of any organic solvent, ionic strength, and temperature, and hence these variables should be strictly controlled during pK_a determination experiments. Estimation of pK_a is a fundamental step during the drug discovery process, and normally involves the long series of routine tests repeated according to standardized protocol.

A lot of different analytical techniques have been employed to determine the pK_a values of various compounds classes, as potentiometry, conductometry, UV–vis spectrometry, NMR, capillary electrophoresis (CE), liquid chromatography, and most recently, the complex semi-experimental or fully theoretical in-silico algorithms. Among this broad arsenal, the CE has turned out to be particularly convenient technique: since: (i) it enables the analysis of minute sample amounts, (ii) CE is a separation technique hence it easily handles the impure and complex samples, (iii) information about concentration of analytes in unnecessary because only the migration times are used, (iv) CE instruments are generally fully automated, which increase the throughput of analysis, (v) the run times are oftentimes in the order of several minutes, and finally (vi) the precise temperature control is usually possible. The comprehensive review of CE-based methodology of pK_a estimation was published in 2004 [1], since that time a vast progress has been made, expressed in total number of about 50 new articles covering this subject area.

The pivotal role of this manuscript is to provide the reader with the new insight into the subject, comprising an easy access to all published contributions for the last decade and the more detailed overview of the major methodological and technical innovations in pK_a determination by CE technique. A particular attention is paid to: the alternative methods for pK_a estimation from CE-derived data, the application of multiplexed and miniaturized instrumentation as well as physicochemically modified capillaries, the internal standard-based method which was proposed and developed as an alternative approach for standard CE application several years ago, the extended algorithms allowing to calculate the pK_a shifts occurring after formation of diastereomeric inclusion complexes with cyclodextrins used as chiral selectors, and other related issues.

2. Theory

The comprehensive presentation of the pK_a theory with a thorough mathematical description can be found in other review articles [1–3], for the current purposes only the most important formulas have been given, essential for the CE-based method implementation.

The effective electrophoretic mobility values of the compound of interest calculated across broad pH range, with maintaining the constant buffers ionic strength and temperature, are the crucial input data in pK_a determination. The μ_{eff} values can be obtained from migration times by implementation of Eq. (1), provided that the suitable neutral electroosmotic flow (EOF) marker is used and the peaks originating from this marker and compound of interest are well separated.

$$\mu_{eff} = \mu_{obs} - \mu_{eof} = \frac{L_{tot} \cdot L_{eff}}{V} \cdot \left(\frac{1}{t_{obs}} - \frac{1}{t_{eof}} \right) \quad (1)$$

where μ_{eff} and μ_{obs}, are the effective and observed electrophoretic mobilities of analyte ($cm^2\,V^{-1}\,s^{-1}$), respectively; μ_{eof} is the mobility of electroosmotic flow ($cm^2\,V^{-1}\,s^{-1}$); L_{tot} and L_{eff} are the total and effective capillary lengths (cm), respectively, V is the separation voltage (V); t_{obs} is the measured migration time of analyte (s), while t_{eof} is the time measured for neutral marker (s).

By plotting the relation between calculated μ_{eff} and pH, the model curve is created, with a characteristic sigmoidal shape. The location of its inflection point on pH scale indicates the sought-after pK_a value, or in the case of multiprotic acids and bases, several inflection points indicate pK_a values for distinct ionization equilibria. In theory, the connection between μ_{eff} and pK_a expressed for monovalent weak acid follows the equation:

$$\mu_{eff} = \left[\frac{10^{-pK_a}}{10^{-pK_a} + 10^{-pH}} \right] \cdot \mu_{A^-} \quad (2)$$

where μ_{A^-} is the effective electrophoretic mobility of a given ion created upon deprotonation.

In general case, any number of equilibria can be considered:

$$\mu_{eff} = \sum_{i=1}^{n} x_i \cdot \mu_{ion,i} \quad (3)$$

where x_i is the mole fraction of ionized species i, and n is the number of all considered equilibria.

In practice, the precise estimation of pK_a value is not a straightforward task, so that various non-linear or linear models may be applied for its finding. The situation becomes more complicated for multivalent acids and bases, as well as in the case when expected pK_a values are especially low or high, being outside a typical pH range of standardized buffer solutions. There is also a number of other prerequisites referring to application of CE which should be fulfilled in order to minimize the uncertainties of pK_a determination. They refer to: (i) the critical buffers selection – low ionic strength is preferred but with ensuring the sufficient buffering capacity – typically from the range 10–100 mM, (ii) capillary conditioning – the suitable rinsing procedure should be optimized prior to the main experiment, (iii) detection mode – the most popular is a (photo)diode array detector (DAD) which however may fail to respond adequately for some compounds, (iv) the choice of convenient EOF marker, capillary type, and data analysis algorithm, to list only the most important ones. Notwithstanding, the aforementioned standard CE-based method allows one to determine pK_a values with a fairly good accuracy around 0.05 pH unit or less, and these values are often in good accordance in literature data derived from other experimental methodologies. It is important however to note, that the values estimated by using Eq. (2) are valid only at specific ionic strength and temperature values (see "thermodynamic pK_a" in further section of this manuscript), and that the other reference methods yield the values burdened with their own uncertainties.

3. General overview

Over 50 scientific papers were published in the last decade covering the field of pK_a determination by CE technique. They have been summarized in Table 1, where the names of used analytes, brief description of the main finding or innovation, publication year, and respective reference number can be found. The most