Journal of Chromatography A, 1446 (2016) 149–157

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Determination of acid dissociation constant of 20 coumarin derivatives by capillary electrophoresis using the amine capillary and two different methodologies

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ARTICLE INFO

Article history:
Received 2 March 2016
Received in revised form 24 March 2016
Accepted 30 March 2016
Available online 2 April 2016

Keywords:
Acid dissociation constant
Capillary coating
Capillary electrophoresis
Coumarin derivatives
Electrophoretic mobility

ABSTRACT

In this work capillary electrophoresis has been used to determine acid dissociation constant of 20 structurally diverse coumarin derivatives. For a majority of compounds pK_a value has been determined for the first time. The obtained values vary between 4.16–9.10 pH unit, pointing to the interesting structure-acidity relationships. The amine permanently coated capillary has been applied for that purpose, because it has turned out to be more effective in pK_a determination than the bare silica and other coated capillaries, ensuring good precision and shorter migration times. A traditional methodology relying on measurements in a broad pH range and fitting of a sigmoidal function has been compared to an alternative simplified approach, reported for the first time, where only two electrophoretic mobility values suffice for pK_a estimation. The first value corresponds to the partially ionized form and it is measured experimentally, while the second one to the totally ionized form – it is measured experimentally (two-values method) or estimated directly from molecular mass (one-value method). We show that despite a limited measurements number, the alternative approach may be consistent with the traditional methodology, yielding the relatively low pK_a deviation. Its reliability has also been confirmed by the analytical predictions, comprising resolution, migration order, migration times and peaks overlapping. Therefore, combination of the amine capillary with the simplified calculation method is an attractive tool for fast and reliable pK_a estimation.

1. Introduction

Capillary electrophoresis (CE) is frequently utilized in determination of acid dissociation constant – K_a, and its logarithmic equivalent – pK_a. [1,2]. It offers high accuracy, automation and little consumption of buffers and samples. In addition, due to high resolving power, it allows for application of impure and complex samples. Obtained pK_a values may be used in physicochemical characterization, pharmacokinetic studies, as well as in typically analytical applications. The acid-base equilibrium plays a paramount role in CE, it determines charge of ionizable analytes at the given pH, and thus influences directly on their migration times [3]. Therefore, acquired knowledge on pK_a values may be employed in design and development of new analytical methods.

The most recognized method for CE-based pK_a determination uses dependency between effective electrophoretic mobility (μ_{eff}) and pH. After application of the non-linear regression pK_a can be found as a fitting parameter [1,2]. For a monoprotic acid ($HA \leftrightarrow H^+ + A^-$) the relation is described by Eq. (1):

$$\mu_{eff} = \left[\frac{\mu_{A^-} \cdot 10^{-pK_a}}{10^{-pK_a} + 10^{-pH}} \right] \quad (1)$$

where μ_{A^-} is a fitting constant equal to the electrophoretic mobility of anion. pK_a value equals to pH indicating inflection point of the obtained sigmoidal curve.

A linear model may also be used [4–6], which is described by Eq. (2):

$$\frac{1}{\mu_{eff}} = \frac{K_a}{\mu_{A^-}} \left(\frac{1}{a_{H^+}} \right) + \frac{1}{\mu_{A^-}} \quad (2)$$

where a_{H^+} is hydrogen ion activity calculated from pH.

Both methods require multiple measurements at different pH values. In the case of non-linear method, pH values should be spaced out evenly and cover at least 5 pH units to register the whole

Abbreviations: EOF, electroosmotic flow marker; IS-CE, internal standard capillary electrophoresis method; OVM, one-value method; TVM, two-values method.

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http://dx.doi.org/10.1016/j.chroma.2016.03.084
0021-9673/

range of mobility change. This approach yields usually relatively low standard error of fitting, within 0.05 pH unit, in particular when measurements at the given pH values are repeated several times.

In recent years a distinct approach has been proposed by Rosés and co-workers [7–12], denoted as an internal standard capillary electrophoresis method (IS-CE). It requires application of an internal standard of accurately known pK_a, close to the predicted value for analyte. Only two analytical runs are needed for pK_a estimation, electrophoretic mobility is measured when analyte and internal standard are partially ionized, and totally ionized. For a monoprotic acid pK_a can be obtained from Eq. (3):

$$pK_{aAN} = pK_{aIS} + \log\frac{\mu_{A^-(AN)} - \mu_{eff(AN)}}{\mu_{eff(AN)}} - \log\frac{\mu_{A^-(IS)} - \mu_{eff(IS)}}{\mu_{eff(IS)}} \quad (3)$$

where "AN" and "IS" refer to parameters calculated for analyte and internal standard, respectively. This method has been tested in terms of chemically different compounds, various electrolyte systems, and temperature variation. Most recently, IS-CE has been implemented in a novel sequential injection–capillary electrophoresis instrument for the high-throughput determination of pK_a regardless of aqueous solubility, number of pK_a values, and structure [13].

In this work we present another theoretically possible approach to pK_a estimation by CE, which to the best of our knowledge, has never been reported. It uses the maximally simplified formula:

$$pK_a = pH + \log\frac{\mu_{A^-} - \mu_{eff}}{\mu_{eff}} \quad (4)$$

where μ_{eff} is an effective electrophoretic mobility of the partially ionized form reached at a given pH. This expression may be easily obtained by rearrangement of Eqs. (1) or (2). It is valid for monoprotic acids, although the analogous expressions for bases and multiprotic compounds can be derived by rearrangement of the known equations valid for the particular types of compounds [3,14]. In this work reliability of this simple alternative methodology has been systematically studied for the first time. It has been done by its comparison to the classical method relying on measurements covering the wide pH range and non-linear regression. Moreover we have proposed a semi-empirical variant of the alternative methodology, yet easier and faster, requiring solely a single electrophoretic mobility value. It assumes that such extremely simple estimation of pK_a is possible via Eq. (4) provided that μ_{A^-} (electrophoretic mobility of anion) may be predicted theoretically. To this end, the model combining μ_{A^-} and molecular mass have been created and utilized. It is to be noted that the relation between electrophoretic mobility and molecular mass has already been investigated for various molecules and used in optimization of separation conditions and determination of maximal charge of unknown molecules [14,15], nonetheless, it has never been used as a direct source of data for pK_a determination.

All methods, classical and alternative ones, have been employed for examination of pK_a of 20 coumarin derivatives, belonging to phenols of common structural motif. The knowledge on their acidity is important since most of them exert strong biological activity, including toxic action as a vitamin K antagonist [16]. Except few cases [17–20], their pK_a values have been determined for the first time. To verify accuracy of pK_a values obtained using the simplified methodology, some analytical predictions have been performed, comprising resolution, migration order, migration times and peaks overlapping. The next aspect of our work is the rationale choice of capillary for pK_a determination. We have studied several different capillaries, including the bare silica and permanently or dynamically coated, and chosen the one ensuring the lowest random errors and fastest analysis.

Table 1
Composition of buffering solutions used in pK_a determination.

pH (measured)	Buffer type	Buffer ingredients	
3.18	Phosphate buffer I	H_3PO_4	NaH_2PO_4
3.97, 4.52, 4.90	Acetic buffer	CH_3COOH	CH_3COONa
5.92, 7.07, 7.53, 8.15	Phosphate buffer II	NaH_2PO_4	Na_2HPO_4
9.18, 9.49, 10.22	Borate buffer	$Na_2B_4O_7{\cdot}10H_2O$	HCl/NaOH

The amounts of the given ingredients have been calculated to ensure the constant ionic strength of 50 mM. The obtained pK_a values refer to these value.

2. Materials and methods

2.1. Materials

All coumarin derivatives: 3-hydroxycoumarin, 4-hydroxycoumarin, 6-hydroxycoumarin, 7-hydroxycoumarin, 4-methyl-7-hydroxycoumarin, 4-phenyl-7-hydroxycoumarin, 4,7-dihydroxycoumarin, 5,7-dihydroxy-4-methylcoumarin, 5,7-dihydroxy-4-phenylcoumarin, 5,7-dihydroxy-4-propylcoumarin, 6,7-dihydroxycoumarin, 7,8-dihydroxycoumarin, 7,8-dihydroxy-4-methylcoumarin, 7,8-dihydroxy-4-phenylcoumarin, 7,8-dihydroxy-6-metoxycoumarin, coumachlor, coumafuryl, coumatetralyl, and warfarin were supplied by Sigma-Aldrich (St. Louis, MO, USA), except 10-hydroxywarfarin which was supplied by LGC Standards (Teddington, UK). All other chemicals were supplied by Avantor Performance Materials Poland. S. A. (Gliwice, Poland). All solutions were prepared in the deionized water (MilliQ, Merck-Millipore Billerica, MA, USA) and filtered through the 0.45 μm regenerated cellulose membrane, then degassed by centrifugation. The concentration of analytes (coumarin derivatives) in the sample was 0.20 mg/mL. Dimethyl sulfoxide (DMSO) was used as the electroosmotic flow (EOF) marker to enable calculation of electrophoretic mobility, its final concentration in the sample was 0.2% (v/v).

2.2. Experimental conditions

Experiments were performed on the P/ACE MDQ Capillary Electrophoresis (CE) System (Beckman Coulter, Brea, CA, USA) equipped with a diode array detector (DAD). The following commercially offered capillaries were used: an unmodified bare fused-silica capillary; a neutral eCAP™ polyacrylamide-coated capillary providing neutralization of EOF; an amine eCAP™ polyamine-coated capillary providing reversion of EOF (supplied by Beckman-Coulter). In addition, the silica and amine capillaries were used to dynamic coating performed with the commercially available kit CEofix™ (Beckman-Coulter). Coating by two ionic-polymer layers (cationic-anionic) was performed for the silica capillary, and by one anionic-polymer layer for the amine capillary, pre-coated with a poly-amine layer. The capillaries were of 60 cm total length, 50 cm effective length, and of 50 μm internal diameter. Sample injection was conducted using forward pressure of 3.45 kPa (0.5 psi) for 5 s. During separations 30.0 kV separation voltage (in normal or reverse polarity) was applied, without or with external pressure of 3.45 kPa (0.5 psi). Pressure was applied in the case of neutral capillary. The capillaries were conditioned at temperature of 25 °C using the liquid cooling system. Ionic strength of all running buffers was kept constant on the level of 50 mM. All pK_a values presented in this work are valid for this ionic strength. The composition of buffering solutions has been given in Table 1. It was reported in the literature that some components of buffers used in pK_a determination, e.g., phosphoric acid, may interact with analytes and distort their electrophoretic mobility [21]. However, no symptoms of these interactions have been observed in this study. The procedure of capillary rinsing/conditioning has been described in Supplemen-