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Thermodynamics of acid-base dissociation of several cathinones and 1-phenylethylamine, studied by an accurate capillary electrophoresis method free from the Joule heating impact

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ABSTRACT

Capillary electrophoresis is often used to the determination of the acid-base dissociation/deprotonation constant (pK_a), and the more advanced thermodynamic quantities describing this process (ΔH°, $-T\Delta S^\circ$). Remarkably, it is commonly overlooked that due to insufficient dissipation of Joule heating the accuracy of parameters determined using a standard approach may be questionable. In this work we show an effective method allowing to enhance reliability of these parameters, and to estimate the magnitude of errors. It relies on finding a relationship between electrophoretic mobility and actual temperature, and performing pK_a determination with the corrected mobility values. It has been employed to accurately examine the thermodynamics of acid-base dissociation of several amine compounds – known for their strong dependency of pK_a on temperature: six cathinones (2-methylmethcathinone, 3-methylmethcathinone, 4-methylmethcathinone, α-pyrrolidinovalerophenone, methylenedioxypyrovalerone, and ephedrone); and structurally similar 1-phenylethylamine. The average pK_a error caused by Joule heating noted at 25 °C was relatively small – 0.04-0.05 pH unit, however, a more significant inaccuracy was observed in the enthalpic and, in particular, entropic terms. An alternative correction method has also been proposed, simpler and faster, but not such effective in correcting ΔH°/$-T\Delta S^\circ$ terms. The corrected thermodynamic data have been interpreted with the aid of theoretical calculations, on a ground of the enthalpy-entropy relationships and the most probable structural effects accounting for them. Finally, we have demonstrated that the thermal dependencies of electrophoretic mobility, modelled during the correction procedure, may be directly used to find optimal temperature providing a maximal separation efficiency.

1. Introduction

Logarithmic equivalent of the acid-base dissociation/deprotonation constant $-pK_a$, is one of the basic physicochemical parameters that characterize organic acids and bases [1]. It determines their solubility in aqueous environment, lipophilicity and membrane permeability, ability to ionic and hydrophobic interactions, activity and functionality in biological systems (including *endo*- and *exo*-genic compounds), as well as particular behaviors in analytical systems, e.g. retention on column in chromatography or migration time in electrophoresis. A thermal dependency of pK_a values allows to predict how these properties change with temperature, e.g. to model selectivity of chromatographic and electrophoretic separations, and to determine more advanced thermodynamic parameters – the standard dissociation enthalpy (ΔH°) and entropy ($-T\Delta S^\circ$) [2–4]. These quantities, in turn, provide a more complete thermodynamic picture of a molecule's deprotonation, thus they are useful in investigation of structural and stereoelectronic factors underlying this process. From the practical point of view, this is especially important for design and development of new chemical structures exhibiting predictable and desirable properties, e.g. drugs.

Capillary electrophoresis (CE) is a powerful separation technique characterized by a huge resolving power, high automation degree, and very low consumption of sample materials and buffers. Aside from efficient separation, CE enables determination of the crucial physicochemical properties, such as pK_a, and their thermal dependencies [5–12]. In comparison to other techniques like poten-

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tiometry, optical spectroscopy, NMR or liquid chromatography, CE uses only a minute amount of sample, handles impure and complex mixtures without a need for applying organic solvents, and as it is commonly assumed, provides very good accuracy [1,13]. In a typical and most accurate approach, pK_a of a compound is determined by plotting the relationship between its electrophoretic mobility, dependent on ionization degree, and pH. This function is of a characteristic sigmoidal shape, and pK_a value can be found as a fitting parameter describing position of the inflection point [1,13].

Electrophoretic mobility can be calculated as:

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

where μ_{ep} is the electrophoretic mobility, L_{tot} and L_{eff} are the total and effective capillary lengths (m), U_{nom} is the nominal (programmed) separation voltage (kV); t_{tot} is the total (observed) migration time of analyte (min); while t_{eof} is the time measured for the neutral marker of electroosmotic flow (EOF).

For a monoprotic base pK_a can be obtained as a fitting parameter, using the following non-linear model [13]:

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

where μ_{C+} is a second fitting parameter – electrophoretic mobility of the cation (totally ionized form).

Nonetheless, determination of pK_a using CE – and in particular its thermal variation, is not free from some intrinsic limitations. One of the most important issues is the problem related to the accuracy of electrophoretic mobility determination [14]. Eq. (1), despite used very commonly in the literature, gives mobility values burdened with some hardly predictable systematic error. It results from two main effects: (i) uncontrolled rise of temperature inside capillary caused by Joule heating generation and insufficient cooling, especially in the inlet/outlet capillary sections devoid of an active cooling; (ii) and a ramping effect, i.e. a gradual increase of voltage at the beginning of separation, lasting around 0.1–0.2 min, which causes a deviation of the average electric field strength from its nominal value used in Eq. (1) [14–18]. As far as the latter effect can be easily eliminated by using a more adequate equation to calculate mobility, taken from our recent work [19]:

$$\mu_{ep(ramp)} = \frac{L_{tot}L_{eff}}{U_{nom}\left(t_{tot} - 0.5t_{ramp}\right)} - \frac{L_{tot}L_{eff}}{U_{nom}\left(t_{eof} - 0.5t_{ramp}\right)} \quad (3)$$

where $\mu_{ep(ramp)}$ is the ramping-corrected electrophoretic mobility, and t_{ramp} is the voltage ramp time set up in a software, normally between 0.1–0.2 min; the elimination of the Joule heating effect is much more troublesome. Notably, the rise of temperature inside capillary may evoke three parallel effects: (i) viscosity change, (ii) pK_a shift of analyte – the most important effect in the context of pK_a determination, and (iii) pK_a shift of buffer – meaningful only for some buffer types, e.g. Tris-HCl. In order to provide high reliability of pK_a values, a suitable correction of these effects is required. It is also worth highlighting that when applying no correction, reliability of the thermodynamic quantities (ΔH° and $-T\Delta S^\circ$) may be much more affected than pK_a values because they are determined directly from the Van't Hoff plot – a function of inverse (uncorrected) temperature.

In our recent work we have proposed a simple method for minimizing the impact of Joule heating on the accuracy of pK_a values [19], effective in correcting the viscosity and pK_a shifts effects. Briefly, the method is based on the estimation of an actual temperature reached at various temperatures of coolant (T_{actual}) using the current values, and subsequently, on plotting the obtained electrophoretic mobilities versus T_{actual}. Then, after function fitting

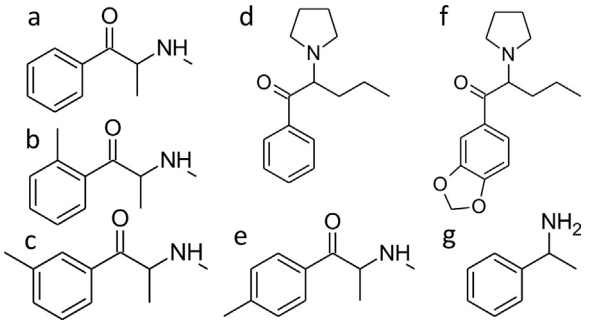

Fig. 1. The chemical structures of: (a) ephedrone, (b) 2-MMC, (c) 3-MMC, (d) PVP, (e) 4-MMC, (f) MDPV, (g) PEA.

(auxiliary polynomial functions), by interpolation we can read correct electrophoretic mobility values corresponding to the chosen temperatures of interest, the same for all buffering systems, see Section 2.2 for more details. In the previous study we used this method for correcting pK_a values obtained for 4-hydroxycoumarin and coumatetralyl (phenolic negatively ionized molecules), and for their complexes with cyclodextrins [19]. This method, however, has never been applied to the amine compounds which are generally known for their large variation of pK_a with temperature [20,21].

The aim of this work was to employ CE to examine thermodynamics of acid-base dissociation of seven structurally-similar amine compounds: six cathinones – 2-methylmethcathinone (2-MMC), 3-methylmethcathinone (3-MMC), 4-methylmethcathinone known also as mephedrone (4-MMC), α-pyrrolidinovalerophenone (PVP), methylenedioxypyrovalerone (MDPV), and ephedrone known also as methcathinone; and structurally similar 1-phenylethylamine (PEA) – see Fig. 1 for the structures. The thermal dependencies of pK_a values were used to determine the standard dissociation/deprotonation enthalpies (ΔH°) and entropies ($-T\Delta S^\circ$). We examined four distinct approaches: (i) without any correction of electrophoretic mobilities; (ii) correcting only the ramping-related effect; (iii) using the full correction method proposed previously (including the ramping and temperature rise effects); (iv) and a simplified correction method of both effects which has never been tested up till now. In the second part we have interpreted the obtained outcomes on the ground of enthalpy-entropy relationships, and with the aid of theoretical DFT calculations, we delineated the dominant structural effects responsible for the observed thermodynamics. In the third part, we demonstrated that the thermal dependency of electrophoretic mobilities, modelled during the correction step, may be directly used to predict the optimal temperature ensuring a maximal separation efficiency.

2. Materials and methods

2.1. Materials

All analytes: 2-MMC, 3-MMC, 4-MMC, PVP, MPDV, ephedrone and PEA were supplied by LGC Standards (Łomianki, Poland). 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 in the sample was 50 μg/mL (aqueous solutions). Dimethyl sulfoxide (DMSO) was used as the EOF marker to enable calculation of electrophoretic mobility, its final concentration in the sample was 0.2% (v/v).

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