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Determination of acid–base dissociation constants of very weak zwitterionic heterocyclic bases by capillary zone electrophoresis

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Dedicated to our colleague and friend, Illya M. Lyapkalo, who suddenly passed away on September 10, 2010.

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ABSTRACT

Thermodynamic acid–base dissociation (ionization) constants (pK_a) of seven zwitterionic heterocyclic bases, first representatives of new heterocyclic family (2,3,5,7,8,9-hexahydro-1*H*-diimidazo[1,2-*c*:2′,1′-f][1,3,2]diazaphosphinin-4-ium-5-olate 5-oxides), originally designed as chiral Lewis base catalysts for enantioselective reactions, were determined by capillary zone electrophoresis (CZE). The pK_a values of the above very weak zwitterionic bases were determined from the dependence of their effective electrophoretic mobility on pH in strongly acidic background electrolytes (pH 0.85–2.80). Prior to pK_a calculation by non-linear regression analysis, the CZE measured effective mobilities were corrected to reference temperature, 25 °C, and constant ionic strength, 25 mM. Thermodynamic pK_a values of the analyzed zwitterionic heterocyclic bases were found to be particularly low, in the range 0.04–0.32. Moreover, from the pH dependence of effective mobility of the bases, some other relevant characteristics, such as actual and absolute ionic mobilities and hydrodynamic radii of the acidic cationic forms of the bases were determined.

1. Introduction

The knowledge of the acid–base dissociation (ionization) constants (pK_a) is essential in a wide range of application and research areas in chemistry, biochemistry and molecular biology. The reason for that is that electroneutral and ionic forms of a compound may exhibit very different physicochemical properties (solubility, lipophilicity) and biological activities, e.g. receptor binding and signal transduction. The pK_a value is an important factor for the estimation of the concentration of individual ionic forms of a compound in the solution, for comprehension of drug passage across cell membrane, for investigation of the biological uptake and metabolism mechanism. Moreover, pK_a explains reactivity, reaction rate and salt creation of chemical substances. In separation sciences, the knowledge of pK_a values of the analyzed compounds is an important factor for the selection of suitable experimental conditions during the development of new electrophoretic and chromatographic methods for their analysis and characterization. Traditionally, potentiometric and spectrophotometric methods are used for the pK_a determination. The main drawback of these classical methods is the difficulty of handling impure compounds. Additionally, spectrophotometric techniques require a compound to have a measurable UV chromophore with absorption that is dependent on pH. In recent years, capillary zone electrophoresis (CZE) has been recognized as an effective method for the pK_a determination in both aqueous and non-aqueous media [1–6] because of its high separation efficiency and resolving power, ability to handle impure compounds and a low analyte and solvent consumption.

The newly synthesized enantiopure zwitterionic bases pertaining to hitherto unknown family of heterocyclic compounds, 2,3,5,7,8,9-hexahydro-1*H*-diimidazo[1,2-*c*:2′,1′-*f*][1,3,2]diazaphosphinin-4-ium-5-olate 5-oxides, were designed to serve as chiral Lewis base catalysts for enantioselective reactions. For estimation of the applicability of these newly synthesized zwitterionic bases, it is necessary to determine their pK_a values because pK_a is one of the most widely used parameters for judging about the relative strength of Lewis bases [7]. For this purpose, a CZE method was chosen, because of its intrinsic advantages mentioned above.

In CZE, the determination of pK_a is based on the measurement of the effective electrophoretic mobility of an ionizable compound in a series of background electrolyte (BGE) solutions of constant ionic strength and variable pH. The pK_a values are obtained by fitting the effective mobility as a function of pH to a particulate model of acid–base equilibria of a solute with the defined number of ionizable groups. In most cases, CZE determination of ionization constants of acids and bases in the pH range from *ca.* 2 to 12 is trouble-free. However, the determination of $pK_a < 2$ brings some complications because of the high conductivity, high ionic strength and very low electroosmotic flow (EOF) of the strongly acidic BGEs in the untreated fused silica capillaries. Nevertheless, utilization of such low pH is useful, and sometimes necessary for the analysis and physicochemical characterization of very weak bases or

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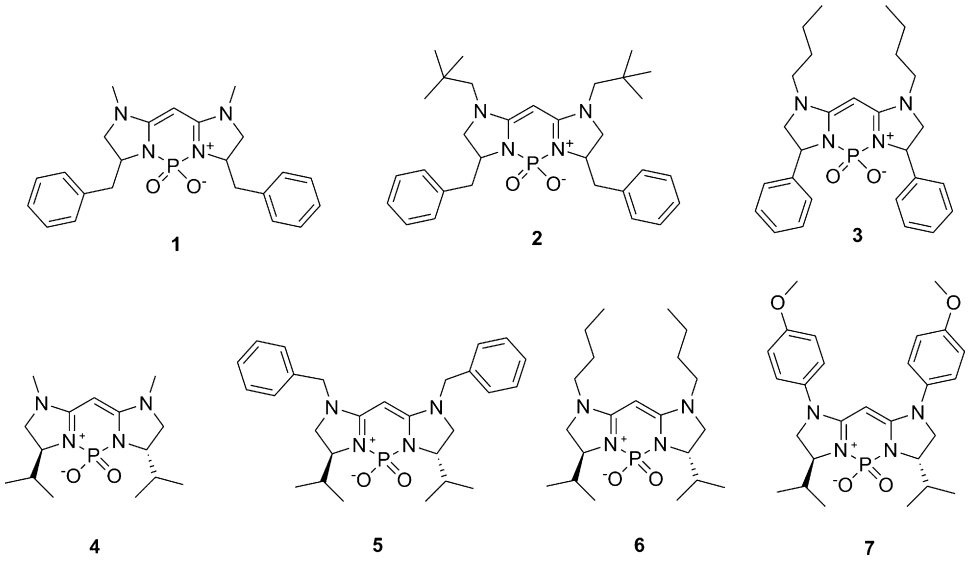

Fig. 1. Molecular structures of the compounds analyzed. For the full names of the compounds see Table 1.

medium strong acids possessing ionogenic groups with low pK_a values in order to enhance the selectivity of their separation and to determine their pK_a values and ionic mobilities. Gluck et al. [8] and Barták et al. [9] used phosphoric acid-based strongly acidic BGEs at pH < 2 in their measurement of the pH dependence of effective mobilities and for the CZE determination of pK_a of analytes. Very acidic BGEs, up to pH 1.1, were applied also for the determination of pK_a values of phosphinic pseudopeptides by CZE [4,10]. To shorten the analysis time for the pK_a determination in low pH region various approaches, such as the successive multiple ionic-polymer layer (SMIL) coating [11] or application of an external air pressure [12–14] have been used. However, an additional pressure driven flow inside the capillary alters the EOF typical piston-like flow profile and may cause peak broadening or even overlapping of EOF marker peak and the analyte peak, if they are migrating closely and/or too high pressure is employed. For that reason, neither of the ways of increasing the velocity of neutral EOF marker was employed in this study.

The aim of this work was to employ CZE for the determination of the thermodynamic acid–base dissociation constants (pK_a) of seven newly synthesized zwitterionic heterocyclic bases (see Fig. 1 and Table 1). To achieve this goal, the pH dependence of the effective electrophoretic mobilities of these compounds was measured by CZE in bare fused silica capillaries in the BGEs within a very low pH range 0.85–2.80. The effective electrophoretic mobilities, measured by CZE at non-constant temperatures and ionic strengths (due to different ambient temperatures, Joule heating and high ionic strength of the extremely acidic BGEs), were first corrected to reference temperature, 25 °C, and to constant ionic strength, 25 mM. Subsequently, these corrected mobilities were used to determine the pK_a values of the studied compounds. Despite the use of extremely low pH BGEs, which resulted in rather long analysis times and wide peaks, the pK_a values of the above zwitterionic heterocyclic bases could be determined with a sufficient precision. According to our best knowledge, the determination of such exceptionally low pK_a values by CZE has not been yet reported.

Table 1
List of compounds analyzed and their relative molecular masses, M_r.

Compound no.	Compound name	M_r
1	(3S,7S)-3,7-dibenzyl-1,9-dimethyl-2,3,5,7,8,9-hexahydro-1H-diimidazo[1,2-c:2′,1′f][1,3,2]diazaphosphinin-4-ium-5-olate 5-oxide	422.0
2	(3S,7S)-3,7-dibenzyl-1,9-bis(2,2-dimethylpropyl)-2,3,5,7,8,9-hexahydro-1H-diimidazo[1,2-c:2′,1′-f][1,3,2]diazaphosphinin-4-ium-5-olate 5-oxide	534.7
3	(3S,7S)-1,9-dibutyl-3,7-diphenyl-2,3,5,7,8,9-hexahydro-1H-diimidazo[1,2-c:2′,1′-f][1,3,2]diazaphosphinin-4-ium-5-olate 5-oxide	478.3
4	(3S,7S)-3,7-diisopropyl-1,9-dimethyl-2,3,5,7,8,9-hexahydro-1H-diimidazo[1,2-c:2′,1′-f][1,3,2]diazaphosphinin-4-ium-5-olate 5-oxide	326.3
5	(3S,7S)-1,9-dibenzyl-3,7-diisopropyl-2,3,5,7,8,9-hexahydro-1H-diimidazo[1,2-c:2′,1′-f][1,3,2]diazaphosphinin-4-ium-5-olate 5-oxide	478.6
6	(3S,7S)-1,9-dibutyl-3,7-diisopropyl-2,3,5,7,8,9-hexahydro-1H-diimidazo[1,2-c:2′,1′-f][1,3,2]diazaphosphinin-4-ium-5-olate 5-oxide	410.5
7	(3S,7S)-3,7-diisopropyl-1,9-bis(4-metoxyphenyl)-2,3,5,7,8,9-hexahydro-1H-diimidazo[1,2-c:2′,1′-f][1,3,2]diazaphosphinin-4-ium-5-olate 5-oxide	510.6

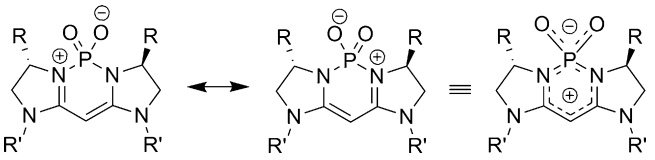

Fig. 2. Resonance structures of the zwitterionic heterocyclic bases.

2. Materials and methods

2.1. Chemicals

All of the chemicals used were analytical reagent-grade. Phosphoric acid, potassium chloride and dimethyl sulfoxide (DMSO) were obtained from Lachema (Brno, Czech Republic) and tris(hydroxymethyl)aminomethane (Tris) from Merck (Hohenbrunn, Germany).

2.2. Analyzed compounds

The analyzed bipolar compounds are the first representatives of a new heterocyclic family of 2,3,5,7,8,9-hexahydro-1*H*-diimidazo[1,2-*c*:2′,1′-*f*][1,3,2]diazaphosphinin-4-ium-5-olate 5-oxides (see Fig. 1 and Table 1).

These compounds feature a conformationally rigid, C_2-symmetrical structure with distinctly asymmetric framework provided by two tertiary homochiral centers originating from naturally occurring amino acids. They were obtained by condensation of diethyl malonimidate with corresponding homochiral diamines derived from the amino acids followed by central ring closure with $POCl_3$ (details of the synthesis will be published elsewhere) and containing elements of chirality in the overall C_2-symmetrical structure.

These structures were originally designed to serve as chiral Lewis base catalysts for asymmetric addition reactions of silylated pronucleophiles R_3Si–Nu such as Me_3SiCN, Me_3SiCF_3, allyl(trimethyl)- or (trichloro)silanes, Mukayama aldol and related reactions. These compounds are formally zwitterionic, with the negative charge being located on the oxygen centers of the PO_2 moiety while the positive charge is evenly distributed over the network of electron-donating nitrogen atoms (see Fig. 2).

The efficiency and scope of applicability of these compounds as Lewis base catalysts depends on the nucleophilic characteristics of their PO_2 moiety. The catalytic mode of activation with these chiral compounds is envisaged via precoordination of the oxygen centre to the silyl group of the pronucleophile thereby triggering a redistribution of the electron density in the resulting chiral hypervalent silicate intermediate ($n-\sigma^*$ activation) [7] and rendering the Nu group more nucleophilic and hence amenable to interaction with an electrophile. This mode of activation is known for anionic promoters as well as for bipolar *O*-nucleophilic Lewis bases such as phosphamides or amine-*N*-oxides.

2.3. Instrumentation and experimental conditions

CZE experiments were performed in a home-made apparatus [15] equipped with a UV photometric detector monitoring absorbance at 206 nm. The measurements were performed in the internally uncoated CE capillary, total/effective length of 306/200 mm, and i.d./o.d. of 50/375 μm. The separations were performed at ambient temperature, 23–26 °C. To minimize the increase of temperature inside the capillary the applied separation voltage was set so that the input power per unit of capillary length was always ~0.4 W/m (see Table 2). This Joule heat caused ca 4 °C temperature increase inside the capillary. A Clarity chromatography station (DataApex, Prague, CR) was used for data acquisition, and the Origin 6.1 program (OriginLab Corp., Northampton, MA, USA) was employed for the non-linear regression analysis. Before the first use and between the series of analyses in different BGEs, the capillary was conditioned by subsequent rinsing with water, 0.1 M NaOH, water and BGE, each rinsing for 5 min. Finally, the capillary was conditioned by a 20 min application of the high voltage to equilibrate the inner surface and to stabilize EOF. Between runs in the same BGE, the capillary was rinsed with the BGE for 2 min. BGEs were prepared from deionized water and filtered through a 0.45 μm pore filter (Millipore, Bedford, MA, USA) before use. BGE solutions composed of phosphoric acid or Tris/phosphoric acid buffers are presented in Table 2. Ionic strength of the BGEs was close to 25 mM except those composed of pure phosphoric acid. The higher ionic strength of phosphoric acid-based BGEs was unavoidable in the very low pH range, 0.85–1.54. The zwitterionic heterocyclic bases studied were dissolved in deionized water at concentration 0.9–1.1 mM and they were hydrodynamically introduced into the capillary followed by the injection of the EOF marker (1 mM DMSO in the BGE) by pneumatically induced pressure, 10 mbar for 5 s each.

2.4. Determination of the effective electrophoretic mobilities

The effective electrophoretic mobilities of analytes, m_{eff}, were determined from measurement of their migration times, t_m, and from migration time of the neutral EOF marker, t_{eof}, at various pH of the BGEs. In all experiments DMSO was used as EOF marker and m_{eff} was calculated from Eq. (1)

$$m_{eff} = \frac{L_t L_{eff}}{U}\left(\frac{1}{t_m} - \frac{1}{t_{eof}}\right) \quad (1)$$

where L_t and L_{eff} are the total and effective capillary length, respectively, and U is the applied separation voltage.

3. Theory of the acid–base equilibria of the studied heterocyclic bases

The studied heterocyclic bases behave as zwitterions with effective charge close to zero in BGEs within almost the whole pH range classically based on water as solvent (see Fig. 2). Only at very acidic pH region, they are present in the form of singly charged cations.

Table 2
pH, composition and ionic strength of the BGEs used, and voltage and input power per unit capillary length of CZE experiments.

pH	H_3PO_4 (mM)	Tris (mM)	Ionic strength (mM)	Voltage (kV)	Input power (W/m)
0.85	1050	0	141.3	1.7	0.40
1.20	500	0	63.1	2.4	0.41
1.36	250	0	43.7	3.0	0.42
1.54	150	0	28.8	3.8	0.41
1.98	55	14	24.5	5.9	0.43
2.17	45	18	24.8	6.8	0.43
2.36	37	20	24.4	7.7	0.42
2.80	30	24	25.6	9.4	0.42

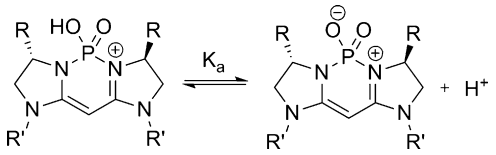

Fig. 3. The acid/base dissociation equilibrium of the heterocyclic bases in acidic pH range.

Their acid/base dissociation equilibrium in acidic pH range is shown in Fig. 3.

This equilibrium can be schematically described as:

$$HAB^+ \rightleftharpoons A^-B^+ + H^+ \quad (2)$$

where HAB^+ represents the cationic protonated form and A^-B^+ is the zwitterionic form of the base.

The corresponding thermodynamic dissociation constant K_a of the acidic form HAB^+ is defined as:

$$K_a = \frac{a_{A^-B^+} a_{H^+}}{a_{HAB^+}} = \frac{c_{A^-B^+} \gamma_{A^-B^+} a_{H^+}}{c_{HAB^+} \gamma_{HAB^+}} \quad (3)$$

where a_{H^+} is the activity of hydrogen ions, $a_{A^-B^+}$ and a_{HAB^+} are the activities of the zwitterionic and cationic form of the heterocyclic bases, respectively. Activities of species A^-B^+ and HAB^+ can be expressed as a product of their molar concentrations $c_{A^-B^+}$ and c_{HAB^+}, and corresponding activity coefficients $\gamma_{A^-B^+}$ and γ_{HAB^+}. The activity coefficient of zwitterionic species A^-B^+ with zero effective charge is assumed to be equal to 1. The value of the activity coefficient of charged species for aqueous solutions at 25 °C can be evaluated according to Debye–Hückel equation [16]:

$$\log \gamma = -\frac{0.509 z^2 \sqrt{I}}{1 + 3.28 a \sqrt{I}} \quad (4)$$

where z is the charge number of the ion, I is the ionic strength of the solution (mol dm^{-3}) and a is the sum of the hydrodynamic radii of the analyte ion and counterion (nm).

The pH of the BGE is related to the activity of hydrogen ions. However, the experimentally determined effective mobilities are related to the molar concentrations of ionic species, therefore it is convenient to introduce so-called "mixed" dissociation constant K_a^{mix} [17]:

$$K_a^{mix} = \frac{c_{A^-B^+} a_{H^+}}{c_{HAB^+}} \quad (5)$$

The substitution of Eq. (5) in Eq. (3) with respect to unity value of $\gamma_{A^-B^+}$ gives:

$$pK_a = pK_a^{mix} + \log \gamma_{HAB^+} \quad (6)$$

The effective mobility of the analyte, which is determined during the CZE experiments, is equal to a sum of the products of molar fractions and ionic mobilities of all ionic forms of the analyte. Thus, the effective mobility of the zwitterionic heterocyclic base, $m_{eff,AB}$, at low pH can be expressed as:

$$m_{eff,AB} = \frac{m_{HAB^+} c_{HAB^+}}{c_{HAB^+} + c_{A^-B^+}} \quad (7)$$

where m_{HAB^+} is the actual ionic mobility of the cationic form of the zwitterionic heterocyclic base. Using Eqs. (5) and (7) we obtain the dependence of the effective mobility of the base, $m_{eff,AB}$, on pH:

$$m_{eff,AB} = \frac{m_{HAB^+}}{1 + 10^{pH - pK_a^{mix}}} \quad (8)$$

This function can be used as the regression function for fitting the pH dependence of the effective mobilities obtained from CZE analyses of the investigated compounds in the BGEs of different pH. The fitted parameters are the actual ionic mobility, m_{HAB+}, and the mixed dissociation constant, pK_a^{mix}, which can be subsequently corrected to the zero ionic strength, i.e. to the thermodynamic dissociation constant pK_a using Eq. (6).

4. Results and discussion

4.1. Correction of mobilities to reference temperature and constant ionic strength

For accurate determination of the acid–base dissociation constants, all CZE experiments should be carried out at constant temperature and ionic strength. However, the home-made device employed in this study was not equipped with any active cooling of the capillary. Thus, it was necessary to determine the actual temperature inside the capillary during a CE experiment and to correct the calculated mobilities to the reference temperature, 25 °C. The correction procedure is thoroughly described in our previous work [18]. Briefly, the separation capillary was filled with 0.02 M potassium chloride aqueous solution and the electric current was measured over a wide range of voltages with the same set-up as used for CZE experiments. From these measurements, the electric resistance of the 0.02 M KCl solution, which is changing with temperature mainly due to the temperature dependence of the viscosity, at a given input power (voltage × current) was calculated. Taking into account the 2% decrease in viscosity per degree Celsius (°C) from 20 to 30 °C in water, the change of solution resistance at given input power applied was recalculated to temperature increment and a calibration curve of the temperature increase inside the capillary, ΔT, versus input power was obtained. The measured effective electrophoretic mobilities were recalculated to the reference temperature of 25 °C assuming the mean increase in mobilities to be 2% per °C:

$$m_{eff,25} = m_{eff,T}[1 - 0.020(T_0 + \Delta T - 25)] \quad (9)$$

where $m_{eff,25}$ and $m_{eff,T}$ are effective mobilities at 25 °C and at actual temperature T inside the capillary ($T = T_0 + \Delta T$), respectively. T_0 is the ambient temperature.

An additional requirement for correction arises because the strongly acidic BGEs (pH < 1.98) had a higher ionic strength than the selected *ca.* 25 mM ionic strength of the Tris-phosphate buffer-based BGEs. Thus, the mobilities obtained in these BGEs and already corrected to reference temperature, 25 °C, had to be recalculated to the values corresponding to the ionic strength of 25 mM. For mobility corrections, the extended Debye–Hückel–Onsager theory was employed, using for the description of the dependence of the ionic mobility of a uni-univalent electrolyte on ionic strength the following equation [17,19–22]:

$$m = m_0 - \left(0.391 \frac{2q}{1 + \sqrt{q}} z_+ z_- m_0 + 3.13 \times 10^{-8} z_+\right) \times \frac{\sqrt{I}}{1 + 3.28 a \sqrt{I}} \quad (10)$$

where m_0 is the mobility at zero ionic strength (m^2 V^{-1} s^{-1}), z_+ and z_- are the charge numbers of the cationic analyte and anionic BGE counterion, respectively. Parameter q is defined as:

$$q = \frac{z_+ z_-}{z_+ + z_-} \frac{m_{0+} + m_{0-}}{z_+ m_{0+} + z_- m_{0-}} \quad (11)$$

where m_{0+} and m_{0-} are the limiting mobilities of cationic analyte and anionic counterion, respectively. For a univalent analyte in a uni-univalent BGE, $q = 0.5$ and Eq. (10) becomes identical to that derived by Survay et al. [16]:

$$m = m_0 - \frac{\left(0.229 m_0 + 3.12 \times 10^{-8}\right) \sqrt{I}}{1 + 3.28 a \sqrt{I}} \quad (12)$$

where a is the sum of the hydrodynamic radii of the analyte ion, r_a, and counterion, r_c (nm).

Value of radius of the BGE counterion $H_2PO_4^-$, $r_c = 0.28$ nm, was obtained from the absolute mobilities presented by Pospíchal et al. [23] using the equation relating absolute mobility of the ion with its radius [20]:

$$r = \frac{ze}{6\pi\eta m_0} \quad (13)$$

where e is the elementary charge and η is the viscosity of the solvent.

In the first cycle of calculation procedure, the approximate value $r_a = 0.25$ nm was used for the radius of cationic forms of the bases. Later on, when the absolute mobilities and hydrodynamic radii of the cationic forms of the bases are obtained (see Section 4.3), the more precise analyte radius, r_a, is calculated, and the corrected parameter a ($a = r_a + 0.28$) is substituted into Eq. (12). This procedure, i.e. correction of mobilities to constant ionic strength, determination of mixed pK_a values, actual and absolute ionic mobilities and calculation of hydrodynamic radii is repeated few times until the changes of the calculated magnitudes become negligible. Eq. (12) has been justified to be valid for univalent ions migrating in aqueous uni-univalent BGEs for ionic strengths up to 75–100 mM [16,19,22]. Mobilities of the compounds analyzed at pH 0.85 were also corrected using Eq. (12) despite the fact that the ionic strength of this BGE exceeded the limit of 100 mM. However, it is assumed that validity of Eq. (12) does not stop stepwise strictly at 75–100 mM ionic strength and that the error in the mobility correction caused by its application at 141 mM ionic strength will not be crucial. Moreover, due to the extremely low pK_a values to be determined, it is necessary to have the values of effective mobilities at pH as low as possible. We assume it is better to have at the lowest pH 0.85 at least the approximate value of the effective mobility than no value at all.

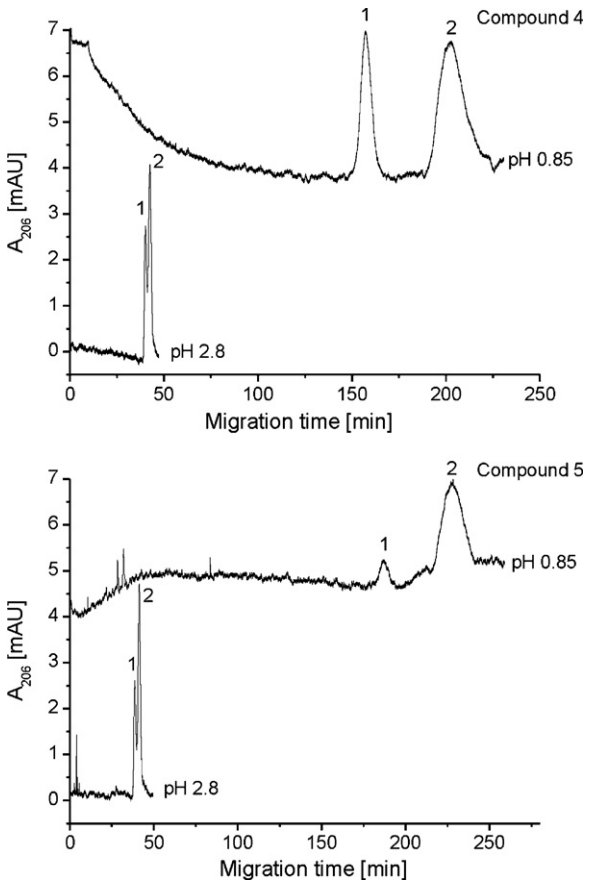

Fig. 4. Electropherograms of two representative heterocyclic bases (compounds **4** and **5**) in the BGEs of lowest (0.85) and highest (2.8) pH. 1, peaks of the bases; 2, peaks of the EOF marker (DMSO). The BGE constituents are given in Table 2. For other experimental conditions see Section 2.3.

4.2. Determination of thermodynamic dissociation constants

The examples of CZE analyses of two heterocyclic bases (compounds **4** and **5**) in the BGEs at the lowest (0.85) and highest (2.80) pH values are shown in Fig. 4. From these electropherograms, the decrease of the differences of the migration times of the analytes and EOF marker, i.e. the decrease of effective mobilities of analytes with increasing pH of the BGE is apparent. The mixed dissociation constants, pK_a^{mix}, of the zwitterionic heterocyclic bases were calculated by a non-linear regression analysis from the fit of experimental effective mobilities (corrected to reference temperature of 25 °C and constant ionic strength of 25 mM, see Section 4.1) to the appropriate regression function, Eq. (8). The dependences of the effective mobilities of the heterocyclic bases on the pH of the BGE are presented in Fig. 5. From the pK_a^{mix} values, the thermodynamic pK_a were calculated according to Eq. (6). The values of thermodynamic pK_a are given in Table 3; they were found to be rather low, in the range of 0.04–0.32. The non-linear regression analysis of the pH dependence of effective mobilities provides also standard deviations of the pK_a values. They have been found to be in the range 0.03–0.07, i.e. in spite of extreme conditions of CZE experiments (very low pH and very high ionic strength of some of the BGEs employed), the pK_a values could be determined with relatively good precision. In general, the differences among the pK_a values of the heterocyclic bases studied are rather low, reflecting the fact that the substituents on the nitrogen atoms are relatively similar and not directly neighboring the diaza-4-phosphinium-5-olate 5-oxide group. A moderate increase of basicity (pK_a values) can be observed in the series of compounds **1**–**3** with increasing length of the linear aliphatic chain on the nitrogen atoms. Slightly increased basicity was found for compound **6** with aliphatic substituents on the N-atoms as compared to the basicity of compound **7** with the aromatic substituents on the N-atoms.

Generally, CZE is widely used for the determination of pK_a values in the pH 2–12 range. In this study, the electrophoretic mobilities, which were used for pK_a calculations, were measured in the BGEs

Table 3
Thermodynamic pK_a values of the zwitterionic heterocyclic bases (compounds **1–7)** and actual ionic mobilities, m_{HAB+}, absolute ionic mobilities, m_{HAB+0}, and hydrodynamic radii, r_{HAB+0}, of the acidic cationic forms of the bases. pK_a, m_{HAB+0} and r_{HAB+0} values are related to reference temperature 25 °C and zero ionic strength, m_{HAB+} is related to the temperature 25 °C and ionic strength 25 mM.

Compound no.	pK_a ± SD	(m_{HAB+} ± SD) × 10^9 ($m^2\,V^{-1}\,s^{-1}$)	m_{HAB+0} × 10^9 ($m^2\,V^{-1}\,s^{-1}$)	r_{HAB+0} (nm)
1	0.04 ± 0.05	25.0 ± 2.5	29.7	0.32
2	0.16 ± 0.04	20.0 ± 1.6	24.6	0.39
3	0.32 ± 0.03	17.1 ± 1.0	21.5	0.44
4	0.14 ± 0.07	22.7 ± 3.0	27.4	0.35
5	0.10 ± 0.05	23.4 ± 2.3	28.0	0.34
6	0.25 ± 0.05	18.7 ± 1.6	23.2	0.41
7	0.06 ± 0.05	24.7 ± 2.4	29.4	0.33

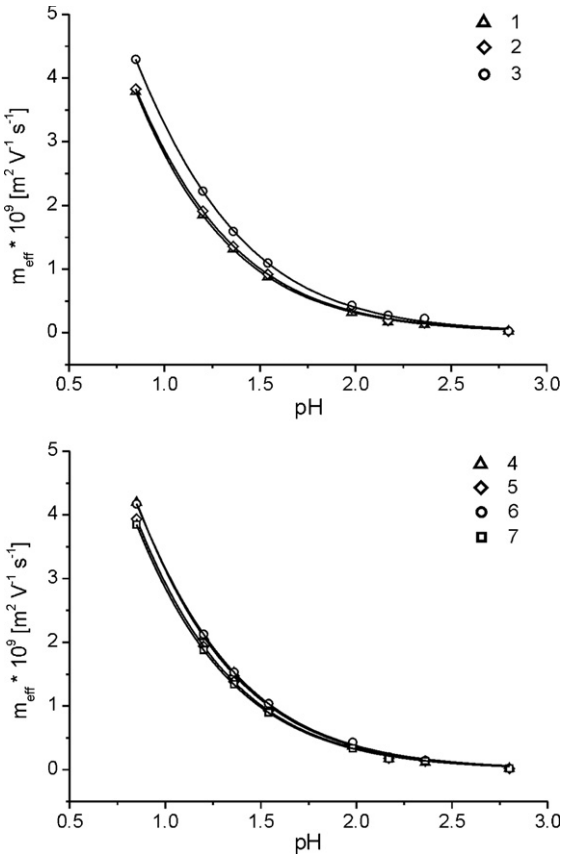

Fig. 5. Dependence of effective mobilities of the zwitterionic heterocyclic bases (corrected to reference temperature 25 °C and constant ionic strength 25 mM), m_{eff}, on pH.

at pH values down to 0.85. However, the estimated pKa values of the heterocyclic bases are still lower than the lowest experimental pH. This is one of the reasons for the increased SD values of the estimated pK_a and actual ionic mobilities (see Table 3). It is impracticable to use BGEs of pH even lower than 0.85, because of the high concentration of the highly mobile H^+ ions in such solutions. The high ionic strengths and conductivities of such BGEs would require strong corrections of the effective mobilties to the reference temperature and constant ionic strength, already behind the validity of the above equations describing the temperature and concentration dependence of the mobility.

4.3. Determination of actual and absolute ionic mobilities and hydrodynamic radii

In addition to pK_a values of the weak zwitterionic heterocyclic bases, from the pH dependence of their effective mobilities also some other relevant qualitative characteristics of the acidic cationic forms of the bases can be obtained. The non-linear regression analysis according to Eq. (8) provides also the actual ionic mobilities of the acidic cationic forms of the bases, m_{HAB+}, see Table 3. These actual ionic mobilities are related to reference temperature 25 °C and 25 mM ionic strength. They can be extrapolated to zero ionic strength according to Eq. (12) resulting in absolute (limiting) mobilities of the cationic forms of the bases, m_{HAB+0}. Knowledge of absolute mobilities also allows calculation of the hydrodynamic radii, r_{HAB+0}, using the Stokes Einstein equation (13). Both absolute ionic mobilities and radii of the cationic forms of the bases are summarized in Table 3. The highest hydrodynamic radii have compounds **3** and **6** (with 1,9-dibutyl substituents), the lowest compounds **1**, **7**, **4** and **5**, whilst compound **2** (with 1,9-bis(2,2-dimethylpropyl) substituents) has an intermediate value. It appears that long alkyl chains have greater impact on hydrodynamic radius than less hydrophobic aryl groups.

Thus, from CZE experiments, a series of relevant characteristics, such as acid base properties (pK_a) and effective mobilities of the weak zwitterionic heterocyclic bases and actual and absolute electrophoretic mobilities and hydrodynamic radii of their acidic cationic forms can be determined.

5. Concluding remarks

The CZE method described in the present study was found to be a suitable and efficient technique for determination of the exceptionally low pK_a values of seven very weak zwitterionic heterocyclic bases in an aqueous medium. Despite the use of BGEs in the low pH range 0.85–2.80 in order to measure the effective mobilities of the zwitterionic heterocyclic bases with the highest practicable proportions of their positively charged forms, the determination of pK_a values of these extremely weak bases was achieved with reasonable precision.

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References

[1] S.K. Poole, S. Patel, K. Dehring, H. Workman, C.F. Poole, J. Chromatogr. A 1037 (2004) 445.
[2] S. Babic, A.J.M. Horvat, D.M. Pavlovic, M. Kastelan-Macan, Trends Anal. Chem. 26 (2007) 1043.
[3] Y. Henchoz, B. Bard, D. Guillarme, P.A. Carrupt, J.L. Veuthey, S. Martel, Anal. Bioanal. Chem. 394 (2009) 707.
[4] D. Koval, V. Kašička, J. Jiráček, M. Collinsová, Electrophoresis 27 (2006) 4648.
[5] V. Šolínová, V. Kašička, D. Koval, M. Česnek, A. Holý, Electrophoresis 27 (2006) 1006.
[6] S. Ehala, J. Míšek, I.G. Stará, I. Starý, V. Kašička, J. Sep. Sci. 31 (2008) 2686.
[7] S.E. Denmark, G.L. Beutner, Angew. Chem. Int. Ed. 47 (2008) 1560.
[8] S.J. Gluck, K.P. Steele, M.H. Benko, J. Chromatogr. A 745 (1996) 117.
[9] P. Barták, P. Bednář, Z. Stránský, P. Boček, R. Vespalec, J. Chromatogr. A 878 (2000) 249.
[10] D. Koval, V. Kašička, J. Jiráček, M. Collinsová, T.A. Garrow, J. Chromatogr. B 770 (2002) 145.
[11] H. Katayama, Y. Ishihama, N. Asakawa, Anal. Chem. 70 (1998) 2254.
[12] R. Jankowsky, M. Friebe, B. Noll, B. Johannsen, J. Chromatogr. A 833 (1999) 83.
[13] Z.J. Jia, T. Ramstad, M. Zhong, Electrophoresis 22 (2001) 1112.
[14] H. Wan, A. Holmen, M. Nagard, W. Lindberg, J. Chromatogr. A 979 (2002) 369.
[15] V. Kašička, Z. Prusík, P. Sázelová, E. Brynda, J. Stejskal, Electrophoresis 20 (1999) 2484.
[16] M.A. Survay, D.M. Goodall, S.A.C. Wren, R.C. Rowe, J. Chromatogr. A 741 (1996) 99.
[17] K. Včeláková, I. Zusková, E. Kenndler, B. Gaš, Electrophoresis 25 (2004) 309.
[18] S. Ehala, J. Dybal, E. Makrlík, V. Kašička, J. Sep. Sci. 32 (2009) 597.
[19] D.M. Li, S.L. Fu, C.A. Lucy, Anal. Chem. 71 (1999) 687.
[20] T. Nhujak, D.M. Goodall, Electrophoresis 22 (2001) 117.
[21] D. Koval, V. Kašička, I. Zusková, Electrophoresis 26 (2005) 3221.
[22] S.A. Allison, H.X. Pei, S. Baek, J. Brown, M.Y. Lee, V. Nguyen, U.T. Twahir, H.F. Wu, Electrophoresis 31 (2010) 920.
[23] J. Pospíchal, P. Gebauer, P. Boček, Chem. Rev. 89 (1989) 419.