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Determination of pKa values of 2-amino-2-oxazolines by capillary electrophoresis

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Abstract

The dissociation constants of new 2-amino-2-oxazolines were determined by capillary electrophoresis (CE) as a new technique. A method based on a linear model has been used in the CE determination. A series of eight 2-amino-2-oxazolines are investigated to determine their ionization constant. Among them, three new oxazolines synthesized are presented. The Ka values were obtained from the plots of reciprocal effective mobility against inverse concentrations of protons. The potentiometric method (PM) was performed as a comparative method. No significant differences were observed between the determined dissociation constants using both methods. Thus, the pKa values have been found to vary between 8.55 and 8.68.
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1. Introduction

The 2-amino-2-oxazolines are heterocyclic compounds with varied interesting pharmacological properties. Indeed, a large number of biologically active compounds belong to this chemical class such as Aminorex (appetite suppressant), Rilmenidine (hypotensive) [1–3]. In continuation of our works on this chemical series, showing a common potential pharmacology activity [4,5], we developed recently an original method [6] for the synthesis of new 2-amino-2-oxazolines. Thus, eight basic substituted 2-amino-2-oxazolines, with a hypotensive effect have been synthesized among which three have not yet been described.

The knowledge of ionization constants is important in the understanding of certain chemical phenomena such as biological uptake, activity and transport [7,8] and in the prediction of migration of these solutes in electromigration methods [9,10]. Hence, the discovery of new compounds requires accurate determination of pKa values. Since new molecules only exist in small quantities and some basic compounds of pharmaceutical interest are only sparingly soluble in water [11], the estimation of

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pKa may be difficult and precludes the classical pKa determinations. Indeed, precise potentiometric titration at low concentrations needs large volumes and a time-consuming preparation of carbonate-free solution. Furthermore, UV–Vis spectrophotometric titration is a suitable method for determination of the pKa at low concentrations, but the neutral and ionic states must give two different spectra.

Recently, capillary electrophoresis (CE) has been introduced as a convenient method for precise measurements of aqueous ionization constants [12–15]. To determine pKa values of compounds, CE offers several advantages over potentiometric and spectroscopic methods [8,12,14,15]: a negligible consumption of the analyte is required, impurities do not disturb the measurements and the exact solute concentration is not necessary, but only migration times. Moreover, close agreement between ionization constants determined by CE and literature values has been observed [16].

In this work, the electrophoretic behaviour of a series of oxazolines has been investigated using CE with direct UV detection at different pH. This allowed the determination of ionization constants from experimental migration time data. The pKa values of the oxazolines studied have been confirmed by a potentiometric method (PM) when their solubility was convenient.

2. Theory

The oxazolines are basic compounds, so their acid–base dissociation constant (Ka) is defined as:

$$\mathrm{Ka} = \frac{(\mathrm{B})(\mathrm{H}^+)}{(\mathrm{BH}^+)} = \frac{\gamma_\mathrm{B}[\mathrm{B}](\mathrm{H}^+)}{\gamma_{\mathrm{BH}^+}[\mathrm{BH}^+]} \qquad (1)$$

Where (H^+), (BH^+) and (B) are, respectively, the activities of protons, protonated and neutral base. $[\mathrm{BH}^+]$ and [B] are the concentrations of protonated and neutral forms. γ is the activity coefficient of ionised (γ_{BH^+}) and neutral (γ_B) species.

Usually, the activity coefficient of the neutral species (γ_B) is assumed to be 1. So, relation (1) can be written as:

$$\mathrm{Ka} = \frac{[\mathrm{B}](\mathrm{H}^+)}{\gamma_{\mathrm{BH}^+}[\mathrm{BH}^+]} \qquad (2)$$

Since the pH influences the electrophoretic behaviour of the substances studied, can be established a relation between pH, pKa and the electrophoretic mobility of compounds (μ_ep).

The electrophoretic mobilities were calculated using the following formula:

$$\mu_\mathrm{ep} = \mu_\mathrm{app} - \mu_\mathrm{eof} = \frac{L_\mathrm{d} L_\mathrm{t}}{V}\left(\frac{1}{t_\mathrm{m}} - \frac{1}{t_0}\right) \qquad (3)$$

where μ_app is the apparent electrophoretic mobility of the solute, μ_eof is the electroosmotic mobility of a neutral marker. μ_ep, μ_eof and μ_app are in $\mathrm{cm}^2/\mathrm{Vs}$. L_d is the distance from the injection point to the detector (cm), L_t is the total length of capillary (cm), V is the applied voltage (volt), t_m and t_0 are the migration times (s) of the analyte and the neutral marker, respectively.

The electrophoretic mobility reaches a maximum value in the fully ionised form of solute ($\mu_\mathrm{ep} = \mu_{\mathrm{BH}^+}$) and is equal to zero in the neutral form. Then, in the pH values surrounding its pKa, the solute presents an intermediate mobility [11] defined as follows:

$$\mu_\mathrm{ep} = (\%\ \mathrm{BH}^+).\mu_{\mathrm{BH}^+} \qquad (4)$$

where% BH^+ is the fraction of a protonated oxazoline.

Moreover, as

$$\%\ \mathrm{BH}^+ = \frac{[\mathrm{BH}^+]}{[\mathrm{B}] + [\mathrm{BH}^+]} \qquad (5)$$

then

$$\mu_\mathrm{ep} = \frac{[\mathrm{BH}^+]}{[\mathrm{B}] + [\mathrm{BH}^+]}\mu_{\mathrm{BH}^+} \qquad (6)$$

So, Eq. (6) can be rearranged to give:

$$\frac{1}{\mu_\mathrm{ep}} = \frac{[\mathrm{B}]}{\mu_{\mathrm{BH}^+}[\mathrm{BH}^+]} + \frac{1}{\mu_{\mathrm{BH}^+}} \qquad (7)$$

According to Eq. (2):

$$\frac{[\mathrm{B}]}{[\mathrm{BH}^+]} = \gamma_{\mathrm{BH}^+}\mathrm{Ka}\frac{1}{(\mathrm{H}^+)} \qquad (8)$$

Thus, Eq. (7) becomes:

$$\frac{1}{\mu_{ep}} = Ka \cdot \frac{\gamma_{BH^+}}{\mu_{BH^+}(H^+)} + \frac{1}{\mu_{BH^+}} \quad (9)$$

This equation is used for linear regression analysis between

$$\frac{1}{\mu_{ep}} \text{ and } \frac{\gamma_{BH^+}}{\mu_{BH^+}(H^+)}.$$

The slope is equal to Ka.

3. Experimental

3.1. Reagents

Sodium borate, sodium acetate, acetic acid, sodium hydroxide, hydrochloridric acid were obtained from Prolabo (Paris, France). Phenoxyethanol and moxisylyte hydrochloride were supplied by Sigma (Saint Quentin Fallavier, France).

All chemicals used in the preparation of buffers and solutions were of analytical reagent grade. Water was distilled (Sartorius, Goettingen, Germany) deionised and filtered through a 0.45 μm nylon membrane.

Five oxazolines (5-cyclohexyloxymethyl-2-amino-2-oxazoline (**2**), 5-methoxymethyl-2-amino-2-oxazoline (**3**), 5-(2-furfuryl)oxymethyl-2-amino-2-oxazoline (**4**), 5-benzyloxymethyl-2-amino-2-oxazoline (**6**), 5-ethoxyethyloxymethyl-2-amino-2-oxazoline (**8**) were previously synthesized by the authors [6]. Three new molecules have been synthesized according the below general procedures. The chemical structure of oxazolines is presented in Table 1.

Table 1
Chemical structures of the 2-amino-2-oxazolines studied

Compounds	Chemical structure
Oxazoline 1	
Oxazoline 2	
Oxazoline 3	
Oxazoline 4	
Oxazoline 5	
Oxazoline 6	
Oxazoline 7	
Oxazoline 8	

3.2. Chemical procedures

Microanalyses were carried out at the Service Central d'Analyse CNRS, Vernaison, France. Melting points were determined with a SM-LUXPOL Leitz hot-stage microscope and are uncorrected. The IR spectra were obtained with a Bruker IFS 25 spectrophotometer. NMR data were recorded with a Bruker AC-200 spectrometer. Chemical shifts (δ ppm) and coupling constants (J Hz) were measured using tetramethylsilane as the internal standard.

The general procedure for the synthesis of 5-alkyloxymethyl-2-amino-2-oxazolines (**1**) and (**7**), according to method A [6] gave:

*5-isobutyloxymethyl-2-amino-2-oxazoline (**1**)*

White powder, heptane; IR (KB_r) ν cm^{-1}: 1688 (C=N), 3361 (NH); ^1H NMR ($CDCl_3$) δ: 4.82(s, 2H, N*H2*), 4.68–4.54 (m, 1H, C5-*H*), 3.76 (dd, 1H, J=12.2, 8.9, C4-H_{2a}), 3.52–3.33 (m, 3H, C4-H_{2b}, C6-H_2), 1.17 (s, 9H, 3 CH_3); ^{13}C NMR ($CDCl_3$) δ: 160.50 (*C*2), 79.72 (*C*5), 73.33 (*C7*), 63.87 (*C6*), 55.01 (*C4*), 27.43 (3 CH_3). Calculated for C8 H16

N2 O2 : C, 55.79; H, 9.36; N, 16.27; Found: C, 55.80; H, 9.40; N, 16.19%.

*5-isopropyloxymethyl-2-amino-2-oxazoline (**7**)*

White powder; IR (KB_r) ν cm^{-1}: 1682 (C=N), 3381 (NH); ^1H NMR ($CDCl_3$) δ: 4.80(s, 2H, N*H2*), 4.70–4.57 (m, 1H, C5-*H*), 3.76 (dd, 1H, J=12.2, 9.2, C4-H_{2a}), 3.60 (dd, 1H, J=12.2, 6.2, C4-H_{2b}), 3.53 (dd, 1H, J=10.5, 7.2, C6-H_{2a}), 3.41 (dd, 1H, J=10.5, 4.1, C6-H_{2b}), 3.36 (d, 1H, J=6.2, $(CH_3)_2CH$), 1.14 (d, 6H, J=6.2, 2 CH_3); ^{13}C NMR ($CDCl_3$) δ: 160.7 (*C*2), 79.2 (*C*5), 72.30 (($(CH_3)_2CH$), 69.9 (*C6*), 54.7 (*C4*), 21.8, 21.9 (2 CH_3). Calculated for C7 H14 N2 O2 : C, 53.15; H, 8.92; N, 17.71; Found: C, 53.18; H, 8.99; N, 17.19%.

The general procedure for the synthesis of 5-aryloxymethyl-2-amino-2-oxazoline (**5**) according to method B [6] gave:

*5-(2-xylyloxy)methyl-2-amino-2-oxazoline (**5**)*

White powder; IR (KB_r) ν cm^{-1}: 1654 (C=N), 3361 (NH); ^1H NMR ($CDCl_3$) δ: 7.31–7.10 (m, 4H, *H*-Ar), 4.82 (s, 2H, N*H2*), 4.77–4.65 (m, 1H, C5-*H*), 4,59 (d, 1H, J=12.0, Ar-C*H2a*), 4,52 (d, 1H, J=12.0, Ar-C*H2b*), 3.77 (dd, 1H, J=12.2, 9.1, C4-H_{2a}), 3.56 (dd, 1H, J=10.5, 6.8, C6-H_{2a}), 3.50 (dd, 1H, J=10.5, 4.1, C6-H_{2b}), 3.40 (dd, 1H, J=12.2, 6.9, C4-H_{2b}), 2.33 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ: 160.7 (*C*2), 136.8, 135.5 (*C*9, *C*10), 130.3, 128.7, 128.0, 125.7, (*C*11, *C*12, *C*13, *C*14), 78.9 (*C*5), 71.8 (Ar *C*H2), 71.65 (*C*6), 54.7 (*C4*), 18.8 (CH_3). Calculated for C12 H16 N2 O2 : C, 65.45; H, 7.27; N, 12.73; Found: C, 65.24; H, 7.33; N, 12.66%.

3.2. Apparatus

All CE experiments were performed on a SpectraPHORESIS 1000 (ThermoQuest, Les Ulis, France) CE Instrument equipped with an autosampler, automatic injector and UV detector. The electropherograms were recorded and integrated with Spectraphoresis software (ThermoQuest).

An open polyimide fused-silica capillary obtained from ThermoQuest [65 cm (length to detector 58 cm)×75 μm ID] was used.

The pH of buffer solutions was measured with a Radiometer PHM 210 using a pHC 3005 combined pH glass electrode (Radiometer, Villeurbanne, France). For potentiometric determination, a Titralab 80 Radiometer was used with a combined pH glass electrode Tacussel XC601 (Radiometer, Villeurbanne, France).

3.3. Buffers and samples preparations

3.3.1. Buffers preparation

For CE, several buffer systems covering the pH range between 7.87 and 9.64 were selected. Each buffer was prepared with sodium borate at 15 m*M* and adjusted to the convenient pH with appropriate amounts of 1 *M* HCl or 0.1 *M* NaOH. For the determination of the electrophoretic mobilities of the fully ionised oxazolines, an 10 m*M* sodium acetate buffer was prepared and adjusted to pH 4.77 with 1 *M* acetic acid. The buffers were first filtered through a 0.45 μm filter and degassed in an ultrasonic bath prior to use.

3.3.2. Samples preparation

For CE, stock solutions of 10^{-2} *M* of all eight oxazolines and of moxisylyte hydrochloride were prepared in methanol. The working solutions were done by diluting the stock solutions in each corresponding buffer. The final concentration for oxazolines 1, 2, 3 and 7 was 5.10^{-4} *M* in borate buffers and in acetate buffers. The final concentrations for oxazolines 4, 5, 6, 8 and moxisylyte hydrochloride were 10^{-4} *M* in each buffer. All oxazoline solutions contained phenoxyethanol at 0.005% (v/v) as a neutral marker. The solutions were always degassed in an ultrasonic bath prior to use.

3.4. Electrophoretic conditions

Every day and before the change of each buffer, the capillary was purged with water for 5 min, with sodium hydroxyde 0.1 *M* for 5 min, followed by electrolyte solution for 20 min. The last step was the application of a voltage of 10 kV for 15 min with the capillary filled with buffer solution. Between each run, the capillary was rinsed successively with NaOH 0.1 *M* for 2 min followed by running buffer for 4 min in order to equilibrate the capillary. The experiments were performed using UV detection at 200 nm and temperature was maintained at 25 °C. A

10 kV separation voltage was applied, allowed to obtained good analysis conditions (maximum current intensity of 26 μA and time analysis lower than 15 min). Samples were injected using the hydrodynamic mode with an injection time of 6 s. Six injections for each sample were run and average electrophoretic mobilities were used in the calculation of pKa.

3.5. Potentiometric method

The compounds studied being very weakly soluble in water, an indirect potentiometric method was used. For each molecule, 5.10^{-4} mole was dissolved in 100 ml of 10^{-2} *M* hydrochloric acid. For the titration by a 10^{-2} *M* sodium hydroxide solution, 90 ml of freshly twice-distilled water were added to 10 ml of the acidic solution. In both cases, the ionic strength of the solution was maintained at 0.15 *M* by adding sodium chloride. For each reagent volume added, the measured pH allowed to obtain a pKa's value and a mean value of pKa was calculated from a series of almost constant values.

4. Results and discussion

4.1. Choice of the electroosmotic marker

Acetone, thiourea and phenoxyethanol were tested as neutral markers of electroosmotic flow (EOF). The best results (high absorbance and symmetrical peaks) were obtained using phenoxyethanol. Table 2 gives the calculated μ_{eof} for different oxazoline solutions containing 0.005% of phenoxyethanol at different pH values. The relative standard deviation of μ_{eof} was lower than 1% (neutral and alkaline conditions) and around of 3% (acidic region). The μ_{eof} values first increase, then are stable and finally decrease from pH 9.34 because of the increase of the buffer viscosity due to the NaOH added.

4.2. Choice of the buffer pH range

Ideally, to measure an unknown pKa value, it is important to cover a wide pH range. However, the eight oxazolines studied constitute an homogenous chemical class. The mean pKa value was expected around 9, according to the pKa values previously measured for seventeen 2-amino-2-oxazolines by Demotes-Mainard et al. [17]. For this reason we have chosen a borate buffer in order to bracket the expected pKa values. A pH range comprised between 7.87 and 9.69 has been used. An acetate buffer at pH 4.77 allowed to measure the maximum electrophoretic mobility of the fully ionised oxazolines.

4.3. Choice of buffer concentrations

The pKa determination depending on the ionic strength of the background electrolytes, the ionic strength must be constant throughout the buffer series. The stability of the ionic strength avoids viscosity changes between the different pH buffers used and allows a better stability of the electroosmotic mobilities. In order to keep the ionic strength constant, the contribution of NaOH or HCl to the ionic strength must be minimized. Therefore, a high buffer concentration (100 m*M*) is often preferred. A concentrated buffer also provides a higher buffering capacity. On the other hand, diluted buffers show the advantage of a low joule heating. To go with these opposite conditions, concentrations of 10 and 15 m*M* have been chosen for acetate and borate buffers, respectively. These weak concentrations allow an acceptable current intensity between 6.5 and 26 μA (Table 3) without joule effect. Although different amounts of NaOH or HCl were added to the buffer solutions to adjust pH, the activity coefficients of the overall buffer series remained almost constant (Table 3).

Table 2
Electroosmotic mobility as a function of electrolyte pH

Buffer pH	4.77	7.87	8.02	8.33	8.57	8.98	9.34	9.69
μ_{eof}	39.7	60.6	64.1	64.6	64.9	64.3	61.4	50.6

μ_{eof}: electroosmotic mobility (10^{-5} cm^2 V^{-1} s^{-1}).

Table 3
Composition of buffers and stock solutions, activity coefficient and current (μA) in the capillary

pH	Buffers	Stock solutions	$-\log \gamma$	Current
4.77	Acetate	$C_2H_3NaO_2$, $3H_2O$, 10 m*M*, $C_2H_4O_2$ 1 *M*	0.093	6.5
7.87	Borate + HCl	$B_4Na_2O_7$, $10H_2O$, 15 m*M*, HCl 1 *M*	0.088	26
8.02	Borate + HCl	$B_4Na_2O_7$, $10H_2O$, 15 m*M*, HCl 1 *M*	0.087	25
8.33	Borate + HCl	$B_4Na_2O_7$, $10H_2O$, 15 m*M*, HCl 1 *M*	0.086	25
8.57	Borate + HCl	$B_4Na_2O_7$, $10H_2O$, 15 m*M*, HCl 1*M*	0.085	23
8.98	Borate + HCl	$B_4Na_2O_7$, $10H_2O$, 15 m*M*, HCl 1 *M*	0.083	18
9.34	Borate + NaOH	$B_4Na_2O_7$, $10H_2O$, 15 m*M*, NaOH 0.1 *M*	0.081	26
9.69	Borate + NaOH	$B_4Na_2O_7$, $10H_2O$, 15 m*M*, NaOH 0.1 *M*	0.085	20

4.4. Calculation of pKa values

In the present paper we studied the determination of the dissociation constants of oxazolines by CE as a new technique as well as by potentiometric titration as a comparison method. Because of its pKa value close to the expected pKa of the oxazolines studied, moxisylyte hydrochloride was used as a reference standard in the pKa determination by CE [18].

The oxazolines 1, 2, 3 and 7 showing a weak UV absorption, only due to the oxazoline moiety as a chromophore group, the concentrations used for these four oxazolines were more higher than those of oxazolines 4, 5, 6 and 8, which presented unsaturated cyclic groups. However, the concentration of the samples must be kept low enough not to affect the pH of the running buffer. We checked that no pH variation exits between the buffer alone and the oxazoline solutions in electrolytes.

The linear regression analysis were then performed on the data from Eq (9). An example with oxazoline 3 is given in Fig. 1. At each of the seven pH values, six replicate determinations were carried out for each oxazoline and the moxisylyte hydrochloride. The *F* values obtained from each compound studied showed significant linear regressions (Table 4). The electrophoretic mobilities ($\mu_{BH+\ calc}$) calculated from Eq. (9) confirmed this linear analysis. Indeed, the values obtained are not significantly different from the experimental electrophoretic mobilities ($\mu_{BH+\ exp}$) determined from Eq. (3) at pH 4.77 (Table 4). The pKa values calculated from the slope (Ka) are given in Table 5. The pKa values determined by CE are close to those determined by PM, with values comprised between 8.55 and 8.68 (Table 5). However, the oxazoline 8 being very weakly soluble in water, the pKa determination was

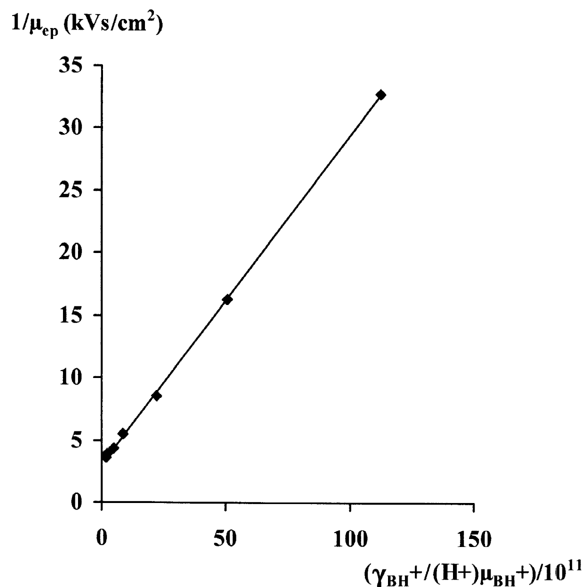

Fig. 1. Plot of the reciprocal electrophoretic mobility (μ_{ep}) as a function of $\gamma_{BH^+}/\mu_{BH^+}(H^+)$.

Table 4
Linear regression parameters obtained from Eq. (9)[a]

Compounds	**a** = Ka ± SD	**b** = $1/\mu_{BH+}$ ± SD	F	$\mu_{BH+calc} = 1/b$	μ_{BH+exp} ± SD
Oxazoline 1	$2.117.10^{-9} \pm 5.372.10^{-11}$	3800.95 ± 316.82	1552.49	$2.63.10^{-4}$	$2.89.10^{-4} \pm 3.358.10^{-7}$
Oxazoline 2	$2.549.10^{-9} \pm 1.363.10^{-10}$	3352.38 ± 861.42	349.66	$2.98.10^{-4}$	$2.69.10^{-4} \pm 2.607.10^{-7}$
Oxazoline 3	$2.634.10^{-9} \pm 2.216.10^{-11}$	3075.24 ± 105.22	14131.12	$3.25.10^{-4}$	$3.59.10^{-4} \pm 2.826.10^{-7}$
Oxazoline 4	$2.824.10^{-9} \pm 7.534.10^{-11}$	3297.06 ± 443.94	1405.31	$3.03.10^{-4}$	$2.89.10^{-4} \pm 6.315.10^{-7}$
Oxazoline 5	$2.487.10^{-9} \pm 7.467.10^{-11}$	3669.41 ± 485.77	1109.13	$2.73.10^{-4}$	$2.62.10^{-4} \pm 3.518.10^{-7}$
Oxazoline 6	$2.496.10^{-9} \pm 5.041.10^{-11}$	3633.27 ± 317.72	2451.94	$2.75.10^{-4}$	$2.70.10^{-4} \pm 4.176.10^{-7}$
Oxazoline 7	$2.093.10^{-9} \pm 3.210.10^{-11}$	3851.11 ± 218.71	4250.91	$2.60.10^{-4}$	$3.08.10^{-4} \pm 3.588.10^{-7}$
Oxazoline 8	$2.395.10^{-9} \pm 6.518.10^{-11}$	3759.54 ± 439.69	1350.07	$2.66.10^{-4}$	$2.52.10^{-4} \pm 5.682.10^{-7}$
Moxisylyte hydrochloride	$1.936.10^{-9} \pm 6.489.10^{-11}$	4857.65 ± 245.82	890.57	$2.06.10^{-4}$	$2.17.10^{-4} \pm 3.261.10^{-7}$

With **a** the slope and **b** the intercept.
[a] Comparison between $\mu_{BH+calc}$ calculated from Eq. (9) and μ_{BH+exp} obtained at pH 4.77 (Fisher–Test, 95% confidence interval, $n=6$ and standard deviation (SD)).

not possible by PM. Nevertheless, its pKa value (8.62) stayed in the range of the values determined by CE and PM for the other oxazolines. The pKa value of moxisylyte determined by CE is equal to 8.71 and is similar to the value (8.72) found in the literature [18]. Thus, the similar pKa values obtained show that the different groups do not change the basic character of the amidine function for this series of molecules.

5. Conclusion

In this investigation, the determination of dissociation constants of eight oxazolines and moxisylyte hydrochloride by capillary electrophoresis has been performed. The results showed that capillary electrophoresis is suitable for the pKa determination of compounds with very low water solubility as particularly observed with oxazoline 8. Moreover, CE allowed to work with very weak quantities of oxazolines (36 nl by injection). The moxisylyte hydrochloride used as a reference product confirmed the accuracy of the method. Finally, the pKa obtained using capillary electrophoresis were in excellent agreement with the values determined by potentiometry.

Table 5
pKa values determined by capillary electrophoresis (CE) and potentiometry (PM)

Compounds	pKa by CE	pKa by PM	Differences
Oxazoline 1	8.67	8.70	−0.03*
Oxazoline 2	8.59	8.60	−0.01*
Oxazoline 3	8.58	8.62	−0.04*
Oxazoline 4	8.55	8.56	−0.01*
Oxazoline 5	8.60	8.61	−0.01*
Oxazoline 6	8.60	8.58	+0.02*
Oxazoline 7	8.68	8.69	−0.01*
Oxazoline 8	8.62	–	
Moxisylyte hydrochloride	8.71	–	−0.01**

*Difference of pKa values between CE and PM.
**Difference of pKa values between CE and literature.

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