Determination of acid-base dissociation constants of newly synthesized arylethanolamine derivatives using capillary zone electrophoresis

IVA KAPUSTÍKOVÁ, MATEJ MARUNIAK, ĽUBICA HAVRANOVÁ-SICHROVSKÁ, LUKÁŠ STANZEL, IVAN MALÍK, EVA SEDLÁROVÁ
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Comenius University in Bratislava, Bratislava, Slovak Republic

Introduction

The acid-base dissociation constant (pK_a) is an important physicochemical parameter which affects drug pharmacokinetics and toxicity (ADMET: absorption, distribution, metabolism, excretion, toxicity). The early-obtained information about these drug properties may decrease the costly development process of a compound with poor pharmaceutical prospects[1, 2]. In the present study, dissociation constants of eight newly synthesized arylethanolamine derivatives, with potential α- and β-adrenolytic properties, are determined.

Determination of pK_a using capillary zone electrophoresis is based on the measurement of the effective electrophoretic mobility of studied substances as a function of separation electrolyte pH. This relationship is then fitted by a sigmoidal curve, where pK_a is the value of pH in the sigmoid inflection point. pK_a values obtained in buffer solution are recalculated to thermodynamic pK_a^T which correspond to water medium[3, 4].

Experimental methods

All chemicals and solvents were of analytical-reagent grade. HEPES, MES, MOPSO, TAPS were purchased from Sigma-Aldrich (Steinheim, Germany). Sodium hydroxide, mesityl oxide and sodium acetate trihydrate from Fluka (Buchs, Switzerland). Acetic and formic acid from Lachema (Brno, Czech Republic). Studied substances (Table 1), with potential α- and β-adrenolytic properties, were synthesized by PharmDr. Matej Maruniak, PhD. at Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Comenius University in Bratislava[5].

Experiments were carried out using an Agilent 3D CE system (Agilent Technologies, Waldbronn, Germany) equipped with a diode array detector. Results were enregistered by the system Agilent ChemStation and processed in the program SigmaPlot v. 10.0 (San Rafael, CA, USA). The pH values of used buffers were measured with a pH meter WTW InoLab pH Level 2 (Praha, Czech Republic) equipped with a combined electrode SenTix 41.

Electrophoretic measurements were performed in a fused-silica capillary of 48.5 cm total length, 40 cm effective length (from injection to detector) and 75 μm internal diameter. Before first using, capillary was rinsed with 0.1 mol.l^{-1} NaOH for 20 min, then with deionised water for 20 minutes. Between consecutive runs, the capillary was rinsed for 1 minute with 0.1 mol. l^{-1} NaOH solution, water and background electrolyte. When background electrolyte was changed, conditioning was extended to 15 min. The sample was injected by the pressure of 40 mbar for 4 s. A constant voltage of 15 kV was applied at 25 °C. Detection was at the wavelength of 254 nm.

Stock solution of $7.5.10^{-3}$ mol.l^{-1} of tested substances was prepared in deionised water. Concentration of mesityl oxide as the electroosmotic flow marker was 2.10^{-6} mol.l^{-1} in water. Working solutions were prepared by diluting the stock solution of analytes and mesityl oxide in each corresponding buffer to the final concentration of $7.5.10^{-4}$ mol.l^{-1} for analytes and 2.10^{-7} mol.l^{-1} for mesityl oxide.

Measurements were performed in the system of ten buffer solutions with a constant ionic strength 0.01 mol.l^{-1} within pH range of 4.5–8.5 (Table 2). Measurement of each compound in each of the background electrolytes was repeated four times. The online program Buffer Calculator was employed to calculate the composition of the series of background electrolytes with various pH[6].

The effective electrophoretic mobilities μ_{eff} ($m^2V^{-1}s^{-1}$) of analytes were calculated from observed migration times of each analyte and migration time of mesityl oxide according to the equation:

$$\mu_{eff} = \left(\frac{1}{t_{mig}} - \frac{1}{t_{EOF}}\right)\frac{L_d L_t}{U} \quad [1]$$

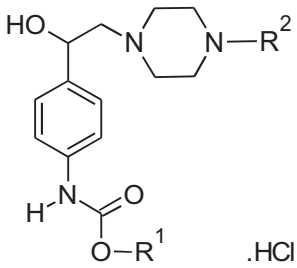
where L_t is the total length of the capillary (m), L_d is the effective length (m), U is the applied voltage (V), t_{mig} the migration time of analyte (s) and t_{EOF} the migration time of the electroosmotic flow marker (s)[3, 4].

The sets of the experimentally obtained effective mobilities were fitted by the sigmoid curve, which is for the monoprotic weak base described by the equation:

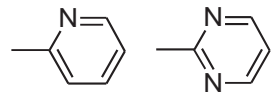
$$\mu_{eff} = \frac{\mu_{BH^+}}{1+10^{pH - pK_a}} \quad [2]$$

PharmDr. Iva Kapustíková, PhD. (✉)
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Comenius University in Bratislava
Odbojárov 10, 832 32 Bratislava, Slovak Republic
e-mail: kapustikova3@uniba.sk

Table 1. Studied substances



R^1: -CH_3; -C_2H_5; -C_3H_7; -C_4H_9

R^2: 

Compound	R^1	R^2	Summary formula	M_r
5m1	-CH_3	pyridine-2-yl	$C_{19}H_{25}N_4O_3Cl$	392.89
5e1	-C_2H_5	pyridine-2-yl	$C_{20}H_{27}N_4O_3Cl$	406.91
5p1	-C_3H_7	pyridine-2-yl	$C_{21}H_{29}N_4O_3Cl$	420.95
5b1	-C_4H_9	pyridine-2-yl	$C_{22}H_{31}N_4O_3Cl$	434.97
5m2	-CH_3	pyrimidine-2-yl	$C_{18}H_{24}N_5O_3Cl$	393.87
5e2	-C_2H_5	pyrimidine-2-yl	$C_{19}H_{26}N_5O_3Cl$	407.89
5p2	-C_3H_7	pyrimidine-2-yl	$C_{20}H_{28}N_5O_3Cl$	421.92
5b2	-C_4H_9	pyrimidine-2-yl	$C_{21}H_{30}N_5O_3Cl$	435.95

Table 2. Composition of background electrolyte system

pH	Background electrolyte	pK_a	Ionic strength (mol.l^{-1})
4.5	CH_3COOH/CH_3COONa	4.75	0.01
5.1	MES/NaOH	6.15	0.01
5.5	MES/NaOH	6.15	0.01
5.8	MES/NaOH	6.15	0.01
6.2	MES/NaOH	6.15	0.01
6.7	MOPSO/NaOH	6.90	0.01
7.0	MOPSO/NaOH	6.90	0.01
7.5	HEPES/NaOH	7.55	0.01
8.0	TAPS/NaOH	8.40	0.01
8.5	TAPS/NaOH	8.40	0.01

where μ_{BH+} is the ionic mobility of the fully protonated base. pK_a of the compound is the pH in the inflexion point of the sigmoidal curve[3, 4].

As the measurements are carried out in a system of buffer solutions of the ionic strength of 0.01 mol.l^{-1}, the obtained values of pK_a had to be converted to the thermodynamic dissociation constants (pK_a^T) which correspond to the aquatic environment of the zero ionic strength:

$$pK_a^T = pK_a + \log \gamma_{BH^+} \quad [3]$$

where for the calculation of the activity coefficients the following formula was used:

$$\log \gamma = -\frac{0{,}5084\, z^2 \sqrt{I}}{1+3{,}281\, a \sqrt{I}} \quad [4]$$

For the ionic strength of 0.01 mol.l^{-1} and a = 0,5 nm, log γ has a value of –0.0437[3, 4].

Results and discussion

Figure 1 shows an example of a typical experiment analysis. The injected mixture contained compound 5m1 and mesityl oxide, as the background electrolyte MOPSO (pH = 6,70) was used.

Measurements of each compound in each of the background electrolytes was repeated four times. The effective electrophoretic mobilities ($m^2V^{-1}s^{-1}$) were calculated using the formula [1] where t_{mig} is the migration time of the studied substance, whereas t_{EOF} denotes the migration of mesityl oxide as the electroosmotic flow maker. pK_a was determined using the dependence of the effective electrophoretic mobilities on the pH of the background electrolyte. This relationship was fitted in the program SigmaPlot v. 10.0 (San Rafael, CA, USA) by the sigmoidal mobility curve [2], where pK_a is the value of pH in the sigmoid inflection point.

Dissociation constants obtained in buffer solvent were recalculated to the thermodynamic values (pK_a^T) for water medium of zero ionic strength [3, 4]. The mobility curve

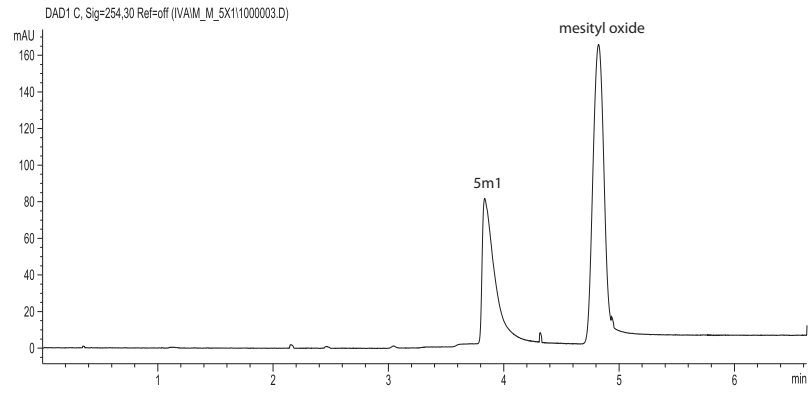


Fig. 1. Electropherogram of compound 5m1 (7.5.10–4 mol.l⁻¹) and mesityl oxide (2.10–6 mol.l⁻¹) in MOPSO/NaOH background electrolyte (pH = 6.70, I = 0.01 mol.l⁻¹), injection by pressure 40 mbar for 4 s, applied voltage +15 kV, temperature 25 °C, detection at 254 nm

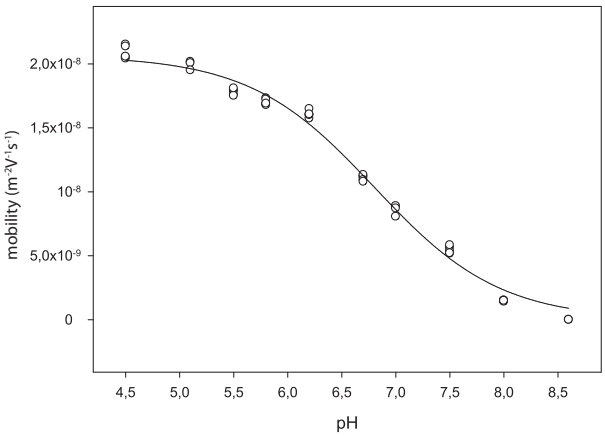


Fig. 2. Mobility curve of substance 5m1

Table 3. Dissociation constants (pK_a), thermodynamic dissociation constants (pK_a^T, standard deviations (s_d) and coefficients of determination (R^2)

Compound	pK_a	pK_a^T	s_d	R^2
5m1	6.87	6.83	0.06	0.995
5e1	6.82	6.78	0.07	0.992
5p1	6.70	6.66	0.09	0.989
5b1	6.82	6.78	0.10	0.981
5m2	6.90	6.86	0.06	0.987
5e2	6.82	6.78	0.06	0.989
5p2	6.81	6.77	0.05	0.987
5b2	6.79	6.75	0.09	0.980

of the selected analyte is shown in Figure 2. The pK_a^T and values of individual compounds are given in Table 3.

The compounds under study were monohydrochlorides of weak bases. The pH range of background electrolyte solutions was chosen in order to guarantee practically full protonation (BH^+) of analytes at the lowest pH and their no protonation (B) at the highest pH. We observed a decrease in the effective mobility with increasing pH. At the lowest pH the mobility of the analyte is maximal, which indicates the predominance of cationic species. As pH increases, a new species with zero electric charge is formed by deprotonation of the analyte and its effective mobility goes to zero. In the case of our molecules, we observed one inflexion point and the pK_a of the compound is the pH in this inflexion point of the sigmoid curve.

One inflexion point in the mentioned pH range confirms that analytes are monohydrochlorides and have one pK_a value. As assumed previously from their chemical structure, pK_a corresponds to protonated piperazine nitrogen which is closer to the arylethanolamine chain. The pK_a values of compounds do not refer about any evident influence of the chemical structure on the acidity of the molecule. Influence of pyridine-2-yl or pyrimidine-2-yl on the pK_a of substances was not proven. Any important influence of R^1 substituents (methyl–butyl) on pK_a value was not observed, either.

Conclusions

Dissociation constants were determined for eight newly synthesized potential drugs. The pK_a values of the compounds under study do not refer about any evident influence of the selected substituents on the acidity of the molecule. The results will be a help to a further study of the substances, first, the prediction of their pharmacokinetics and also in the optimization of the assay conditions of other physicochemical research.

An advantage of the capillary zone electrophoresis has been a need of a small quantity of the sample without the necessity of high purity, and the possibility to operate in an aqueous medium even in the case of low water solubility of studied substances.

This work was supported by the following grants: FaF UK/63/2015; FaF UK/28/2015; FaF UK/29/2015; FaF UK/44/2015; Grant UK/346/2015; Comenius University in Bratislava Science Park supported by the Research and Development Operational Programme funded by the ERDF – Grant number: ITMS 26240220086.

Conflicts of interest: none.

References

1. **Testa B., van de Waterbeemd H., Folkers G., Guy R.** Pharmacokinetic optimization in drug research. Weinheim: Wiley-VCH 2001.
2. **Babić S., Horvat A. J. M., Mutavdžić Pavlović D., Kaštelan-Macan M.** Determination of pK_a values of active pharmaceutical ingredients. Trends Anal. Chem. 2007; 26, 1043–1061.
3. **Fuguet E., Reta M., Gibert C., Rosés M., Bosch E., Ràfols C.** Critical evaluation of buffering solutions for pK_a determination by capillary electrophoresis. Electrophoresis 2008, 29, 2841–2851.
4. **Šlampová A., Křivánková L., Gebauer P., Boček P. J.** Standard systems for measurement of pK_a values and ionic mobilities 2. Univalent weak bases. J. Chromatogr. A 2009; 1216, 3637–3641.
5. **Maruniak M.** Preparation, physico-chemical properties and biological activity evaluation of cardiovascularactive potential drugs from the group of phenylcarbamic acid derivatives (Arylethanolamines, Aryloxyaminopropanols). Dissertation thesis. Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Comenius University in Bratislava 2012.
6. **Robinson D.** Buffer calculator. http://dbr.csoft.net/chem/bufcalc.php (24. 6. 2015).