

Determination of Acid Dissociation Constants of Poorly Water-Soluble Nicotinic Ligands by Means of Electrophoretic and Potentiometric Techniques

Gabriella Roda*, Clelia Dallanoce, Veniero Gambaro, Giovanni Grazioso, Vincenzo Liberti, Carlo Matera, Marta Quadri and Marco De Amici

Department of Pharmaceutical Sciences, Division of Medicinal Chemistry "Pietro Pratesi", University of Milan, Via L. Mangiagalli 25 20133, Milan, Italy

Abstract

Objectives: Acid-base dissociation constants of a series of poorly water-soluble nicotinic ligands designed as anti-inflammatory agents were determined in order to characterize the pharmacokinetic profile of this kind of ligands.

Methods: pK_a values were assessed by means of potentiometric and electrophoretic methods and investigated by computational protocols.

Results: Both electrophoretic and potentiometric measurements produced reliable results. However, with the electrophoretic technique only an average value for close pK_a s was found, whereas the potentiometric method allowed determination of each pK_a value in water-cosolvent mixtures. A theoretical treatment with various prediction programs - i.e. ADME Boxes v. 4.1, ACD/ pK_a DB and ACD/ pK_a GALAS - led in most cases to values which were not in accordance with the experimental ones.

Conclusion: Electrophoretic and potentiometric techniques showed complementary features. Indeed, with capillary electrophoresis, the problem associated with the low water solubility of the studied samples could be easily overcome, although this technique did not allow to measuring all dissociation constants. In contrast, application of the potentiometric method afforded all the theoretical pK_a values, although we had to perform the titrations in water-cosolvent mixtures, a less precise, more laborious and time-consuming approach.

Keywords: Dissociation constants; Capillary electrophoresis; Potentiometric titration; Nicotinic ligands

Introduction

The cholinergic anti-inflammatory pathway is a physiological mechanism modulating host inflammatory responses and immune system through cholinergic transmission mediated by $\alpha 7$ nicotinic acetylcholine receptors (nAChRs) expressed on macrophages, human microvascular endothelial cells and other cytokine-producing cells [1,2]. The neurotransmitter acetylcholine interacts with $\alpha 7$ nAChRs, down-regulating pro-inflammatory cytokine synthesis and preventing tissue damage [3]. Hence, the $\alpha 7$ receptor subtype is placed at the apex of key CNS and peripheral cellular pathways that are involved in anti-inflammatory processes as well as cell survival. Given these roles, selective activation of $\alpha 7$ nAChR is a viable and promising therapeutic strategy not only for a variety of disorders involving cognitive deficits and neurodegeneration but also for inflammatory-related diseases and conditions [4,5]. In this framework, CAP 55 (1) emerged as a model cholinergic compound due to its significant anti-inflammatory activity in inhibiting both endothelial cell activation and tumour necrosis factor (TNF) production [6].

As part of an ongoing research program on the study of novel heterocyclic derivatives targeting nAChR subtypes [7-10], we designed and prepared the set of compounds 2-9, in which the Δ^2 -isoxazoline moiety of reference ligand 1 was replaced by the 1,2,3-triazole ring (Figure 1). Along with their pharmacodynamic profile, ligands of putative pharmacological significance must be investigated also for their physicochemical features such as solubility, lipophilicity, hydrogen bonding capacity and charge, which affect their *in vivo* pharmacokinetic behaviour. The above mentioned parameters are easily calculated from the acidic dissociation constant of a compound under study, and the knowledge of pK_a values is crucial in view of predicting the interactions of small molecules with their protein counterpart [11,12]. In addition,

biologically active derivatives are often fully or partially ionized at physiological pH and the presence of ionisable groups is often essential in directing their pharmacological response.

Traditionally, potentiometric titrations are the standard method for pK_a determination [12]: the sample is titrated with acid or base using a pH electrode to monitor the course of the titration: pK_a values are calculated from the change of shape of the sample titration curve compared with that of a blank titration [13]. Since 1990 capillary electrophoresis has been proposed as an alternative method for the determination of ionization constants [12], which is based on the change of the electrophoretic mobility of the ionisable sample as a function of pH. In its uncharged state, the sample has no effective mobility, while in its fully ionized state it has a maximum mobility. Intermediate mobility is a function of the dissociation equilibrium, and pK_a values are determined by regression analysis [14]. In a previous paper, we reported the measure of the acid-base dissociation constants for a set of nAChR ligands with both potentiometric and electrophoretic methods [15]. Herein, we describe a parallel study on the determination of the dissociation constants of the group of new

***Corresponding author:** Gabriella Roda, Dipartimento di Scienze Farmaceutiche, Sezione di Chimica Farmaceutica "Pietro Pratesi", Università degli Studi di Milano, Via L. Mangiagalli 25, 20133, Milano, Italy, Tel: 02503 19328; E-mail: gabriella.roda@unimi.it

Received April 24, 2015; Accepted July 15, 2015; Published July 17, 2015

Citation: Roda G, Dallanoce C, Gambaro V, Grazioso G, Liberti V, et al. (2015) Determination of Acid Dissociation Constants of Poorly Water-Soluble Nicotinic Ligands by Means of Electrophoretic and Potentiometric Techniques. Pharm Anal Acta 6: 394. doi:10.4172/21532435.1000394

Copyright: © 2015 Roda G, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

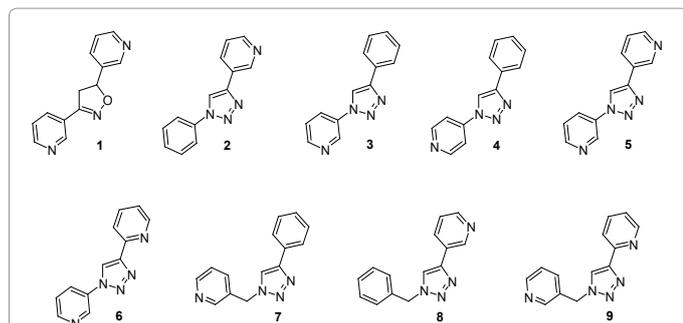


Figure 1: Chemical structures of model and target compounds 1-9 investigated in this study.

nicotinic ligands 2-9. In these compounds, the substitution of the Δ^2 -isoxazoline ring of reference compound 1 with the 1,2,3-triazole moiety caused a decrease of their solubility in water, thus making the pK_a determination more difficult. We determined the pK_a values of target ligands by means of capillary electrophoresis and potentiometric titrations in water-cosolvent mixtures, and compared the features of the two applied techniques. The calculated values were analysed with the predictor software ADME Boxes v. 4.1 by Pharma-algorithms, a program which has been developed in collaboration with Sirius, the company which produces the apparatus for the potentiometric titrations. Our theoretical investigation included ACD/pK_a DB, by Advanced Chemistry Development Inc., which is one of the most reliable commercial prediction programs [16], extensively used by the majority of pharmaceutical and API companies. We also made use of the ACD/pKa GALAS software, which permit the calculation of charge influences of ionized groups to neighboring ionization centers.

Experimental

Chemicals

Compounds 2-9 have been prepared in our laboratory and their synthesis and biological activity will be reported in a due course. Phosphoric acid (H_3PO_4), formic acid (HCOOH), acetic acid (CH_3COOH), boric acid (H_3BO_3), 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS), sodium hydroxide, CO_2 free potassium hydroxide, potassium chloride, potassium hydrogen phthalate (KHP), hydrochloric acid 0.5 M, acetone and methanol were purchased from Sigma Aldrich. All reagents used were of analytical grade and all reagents and buffers were prepared with water obtained from a Milli-Q water purification system (Millipore).

Apparatus

Capillary electrophoretic experiments were carried out using a Beckman Coulter Proteome Lab PA 800 system equipped with a diode-array detector scanning from 190 to 600 nm. 32 Karat software was employed for instrumental control, data acquisition and data analysis. Electrophoretic separations were performed under the conventional operating conditions (anodic injection) in an uncoated fused-silica capillary of 32 cm total length, 21 cm effective length and 50 μm i.d. (Composite Metal Service Ltd). Before first use, new capillaries were conditioned as follows: 30 min with 0.1 M NaOH, 30 min with water and 30 min with the running buffer. Between runs at different pH values the capillary was activated with 0.1 M NaOH for 3 min, rinsed with water for 3 min and with background electrolyte (BGE) for 5 min. Between runs at the same pH value the capillary was only washed with water for 3 min and equilibrated with BGE for 5 min. Activation,

rinse and equilibrations steps were all carried out with a pressure of 20 psi. All injections were performed in the hydrodynamic mode (10 s, 0.5 psi). The capillary was operated at 8 kV, while maintaining its temperature at 25 °C and the detection wavelength was 280 nm. Three replicate injections were carried out for each compound at each pH value and all of them were used for the calculation of pK_a values. The pH values of the running buffers were measured with a MP 220 pH meter (Mettler Toledo) equipped with an electrode In Lab 418 (Mettler Toledo), daily calibrated. Graph Pad Prism Version 5.0 software (Graph Pad Software) was used to perform non-linear regression analysis of the electrophoretic data.

Potentiometric titrations were carried out with the Sirius GL pKa instrument coupled with a computer-aided system for the evaluation of pK_a values (Sirius Refinement Pro software version 1.0). Electrode standardization was performed every day following a two-step procedure: a) a single point calibration, in which the electrode is placed in a pH 7 buffer solution and after which the Nernst equation is applied to set an operational pH scale used for the pH readings during titrations; b) an aqueous blank titration, in which 20 mL of ISA water (ionic strength adjusted water, i.e. 0.15 M KCl unbuffered solution) are titrated over a wide pH range (typically from pH 1.8 to 12.2) under inert atmosphere at constant temperature (25°C) with a 0.5 M KOH solution. The experimental titration curve was fitted to the theoretical curve using the Sirius Four-Plus equation, which relates pH (operational pH, the pH scale derived from the pH 7 buffer solution) to p[H] (concentration pH, a scale which takes liquid junction potentials and other deviations from ideal behaviour into account). This fitting was performed by means of the Refinement Pro software using the following equation:

$$pH = \alpha + S(-\log[H^+]) + j_H [H^+] + j_{OH} K_w / [H^+] \quad (1)$$

in which α corresponds to the negative logarithm of the activity coefficient of H_3O^+ at the working temperature and ionic strength; S stands for the Nernst slope; the j_H term corrects pH readings for the non-linear pH response due to liquid junctions and asymmetry potentials in acidic solutions (pH 1.5-2.5); the j_{OH} term corrects for non-linear effects at high pH (pH > 11) [17], and K_w stands for the aqueous constant as a function of ionic strength and temperature [18]. Four-Plus terms α , S, j_H , j_{OH} are characteristic of the electrode performance. In blank titrations, the "sample" is the CO_2 dissolved in water and the difference curve obtained after the effect of CO_2 has been subtracted, giving an indication of the quality of pH electrode response at different points on the pH scale. Base titrant (0.5 M KOH) was prepared from CO_2 -free ampoules of KOH in order to minimize the concentration of CO_2 in the solution. This reagent was standardized by titration with a weighted amount (0.15 to 0.19 g) of potassium hydrogen phthalate dissolved in 20 ml of ISA water. For a better result, three KHP titrations were performed and combined in a Multi Set by means of the Refinement Pro software to determine the mean value of the KOH concentration factor. A volumetric standard solution (0.5 M) of hydrochloric acid was employed as acid titrant. This reagent was standardized by means of a blank titration.

Electrophoretic measurements

For electrophoretic experiments, samples containing one substance dissolved in a water/methanol/acetone 94/5/1 v/v/v mixture were prepared. Acetone was used to determine electro-osmotic flow (EOF) and methanol was added to improve the solubility of the tested compounds. Sample concentrations were very low (0.02 mg/mL) because all compounds were poorly soluble in water. Twenty-

one buffers, set at an ionic strength of 20 mM, were prepared from pH 2.0 to pH 12.0 with an increment of 0.5 pH units according to Geiser et al. [19]. The buffers were degassed in an ultrasonic bath prior to use and were replaced every ten runs, thus avoiding electrolytic phenomena. The analyte apparent mobility is the sum of the effective mobility (μ_{eff}) and the mobility of EOF (μ_{EOF}). Therefore, for measuring μ_{eff} it was necessary to determine μ_{EOF} , i.e. the mobility of a neutral electro-osmotic flow marker (acetone). Practically, the analyte effective electrophoretic mobility was calculated according to equation (2):

$$\mu_{\text{eff}} = \mu_{\text{app}} - \mu_{\text{EOF}} = \frac{L_{\text{tot}} L_{\text{eff}}}{V} \left(\frac{1}{t_{\text{app}}} - \frac{1}{t_{\text{EOF}}} \right) \quad (2)$$

where t_{app} and t_{EOF} are the migration times (s) of the analyte and the neutral marker, respectively; V is the applied voltage (V), L_{tot} the total capillary length (cm) and L_{eff} the capillary length from the injection point to the detector (cm). μ_{eff} , μ_{EOF} and μ_{app} are all expressed in $\text{cm}^2\text{V}^{-1}\text{s}^{-1}$. The calculated values of μ_{eff} were reported as a function of pH giving rise to a sigmoidal curve, according to the model equations for pK_a determinations [12]. A non-linear regression was performed to determine pK'_a values which were dependent on the BGE concentration. These values were corrected by introducing an activity coefficient, which gave the “true” pK_a value, independent of experimental procedures [19].

Potentiometric measurements

As compounds 2-9 were water insoluble, the samples were titrated in a mixture of water and a cosolvent. From these titrations, apparent pK_a values in the presence of cosolvent were calculated. Three titrations were conducted at different cosolvent concentrations and the different datasets obtained were combined in the RefinementPro software to create a MultiSet; the apparent pK_a values were extrapolated to “zero cosolvent” to obtain the aqueous pK_a using the Yasuda-Shedlovsky procedure [20]. Methanol was the solvent of choice because its general effect on pK_a has been extensively studied. Moreover, CO_2 is poorly soluble in methanol, leading to more reliable titration data. For calculation of apparent pK_a values, Four-Plus pH electrode standardization parameters, which are corrected at the same water-cosolvent ratio as the sample titration, must be used in the refinement of the data. The aqueous Four-Plus values were automatically adjusted to the correct values by the Refinement Pro software. The adjustment was calculated by applying data from files built into the software, in which the performance of pH electrodes over a wide range of water-cosolvent ratios in 0.15 M KCl at 25°C were determined. A weighted amount of analyte (2 mg) was dissolved in ISA water containing different amounts of methanol (10, 20, 30%) and the pH was lowered to 1.8 with 0.5 M HCl before titration, which was performed with 0.5 M KOH up to pH 12.2. For each sample, three determinations were carried out in the same vial and the results were combined in a MultiSet and extrapolated to “zero cosolvent” by means of the Refinement Pro software. The potentiometric pK_a values, obtained at 0.15 M ionic strength, were corrected to 0.02 M ionic strength to compare them with the electrophoretic values using an empirical expression described in the literature [21].

Prediction of pK_a values

Theoretical pK_a values were calculated applying three prediction programs. ADME Boxes v. 4.1 software (trial version, Pharma-algorithms) is a ionization predictor based on more than 12,000 compounds training set, which, from the structural formula of new compounds, is able to calculate the dissociation constants of their ionization centres. The ACD/ pK_a Classic software (trial version,

Advanced Chemistry Development, Inc.) is based on Hammett equations derived from a library of more than 30,000 experimental pK_a values for approximately 16,000 compounds in aqueous solutions and of more than 2,000 derivatives in non-aqueous solvents [16,22,23]. The ACD/ pK_a GALAS (Global, Adjusted Locally According to Similarity) software makes use of an algorithm in which the pK_a microconstants of all possible ionization centres are predicted, then corrected according to the chemical environment of the reaction centre. Predictions are performed by means of a database containing 4600 ionization centres [24,25].

Results and Discussion

In the set of investigated compounds, derivatives 2, 3, 4, 7, 8 are mono-bases while 5, 6 and 9 are di-bases. CE measurements yielded electrophoretic mobilities which were plotted versus the pH of the running buffer; μ_{eff} values were positive according to the cationic nature of the ionized compounds under study. In (Figure 2) a representative plot of μ_{eff} against pH for compound 2 is illustrated. The results obtained from the potentiometric and the electrophoretic methods are gathered in Table 1 together with the values resulting from application of the three different computational prediction programs.

Confidence intervals associated with electrophoretic and potentiometric determinations were around 0.2, while confidence intervals related to software predictions ranged from 0.4 to 0.8. Experimental and theoretical pK_a values were also compared graphically (Figure 3). Electrophoretic pK_a values were in the same range as those determined potentiometrically, however we found a significant difference when experimental potentiometric values were compared with theoretical ones. On the other hand, experimental pK_a values measured by capillary electrophoresis fell in the same range of the theoretical values from the ACD/ pK_a Classic software and the ACD/ pK_a GALAS software but not with those calculated by applying the ADME Boxes algorithm. With the potentiometric method, pK_a values were the result of a sample titration in water-cosolvent mixtures with

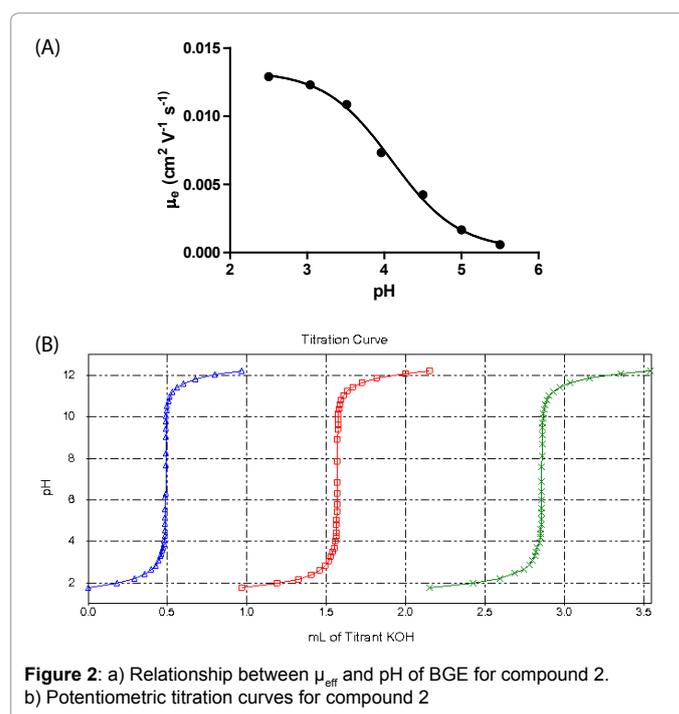


Figure 2: a) Relationship between μ_{eff} and pH of BGE for compound 2. b) Potentiometric titration curves for compound 2

an increasing amount of cosolvent, followed by extrapolation of the pK_a value at zero cosolvent concentration. Indeed, the very low water solubility at the required concentration did not allow determination of the pK_a values in water owing to sample precipitation during titration. Given the insufficient amount of compound 9 for a measure with the potentiometric option, we were unable to estimate the two pK_a values of this derivative by means of this technique, which, on the contrary, was successful for achieving both pK_a values in the case of di-bases 5 and 6. Worth mentioning, the two dissociation constants were too close to be discriminated with the electrophoretic titration protocol, which provided only an average value.

On the other hand, the very low water solubility of title derivatives 2-9 was not a limitation when we applied the electrophoretic method. Indeed, in this instance the required sample concentration is rather low due to the intense UV absorbance of the investigated derivatives. If the graphical comparison illustrated in Figure 3 is taken into account, we can conclude that the values attained with the two experimental protocols were to a large extent in mutual accordance. Worth noting, the theoretical prediction programs ADME Boxes and ACD pK_a GALAS provided significantly higher values than the experimentally determined ones. On the contrary, the ACD pK_a Classic software led to theoretical values which, in most cases, were dramatically lower than the experimental values, and this was especially true for the first dissociation constant of both compounds 6 and 9.

Conclusions

In the present study, we used two different approaches, the potentiometric as well as the electrophoretic experimental protocol, to measure the acid-base dissociations constants of eight poorly water-soluble weak bases targeting the nicotinic receptor system. Electrophoretic and potentiometric measurements, which gave

reproducible and comparable results, showed complementary features. Indeed, with capillary electrophoresis, the problem associated with the low water solubility of the studied samples could be easily overcome, although this technique did not allow to measuring all dissociation constants. In contrast, application of the potentiometric method afforded all the theoretical pK_a values, although we had to perform the titrations in water-cosolvent mixtures, a less precise, more laborious and time-consuming approach. Furthermore, the parallel use of three different computational packages produced a set of results, which, overall, were not matching the experimental data, and, therefore, appeared to be unsuitable for a reliable theoretical prediction of the dissociation constants of our set of derivatives.

Acknowledgments

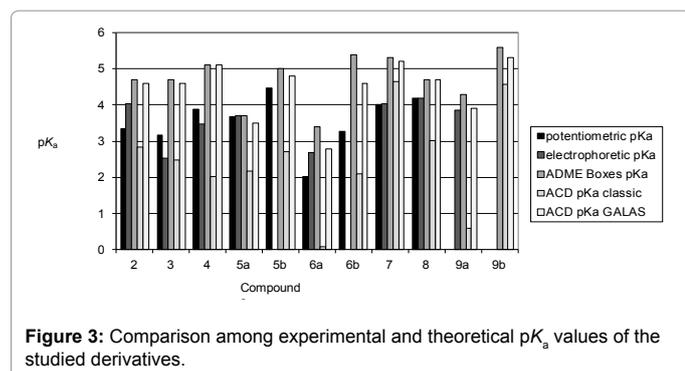
This research was financially supported by the Italian Ministry of Education, University and Research (PRIN grant 2009-R7WCZS to M.D.A.)

References

- Wang H, Yu M, Ochani M, Amella CA, Tanovic M, et al. (2003) Nicotinic acetylcholine receptor 7 subunit is an essential regulator of inflammation. *Nature* 421: 384-388.
- Pavlov VA, Tracey KJ (2006) Controlling inflammation: the cholinergic anti-inflammatory pathway. *Biochem Soc T* 34: 1037-1040.
- Wang DW, Zhou RB, Yao YM (2009) Role of cholinergic anti-inflammatory pathway in regulating host response and its interventional strategy for inflammatory diseases. *Chin J Traumatol* 12: 355-364.
- Bencherif M, Lippiello PM, Lucas R, Marrero MB (2011) Alpha7 nicotinic receptors as novel therapeutic targets for inflammation-based diseases. *Cell Mol Life Sci* 68: 931-949.
- Filippini P, Cesario A, Fini M, Locatelli F, Rutella S (2012) The Yin and Yang of non-neuronal 7-nicotinic receptors in inflammation and autoimmunity. *Curr Drug Targets* 13: 644-655.
- Saeed RW, Varma S, Peng-Nemeroff T, Sherry B, Balakhaneh D, et al. (2005) Cholinergic stimulation blocks endothelial cell activation and leukocyte recruitment during inflammation. *J Exp Med* 201: 1113-1123.
- Grazioso G, Pomè DY, Matera C, Frigerio F, Pucci L, et al. (2009) Design of novel 7-subtype-preferring nicotinic acetylcholine receptor agonists: Application of docking and MM-PBSA computational approaches, synthetic and pharmacological studies. *Bioorg Med Chem Lett* 19: 6353-6357.
- Dallanoce C, Magrone P, Matera C, Frigerio F, Grazioso G, et al. (2011) Design, synthesis, and pharmacological characterization of novel spirocyclic quinuclidinyl- Δ^2 -isoxazoline derivatives as potent and selective agonists of $\alpha 7$ nicotinic acetylcholine receptors. *ChemMedChem* 6: 889-903.
- Dallanoce C, Frigerio F, Grazioso G, Matera C, Visconti GL, et al. (2011) New spirocyclic Δ^2 -isoxazoline derivatives related to selective agonists of $\alpha 7$ neuronal nicotinic acetylcholine receptors. *Eur J Med Chem* 46: 5790-5799.
- Dallanoce C, Matera C, Pucci L, Gotti C, Clementi F, et al. (2012) Synthesis and binding affinity at $\alpha 4\beta 2$ and $\alpha 7$ nicotinic acetylcholine receptors of new analogs of epibatidine and epiboxidine containing the 7-azabicyclo[2.2.1]hept-2-ene ring system. *Bioorg Med Chem Lett* 22: 829-832.
- Kibbey CE, Poole SK, Robinson B, Jackson JD, Durham D (2001) An integrated process for measuring the physicochemical properties of drug candidates in a preclinical discovery environment. *J Pharm Sci* 90: 1164-1175.
- Poole SK, Patel S, Dehning K, Workman H, Poole CF (2004) Determination of acid dissociation constants by capillary electrophoresis. *J Chromatogr A* 1037: 445-454.
- Adveef A (1992) pH-Metric log P. Part 1. Difference Plots for Determining Ion-Pair Octanol-Water Partition Coefficients of Multiprotic Substances. *Quant Struct Act Relat* 11: 510-517.
- Gluck SJ, Cleveland JA (1994) Capillary zone electrophoresis for the determination of dissociation constants. *J Chromatogr A* 680: 43-48.
- Roda G, Dallanoce C, Grazioso G, Liberti V, De Amici M (2010) Determination of acid dissociation constants of compounds active at neuronal nicotinic acetylcholine receptors by means of electrophoretic and potentiometric techniques. *Anal Sci* 26: 51-54.

	pK_a by potentiometry	pK_a by CE	pK_a by ADME Boxes	pK_a by ACD/ pK_a Classic	pK_a by ACD/ pK_a GALAS
2	3.339	4.037	4.70	2.85	4.60
3	3.164	2.534	4.70	2.47	4.60
4	3.875	3.480	5.10	2.02	5.10
5	3.680 4.481	3.708	3.70 5.00	2.17 2.70	3.50 4.80
6	2.033 3.271	2.685	3.40 5.40	0.09 2.10	2.80 4.60
7	4.002	4.044	5.30	4.66	5.20
8	4.197	4.192	4.70	3.02	4.70
9	-	3.858	4.30 5.60	0.59 4.56	3.90 5.30

Table 1: Measured and predicted pK_a values for tested compounds 2-9.



16. Meloun M, Bordovská S (2007) Benchmarking and validating algorithm that estimate pK_a values of drugs based on their molecular structure. *Anal Bioanal Chem* 389: 1267-1281.
17. Kahle C, Holzgrabe U (2004) Determination of binding constants of cyclodextrin inclusion complexes with amino acids and dipeptides by potentiometric titration. *Chirality* 16: 509-515.
18. Sweeton FH, Mesmer RE, Baes CF (1974) Acidity measurements at elevated temperatures. VII. Dissociation of water. *J Sol Chem* 3: 191-214.
19. Geiser L, Henchoz Y, Galland A, Carrupt P-A, Veuthey J-L (2005) Determination of pK_a values by capillary zone electrophoresis with a dynamic coating procedure. *J Sep Sci* 28: 2374-2380.
20. Avdeef A, Box KJ, Comer JEA, Gilges M, Hadley M, et al. (1999) PH-metric log P 11. pK_a determination of water-insoluble drugs in organic solvent-water mixtures. *J Pharm Biomed Anal* 20: 631-641.
21. Avdeef A, Bucher JJ (1978) Accurate measurements of the concentration of hydrogen ions with a glass electrode: calibration using the Prideaux and other universal buffer solutions and a computer-controlled automatic titrator. *Anal Chem* 50: 2137-2142.
22. Liao CZ, Nicklaus MC (2009) Comparison of nine programs predicting $pK(a)$ values of pharmaceutical substances. *J Chem Inform Model* 49: 2801-2812.
23. Adams DJ and Morgan LR (2011) Tumor Physiology and Charge Dynamics of Anticancer Drugs: Implications for Camptothecin-based Drug Development. *Curr Med Chem* 18: 1367-1372.
24. O'Neil MJ, Smith A, Heckelman PE, Budavari S (2001) The Merck Index. An Encyclopedia of Chemicals, Drugs, and Biologicals (13th Edn) Merck & Co., Inc., Whitehouse Station, NJ.
25. Moffat AC, Jackson JV, Moss MS, Widdop B (1986) Clarke's Isolation and Identification of Drugs (2nd Edn) The Pharmaceutical Press, London.

Citation: Roda G, Dallanoce C, Gambaro V, Grazioso G, Liberti V, et al. (2015) Determination of Acid Dissociation Constants of Poorly Water-Soluble Nicotinic Ligands by Means of Electrophoretic and Potentiometric Techniques. *Pharm Anal Acta* 6: 394. doi:[10.4172/21532435.1000394](https://doi.org/10.4172/21532435.1000394)

Submit your next manuscript and get advantages of OMICS Group submissions

Unique features:

- User friendly/feasible website-translation of your paper to 50 world's leading languages
- Audio Version of published paper
- Digital articles to share and explore

Special features:

- 400 Open Access Journals
- 30,000 editorial team
- 21 days rapid review process
- Quality and quick editorial, review and publication processing
- Indexing at PubMed (partial), Scopus, EBSCO, Index Copernicus and Google Scholar etc
- Sharing Option: Social Networking Enabled
- Authors, Reviewers and Editors rewarded with online Scientific Credits
- Better discount for your subsequent articles

Submit your manuscript at: <http://www.editorialmanager.com/virology>