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pK_a Constant of Varenicline

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ABSTRACT: The pK_a constant of varenicline using a partial agonist of the nicotinic $\alpha4\beta2$ acetylcholine receptor was determined potentiometrically. The pK_a constant of varenicline was calculated as 9.22 ($\pm$ 0.13) at I = 0.15 (NaCl), 25 $\pm$ 1 °C using the Irving−Rossotti method. The experimental results were compared with theoretical ones obtained via the SPARC pK_a calculation program. The distribution of species of the drug molecule at various pH values was demonstrated to provide experimental data of its pK_a, to be used in commenting on its biological uptake, the binding of these molecules to environmental matrices, forming chelates with metallic cations, and its analysis.

INTRODUCTION

Varenicline (7,8,9,10-tetrahydro-6,10-methano-6*H*-pyrazino-[2,3-*h*][3]benzazepine; Figure 1) is a partial agonist of the nicotinic $\alpha4\beta2$ acetylcholine receptor, with demonstrated efficacy as a smoking cessation agent,[1] and was approved by Therapeutic Goods Administration (Australia) in 2007. Varenicline is a simple, small organic molecule, providing limited structural entities for biotransformation reactions. In fact, a large percentage of the administered dose of varenicline is excreted unchanged.

Absorption is virtually complete after oral administration, and systemic availability is high.[2] The oral bioavailability of varenicline tartrate is unaffected by food or time-of-day dosing. Renal elimination of varenicline tartrate is primarily through glomerular filtration along with active tubular secretion via the organic cationic transporter, OCT2. Plasma protein binding of varenicline tartrate is low (< 20 %). Most of the active compound is excreted renally (92 to 93 %). A small proportion is glucuronidated, oxidated, *N*-formylated, or conjugated to a hexose.[3] The elimination half-life is about 24 h.

The dissociation constant (pK_a) of a drug molecule is a key parameter in absorption, distribution, metabolism, excretion, and toxicity researches because it governs the solubility, absorption, distribution, and elimination of substances. Also, the pK_a values constitute important data for a thorough understanding of certain chemical phenomena such as biological uptake and the binding of these molecules to environmental matrices and forming chelates with metallic cations. Among these techniques, the potentiometric method is a high precision technique to determine the pK_a values of substances. It is commonly used due to its accuracy and the commercial availability of fast, automated instruments.[4] The pK_a is also important in choosing the optimum conditions in the development of analysis methods for drug molecules. Acid dissociation constant determination studies were performed for many drugs like irinotecan HCl[5,6] and epirubicin HCl, leucovorin, 5-fluorouracil, montelukast sodium,[7] levodropropizine, mupirocin,[8] and many other drugs such as antiinflammatories, antibiotics, β-blockers, and so forth.

No potentiometric study on the pK_a constant of varenicline currently exists in the literature. The pK_a constant for varenicline is given as 9.2, in the product information sheet of the producer,[9] without further experimental knowledge.

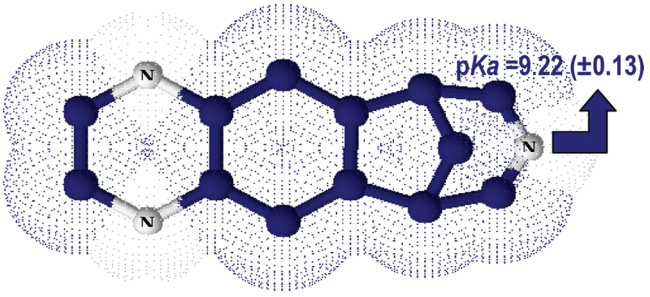

Figure 1. 3D molecular structure of varenicline.

In this study, the pK_a constant of varenicline has been determined in aqueous media, potentiometrically, using the Irving−Rossotti method.[10] The ionized and nonionized species in various pH values were determined.

EXPERIMENTAL SECTION

Varenicline tartarate of analytical purity (99.9 %) was kindly provided by Pfizer (Turkiye). $NaClO_4 \cdot H_2O$, $HClO_4$, 0.1000 mol·L^{-1} NaOH (Titrisol), and tartaric acid were purchased from Merck (Darmstadt, Germany). All of the reagents were of analytical grade. Titrations were performed using an Isolab digital buret with 0.01 mL sensitivity, WTW 340I model pH meter with a WTW SenTix 41combined glass electrode, and a temperature sensor. The pH meter was calibrated with certificated WTW pH buffers of pH 4.01, 7.00, and 10.01. The potentiometric calibration is performed, and the carbonate content of NaOH was calculated using the Gran method.[11,12] To achieve this, 0.1000 mol·L^{-1} standard HCl (Titrisol) was titrated with 0.1000 mol·L^{-1} NaOH (Titrisol) solution, bubbling with N_2, to exclude CO_2 from the system. Fifty plots were used in calibration. Titrations were performed with three repetitions. The

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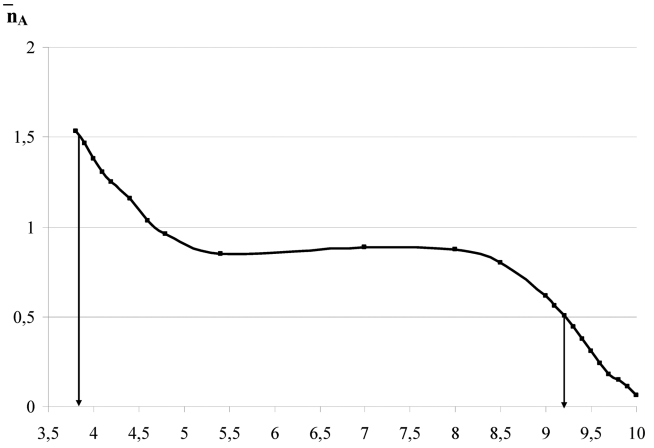

Figure 2. A sample pH—$\bar{n}_A$ graph of varenicline tartarate. pK_a of varenicline = 9.20, pK_a originating from tartarate = 3.85 (I = 0.154, 25 ± 1 °C).

potential was allowed to stabilize after each addition of acid, and the potential values obtained were used to calculate the standard potential of the cell, E_0, and the slope. A computer program named GLEE was used to obtain the standard potential and slope via the Gran plot, for calculating the carbonate content of NaOH solution and correction of its concentration.[12] The mean of the slopes was 58.5 ± 0.34 mV·pH^{-1}, and the slope factors were ≥0.99. The carbonate impurity was determined as ≤ % 0.40. All solutions were prepared using freshly distilled water. Varenicline tartarate and tartaric acid solutions were prepared just prior to each titration. All titrations for analysis and calibration were performed at 25 ± 1 °C, 0.154 mol·L^{-1} ionic strength, under N_2 gas.

(a) 50.00 mL solutions each including 0.154 mol·L^{-1} NaCl (to provide an ionic strength equivalent to 0.9 % isotonic NaCl solution), 1.00·10^{-2} mol·L^{-1} HCl, and 4.00·10^{-4} mol·L^{-1} varenicline tartarate;

(b) 50.00 mL solutions each including 0.154 mol·L^{-1} NaCl (to provide an ionic strength equivalent to 0.9 % isotonic NaCl solution), 1.00·10^{-2} mol·L^{-1} HCl, and 4.00·10^{-4} mol·L^{-1} tartaric acid;

(c) Blank solutions: 50.00 mL solutions each including 0.154 mol·L^{-1} NaCl (to provide an ionic strength equivalent to 0.9 % isotonic NaCl solution) and 1.00·10^{-2} mol·L^{-1} HCl.

Solutions a and b were titrated along with their blanks (c), using 0.0977 mol·L^{-1} NaOH. Carbon dioxide dissolved in titration solutions was purged out with nitrogen gas, before and during titrations. All titrations were performed at 25 ± 1 °C, I = 0.154 (NaCl). The same procedure was applied also at a 8.00·10^{-4} mol·L^{-1} concentration for solutions a and b.

The titration curves were plotted versus milliliter values. Using the titration values, $\bar{n}_A$ values were calculated according to the Irving—Rossotti method.[10] For this purpose, the average formation ratio for the proton—ligand complex, $\bar{n}_A$ (which can be denoted as the total proton concentration bounded to ligand/free ligand concentration) was determined for the ligands at various pH values according to the literature. Each volume of NaOH spent to form potentiometric titration curves of acid + ligand and acid (as its blank) was used to calculate the average values $\bar{n}_A$ (eq 1),

$$\bar{n}_A = y + \frac{(V_1 - V_2)(N + E^o)}{(V^o + V_1)T_L^o} \quad (1)$$

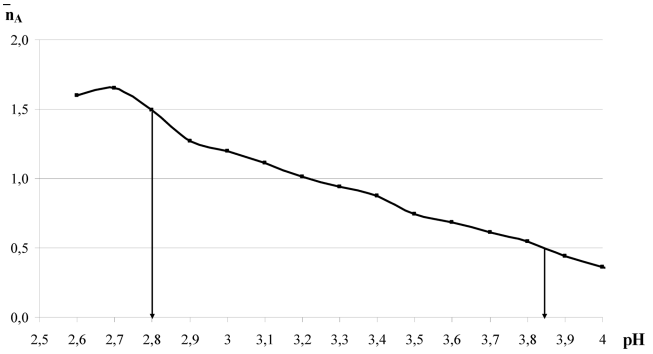

Figure 3. A sample pH—$\bar{n}_A$ graph of tartaric acid. pK_{a1} = 2.80, pK_{a2} = 3.85 (I = 0.154, 25 ± 1 °C).

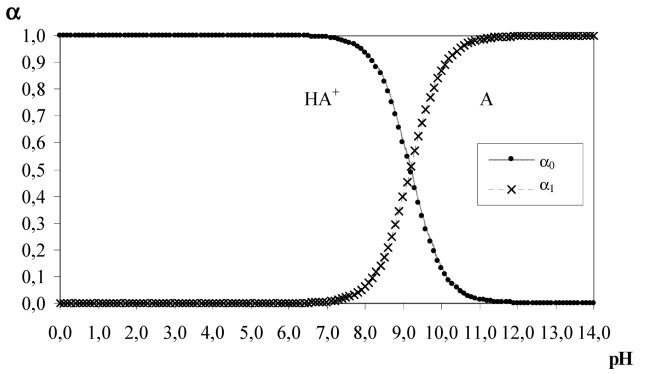

Figure 4. Distribution of ionized and nonionized species of varenicline at various pH values (I = 0.154, 25 ± 1 °C). α_0 = relative abundance of HA^+ form, α_1 = relative abundance of A^- form.

where V^o = 50.0 mL (initial volume), N = 0.0977 mol·L^{-1} (standardized concentration of titrisol NaOH solution), T_L^o = 4.00·10^{-3} mol·L^{-1} (molar concentration of ligand in initial solution that will be titrated), E^o = exact calculated molarity of $HClO_4$ in initial solution that will be titrated, y = 0 for varenicline, 2 for tartaric acid (the number of dissociable protons initially present on the ligand), 2 for varenicline tartarate, V_1 and V_2 represent the volumes of NaOH added, to reach the same pH reading in both blank acid and acid + ligand titrations. The titration of blank ($HClO_4$) solution is crucial for the calculation of the exact concentration of $HClO_4$ and thus for the correct calculation of log K and pK_a. The $HClO_4$ titration is also used in measuring $V_1 - V_2$, which is the displacement of the ligand curve relative to the acid curve in each pH along the volume axis, as an indication of proton dissociation. Using the Irving—Rossotti method, $\bar{n}_A$ values were plotted as a function of pH, that is, $\bar{n}_A = f$(pH) (Figures 2 and 3). The pK_a constant was determined from the pH axis, corresponding to $\bar{n}_A$ = 0.5 on $\bar{n}_A = f$(pH) curve. Distributions of ionized and nonionized species of the drug were calculated, using the pK_a value (Figure 4). The experimental results were compared with the literature and the theoretical results obtained using the SPARC pK_a calculation program.[13]

RESULTS AND DISCUSSION

The pK_a result was confirmed by carrying out titrations in two different concentrations (4.00·10^{-4} mol·L^{-1}, 8.00·10^{-4} mol·L^{-1}) for varenicline. $\bar{n}_A = f$(pH) curves for varenicline

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Table 1. Experimentally Found pK_a Value of Varenicline (I = 0.15, 25 ± 1 °C), together with Literature Data and the Values Predicted by SPARC

experimental results (potentiometric)		SPARC pK_a estimation		literature values	
pK_{a1} (log K_2[a])	pK_{a2} (log K_1[a])	pK_{a1} (log K_2[a])	pK_{a2} (log K_1[a])	pK_{a1} (log K_2[a])	pK_{a2} (log K_1[a])
		Varenicline			
9.22 (± 0.13)[a] (ΔG° = −52607.92)[b]		9.35		9.2[9]	
		Tartaric Acid			
2.83 (± 0.09)[a]	3.85 (± 0.05)[a]	2.86	4.52	2.96[14]	4.24[14]

[a] n = 6 for varenicline, n = 3 for tartaric acid, n: number of repeats. [b] ΔG° values are given in kJ·mol^{-1}.

and tartaric acid are given in Figures 2 and 3. The dissociation equilibria of varenicline and tartaric acid are given in eqs 2, 3, and 4.

$$C_{13}H_{13}N_3H^+ + H_2O \rightleftarrows C_{13}H_{13}N_3 + H_3O^+ \quad (2)$$
$$pK_a$$

$$C_4H_6O_6 + H_2O \rightleftarrows C_4H_5O_6^- + H_3O^+ \quad (3)$$
$$pK_{a1}$$

$$C_4H_5O_6^- + H_2O \rightleftarrows C_4H_4O_6^{2-} + H_3O^+ \quad (4)$$
$$pK_{a2}$$

The pK_{a1} and pK_{a2} values of tartaric acid were found as 2.83 (± 0.09) and 3.85 (± 0.05) (n = 3). These pK_a values coincided exactly with the second and third pK_a values which appeared in the calculation of varenicline tartarate titration, and it was understood that they originated from tartaric acid. Under the light of the calculations of experiments, the pK_a constant of varenicline was determined as 9.22 (± 0.13) (n = 6) (Figure 2). This pK_a value is attributed to the secondary aromatic amine in the molecule. The obtained results were in accordance with the literature[14] and the value of 9.35, which had been found theoretically using the SPARC computer program.[13] The SPARC program uses algorithms based on fundamental chemical structure theory that combines principles of quantitative structure activity relationships (QSAR), linear free-energy relationships (LFER), and perturbation theory from quantum chemistry.[4] This program estimates the macroscopic and microscopic pK_a of any organic compound solely from its chemical structure. SPARC is available free of charge on the Internet.[13] The experimentally found pK_a value of varenicline, together with literature data and the values predicted by SPARC are given in Table 1. This is the first potentiometric study on the pK_a constant of varenicline.

The Gibbs free energy change ΔG° for the dissociation process of varenicline in water has been calculated using eq 5, where the gas constant R is 8.314 J·K^{-1}·mol^{-1}, T is 298 K, and K_a is a dissociation constant for the ligand (Table 1).

$$\Delta G^\circ = -2.303RT \log K_a \quad (5)$$

The distribution of species of varenicline has been shown in Figure 4. Performing the experiments in 0.9 % isotonic NaCl solution aided the imitation of the biological conditions that it is delivered to the organism, to relate its pK_a with its transfer from the membranes. It was known that 100 % of varenicline exists in the ionized form in the stomach (pH = 1.0 to 4.0) and duodenum (pH ≈ 6.0) and 98 % in blood (pH = 7.4). The nonionized moieties of the drug molecules are able to pass the membranes easily, while the ionized forms are not. However, since varenicline has a high bioavailability, when the pK_a constants are considered along with the absorption of the drug molecule, the good penetration of the ionized moiety to body compartments seems to arise merely because it passes through the porous membranes of vessels.

The pK_a of varenicline will be important data in the development of quantification and separation methods. To know which form of a drug molecule exists in which ratio in a definite solvent media is no doubt of great use in choosing the right separation technique during the quantification procedures.

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