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Study of protonation equilibrium of lorazepam by potentiometry and multi-wavelengths spectrophotometry

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Abstract

The acid-base equilibrium of lorazepam was studied by means of potentiometry and multiwavelengths spectrophotometry. The stoichiometric equilibrium constants were determined at 25 °C and constant ionic strength 0.1 mol L^{-1} (NaCl). The acidity constants pK_1=1.58±0.05 and pK_2=11.67±0.07 were found by potentiometry, and pK_1=1.54±0.02 and pK_2=11.61±0.03 by multi-wavelengths spectrophotometry. Potentiometric determination of equilibrium constants was performed by application of the formation function method. Data analysis program applied for multi-wavelengths spectrophotometric determination of acidity constants.

Keywords: Lorazepam; Acidity constants; Potentiometry; Spectrophotometry.

1. Introduction

Acidity constants can be a key parameter for understanding and quantifying chemical phenomena such as reaction rates, biological activity, biological uptakes, biological transport and environmental fate [1]. There have been several methods of the determination of acidity constants, including the use of potentiometric titration, spectrophotometry, capillary electrophoresis, and so on.

Lorazepam (Scheme 1), {7-chloro-5-(2-chlorophenyl)-3-hydroxy-2,3-dihydro-2H-1,4-benzodiazepin-2-one, is one of the 1,4-benzodiazepine derivatives are most widely used for treatments of anxiety, relief of insomnia and sleep disturbances. They have sedative, hypnotic, muscle relaxant, anticonvulsant and amnesic properties [2]. The characteristic of lorazepam such as acidity constants are important for a through understanding of biological activity of this drug. The acidity constants values of lorazepam were reported, pK_1=1.3 and pK_2=11.5, previously [3]. The first acidity constant corresponds to the protonation of the nitrogen in the azomethine group. In the present work the acid-base properties of lorazepam was studied by potentiometry and UV spectrophotometry. Since this drug contains two proton-binding sites, the acid-base properties are depicted by equilibrium constants, K_1 and K_2, respectively.

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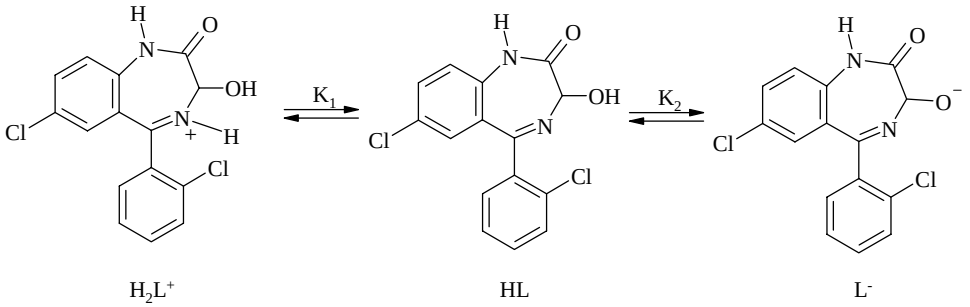

Scheme 1. Protonation Scheme of lorazepam.

2. Experimental

2.1. Chemicals

Lorazepam, hydrochloric acid, sodium chloride and sodium hydroxide were purchased from Merck. All the reagents were of analytical-reagent grade. Stock standard solution of lorazepam, 5×10^{-3} mol L^{-1} was prepared by dissolving the compound in methanol. This solution was stored in the dark at 4 °C and was found to be stable for at least four weeks changing in the its spectral profile. All the solutions were prepared in deionized water. Standardization of HCl and NaOH was performed potentiometrically. The ionic strength was kept constant (0.1 mo L^{-1}) by addition of NaCl, and all measurements were performed at 25 °C. The conversion of measured pH values into pc_H for I=0.1 mol L^{-1} was done by using the following relation [4]:

$$pc_H = -\log[H_3O^+] = pH - 0.04 \qquad (1)$$

2.2. Apparatus and software

A Perkin Elmer (Lambda 25) spectrophotometer controlled by a computer and equipped with a 1-cm path length quartz cell was used for ultraviolet spectra acquisition. Spectra were acquired between 260 and 380 nm (1 nm resolution). A Metrohm 692 pH-meter furnished with a combined glass-saturated calomel electrode. The electrode was calibrated with standard buffer solutions (pH 3.00-9.00). The data were treated in an AMD 2000 XP (512 Mb RAM) microcomputer using MATLAB software, version 6.5 (The MathWorks) or for processing by using DATAN package [5].

2.3. Determination of acidity constants

2.3.1. Potentiometric determination of acidity constants

The acidity constants were determined from the data obtained by potentiometric titration. Aliquot (25 mL) containing 3×10^{-3} mol L^{-1} lorazepam and 3.54×10^{-3} mol L^{-1} HCl were titrated by 0.1246 mol L^{-1} NaOH. The constant ionic strength of 0.1 mol L^{-1} was kept by NaCl as the auxiliary electrolyte.

2.3.2. Spectrophotometric determination of acidity constants

For the spectrophotometric lorazepam titrations, absorption spectra were measured with an automatic titration set-up consisting of a computer interfaced to a spectrophotometer. After each

pH adjustment, solution is transferred into the cuvette and the absorption spectrum is recorded in the wavelength range of 260 to 380 nm.

3. Results and discussion

The neutral nonionic form of lorazepam is rearranged spontaneously to the neutral nonionic due to the protolysis of hydroxyl group and the proton acceptance of the nitrogen in the azomethine group. However, both >N-H group [3] and –O-H group [6] were suggested to be potential deprotonation sites. Acid-base equilibrium of lorazepam as shown in Scheme 1, where H_2L^+, HL and L^- represent a cationic, neutral nonionic and ionic species of lorazepam, respectively. The corresponding equilibrium constants are as follows:

$$K_1 = \frac{[H^+][HL]}{[H_2L^+]} \qquad (2)$$

$$K_2 = \frac{[H^+][L^-]}{[HL]} \qquad (3)$$

3.1. Potentiometric determination of acidity constants

Potentiometric determination of equilibrium constants was performed by application of the formation method [7]. Popovic et al. used this method for study of acid-base equilibria of fleroxacin [8]. The method is based on the determination of $\bar{n}$, i.e. the average number of protons bound to the free base. In the case examined it is given by:

$$\bar{n} = \frac{2[H_2L^+] + [HL]}{[H_2L^+] + [HL] + [L^-]} \qquad (4)$$

by combining Eqs. (2) and (3) and (4), the linear dependence was obtained:

$$\frac{\bar{n}-1}{\bar{n}}[H^+] = \frac{1}{K_1}\frac{2-\bar{n}}{\bar{n}}[H^+]^2 - K_2 \qquad (5)$$

on the basis of which K_1 and K_2 can be determined from the slope and intercept, respectively. The determination of the $\bar{n}$ from experimental data was calculated according to the equation:

$$\bar{n} = \frac{c_{HL} + c_{HCl} - c_{NaOH} - [H^+] + [OH^-]}{c_{HL}} \qquad (6)$$

where c_{HL}, c_{HCl} and c_{NaOH}, correspond to the stoichiometric concentrations of lorazepam, hydrochloric acid and sodium hydroxide solution respectively, and $[H^+]$ and $[OH^-]$ were obtained from pH measurements. The dependence of formation function, $\bar{n}$ versus pH shown in Fig. 1a. Linear dependence of $\frac{\bar{n}-1}{\bar{n}}[H^+]$ versus $\frac{2-\bar{n}}{\bar{n}}[H^+]^2$ gives $K_1=2.63\times10^{-3}$ ($pK_1=1.58\pm0.05$), and $K_2=2.14\times10^{-12}$ ($pK_2=11.67\pm0.07$) (Fig. 1b).

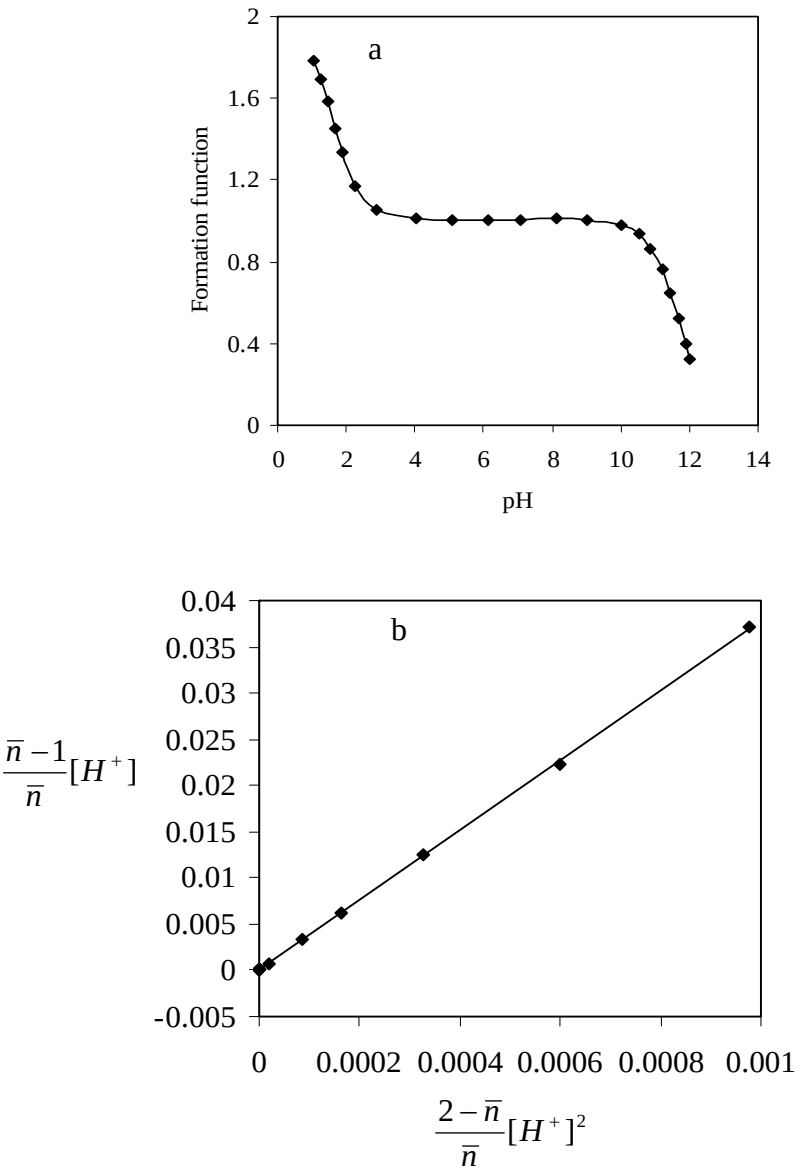

Fig.1. (a) Formation function dependence on pH, (b) Graphical presentation of Eq. (5) for potentiometric evaluation of acidity constants K_1 and K_2.

3.2. Spectrophotometric determination of acidity constants

DATAN program applied for determination of protolytic equilibria. Output of DATAN program are acidity constant values, number of principal components, concentration distribution diagrams and pure spectrum of each assumed species. The theory and application of the physical constraints method was discussed by Kubista et al., in several papers [5, 9-16]. The absorption spectra of lorazepam obtained in pH range from 1 to 13. The principal component analysis of all absorption data matrix obtained at various pH shown at least three significant factors. This claim is, also, supported by the statistical indicators method that has been recently developed by Elbergali et al. [17], which has predicted three distinguishable components in the samples. These factors could be attributed to the two dissociation equilibria of lorazepam.

The acidity constant values of lorazepam were investigated spectrophotmetrically at 25°C and ionic strength of 0.1 mol L^{-1}. Protolytic equilibria of lorazepam were evaluated using DATAN program using the corresponding absorption spectra-pH data. In this study, we obtained acidity constant values of lorazepam, 1.61±0.03 and 11.58±0.04 for pK_1 and pK_2, respectively. The most important features of distribution diagram are the pH limit of evolving and disappearance of components. So according to distribution diagram it is may conclude that at smaller pH than 1.5 assigned to H_2L^+ form because this form is dominated at this range. At pH 3-10.5 interval the HL form is dominated and the L^- form appeared at pH>11.5. The results obtained from this report, are nearly the same as the previously obtained values [3, 6] and the differences are within the experimental and measurement errors.

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