Multiwavelength Spectrophotometric Determination of Acidity Constants of Newly Synthesized 1,2,4-Triazole Derivatives in Ethanol−Water Mixtures

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The acid−base properties of 4-benzyl-5-(pyridin-2-yl)-4*H*-1,2,4-triazole-5(4*H*)-thione, (4-benzyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazole-5(4*H*)-thione, and 4-benzyl-5-(pyridin-4-yl)-4*H*-1,2,4-triazole-5(4*H*)-thione in ethanol/water mixtures have been studied at 25 °C and for an ionic strength of 0.1 M by a multiwavelength spectrophotometric method. For evaluation of the pH−absorbance data, a resolution method based on the hard modeling was used. The acidity constants of each dissociation equilibria were estimated by fitting the whole spectral collected data to an established factor analysis model. The EQUISPEC program was used for determination of acidity constants. The corresponding pK_a values in ethanol−water mixtures were determined. There is a linear relationship between acidity constants and the mass fraction of ethanol in the solvent mixtures. The effect of solvent on acid−base behavior was discussed.

Introduction

1*H*-1,2,4-Triazoles display important pharmacological activities such as antiasthmatic,[1] antiviral (ribavirin),[2] antifungal (fluconazole),[3] antibacterial,[4] and hypnotic[5] (triazolam) drugs. Furthermore, various 1,2,4-triazole derivatives have been reported as fungicidal[6] and pesticidal[7] agents. In addition, it was reported that compounds having triazole moieties such as vorozole, letrozole, and anastrozole appear to be very effective aromatase inhibitors, which prevent breast cancer.[8,9] 1,2,4-Triazole and its derivatives are very interesting ligands because they combine the coordination geometry of both pyrazoles and imidazoles with regard to the arrangement of their three heteroatoms. Transition metal complexes of 1,2,4-triazole derivatives are intriguing from both theoretical and practical viewpoints and are the subject of several magnetic studies.[10,11]

Drug pharmaceutics and the determination of useful dosage forms and regimes depend upon an understanding of drug dissociation and the extent of dissociation that will occur in the systems of the body. Depending on the route of the administration of the drug and the location of the target site, the pH of the environments that the compound is exposed to may vary considerably. The affinity of the drug molecule for the target of interest and its ability to partition into a lipophilic environment at different pH values has to be quantified for a proper prediction of its ability to interact with the biological target and hence to be efficacious. The most important physicochemical characteristics of drugs are their acidity or basicity expressed by their pK_a values, their hydrophobicity, and its dependence on pH. Protonation constants or acid dissociation constants are very important both in the analysis of drugs and in the interpretation of their mechanisms of action as they are the key parameters for predicting the extent of the ionization of a molecule in solution at different pH.

Spectrophotometric methods in combination with suitable chemometric tools can be used for the determination of acid dissociation constants pK_a even for barely soluble drugs.[12−18] When the components involved in the protonation equilibria have distinct spectral responses, their concentrations can be measured directly, and determination of the protonation constant is trivial. In many cases, however, the spectral responses of two and sometimes even more components overlap considerably, and analysis is no longer straightforward. In such cases, much more information can be extracted if multivariate spectrophotometric data are analyzed by means of an appropriate multivariate data analysis method. Hard modeling methods include traditional least-squares curve fitting approaches, based on a previous postulation of a chemical model. The postulations are a set of species defined by their stoichiometric coefficients and formation constants, which are refined by least-squares minimization.

In spectrophotometric titrations, linear or near-linear dependence of concentration profiles and the existence of minor species cause difficulties in the evaluation of the data. These difficulties reside, on the one hand, in the calculation of absorption spectra, and on the other hand, the corresponding equilibrium constants are not or are only poorly defined. In second-order global analysis, the spectrophotometric titration data with different initial concentrations are simultaneously analyzed. In this way, the concentration matrix is augmented to full rank, and second, conditions for the significant formation of each species can be obtained. The resulted equilibrium constants and absorption spectra are notably better defined. To overcome such problems, some programs such as SPECFIT,[19] SQUAD,[20,21] and EQUISPEC[22] are developed.

EQUISPEC is a computer program using the matrix-based MATLAB environment for second-order global analysis of spectrophotometric equilibrium data. It is capable of analyzing pH spectrophotometric titration data of solutions containing multiprotonic acids (HnL) in the presence or absence of other complexing agents (e.g., M). The details and advantages of this program are clearly described by Dyson et al.[22]

In this study, we have evaluated the information on the acidity constants of three 1,2,4-triazole derivatives: 4-benzyl-5-(pyridin-2-yl)-4*H*-1,2,4-triazole-5(4*H*)-thione, 4-benzyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazole-5(4*H*)-thione, and 4-benzyl-5-(pyridin-4-yl)-4*H*-1,2,4-triazole-5(4*H*)-thione. The empirical determination of

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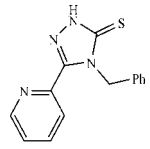
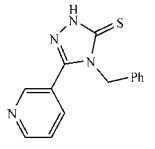
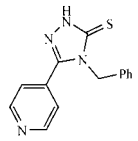
Scheme 1. Chemical Structure of Three 1,2,4-Triazole Derivatives



4-benzyl-3-(pyridin-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione (P2T)



4-benzyl-3-(pyridin-3-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione (P3T)



4-benzyl-3-(pyridin-4-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione (P4T)

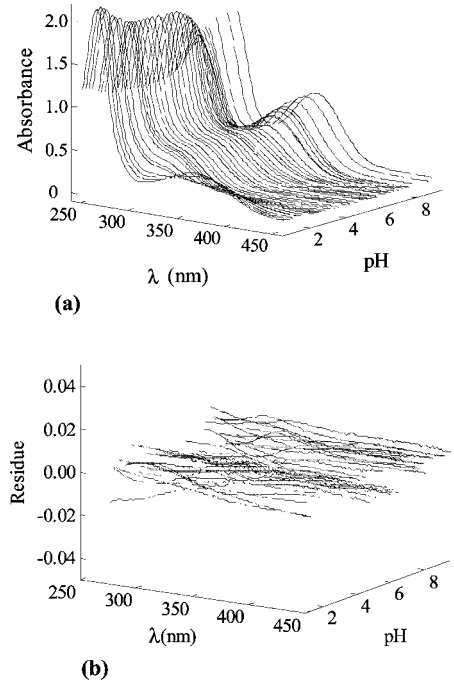


Figure 1. Absorption spectra of the protonation equilibria of P4T ($1.0 \cdot 10^{-4}$ M) in 40 wt % ethanol to water in dependence on pH at 25 °C: (a) 3D absorbance response surface representing input data; (b) the 3D overall diagram of residuals representing a response surface indicating the quality of a goodness-of-fit.

protonation constants was compared with their computational prediction based on knowledge of chemical structures.

Experimental Section

Chemicals. 4-Benzyl-5-(pyridin-2-yl)-4*H*-1,2,4-triazole-5(4*H*)-thione (Scheme 1, P2T), 4-benzyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazole-5(4*H*)-thione (Scheme 1, P3T), and 4-benzyl-5-(pyridin-4-yl)-4*H*-1,2,4-triazole-5(4*H*)-thione (Scheme 1, P4T) were synthesized and purified in our research laboratory.[23,24] Stock solutions ($1.0 \cdot 10^{-3}$ M) of P2T, P3T, and P4T were prepared in ethanol/water solvent mixtures and diluted to desired concentration (usually $5.0 \cdot 10^{-4}$ M − $1.0 \cdot 10^{-4}$ M) by the same solvent composition. Hydrochloric acid, sodium hydroxide, and potassium chloride were analytical grade commercial products from Merck Company and were used as received. Ethanol (HPLC grade, Merck Co.) and triply distilled water were used for the preparation of different ethanol/water solvent mixtures.

Apparatus. The electronic absorption spectra were recorded using an HP-Agilent 8453 spectrophotometer equipped with a water thermostable cell holder, a sipper, and a flow cell (80 μL, 1.0 cm). A Huber thermostat was used to keep temperature at (25 ± 0.5) °C. The pH measurements were made using a Metrohm 691 pH meter equipped with a glass calomel combined electrode.

Procedure. To record electronic absorbance spectra as a function of pH, the pH of the solution containing each triazole compound (usually 50 mL of $5.0 \cdot 10^{-5}$ M − $1.0 \cdot 10^{-4}$ M) was adjusted in the range of 1.2 to 12 by adding hydrochloric acid and potassium hydroxide solutions. Then, the solution transferred through the flow cell, and the absorbances at (240 to 600) nm were recorded at 1 nm increments. All experiments were carried out at the temperature (25 ± 0.5) °C and constant ionic strengths (0.1 M KCl). After each pH adjustment, the spectra were recorded and data were collected for subsequent treatment.

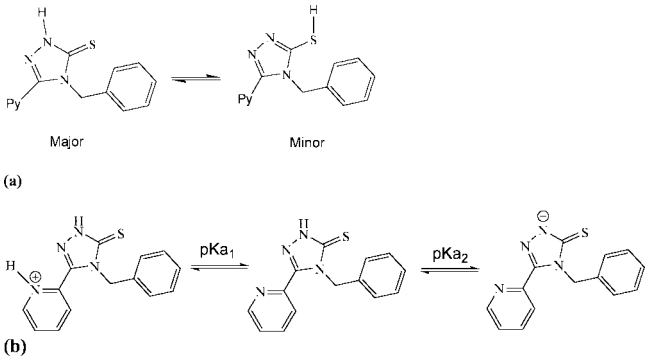
The calibration of the pH meter was done in the usual way using two standard buffer solutions (Methrohm) in aqueous media. Then, the pH values in ethanol/water solvent mixtures were corrected by using the equation pH* = pH(R) − δ, where pH* is the corrected reading and pH(R) is the pH-meter reading obtained in a partially aqueous organic solvent. The δ is written for $E_j - \log {}_m\gamma_H$, where E_j is the liquid junction potential and $\log {}_m\gamma_H$ is the medium effect for the proton (H^+) in mixed solvent. The evaluation method and the values of δ at different ethanol/water mixed solvents were reported previously.[25–27] For ethanol/water mixed solvent, up to 60 wt % of ethanol, the value of δ is small (< 0.20). Douheret reported ± 0.02 to ± 0.03 deviations on pK_a values as an effect of using the mentioned method for pH correction.[27]

Software. Determination of acidity constants was performed by regression analysis of the UV/vis spectra using the EQUISPEC program[22] running in the MATLAB 7.2 (The MathWork Co.) environment. Calculations were perform by an iterative procedure by using the Newton−Gauss−Levenberg/Marquardt (NGL/M) algorithm of nonlinear least-squares fitting.[28,29] The chemical structures were drawn by using ChemBioDraw ultra (Ver.11, Cambridgesoft). MARVIN (Ver. 5.0),[30] SPARC online calculator (Ver. 4.1),[31] and ACD/pK_a predictor[32] are programs for making predictions based on the structural formula of triazole compounds. By entering the compound topological structure descriptors graphically, pK_a values of organic compounds are predicted using approximately hundreds of Hammett and Taft equations and quantum chemistry calculus.

Results and Discussion

The electronic absorption spectra of P2T, P3T, and P4T at binary solvent mixtures at various pH values at (240 to 600) nm were recorded. Due to the dependency of pK_a values on temperature, all spectra were recorded while the temperature was set at (25 ± 0.5) °C using a Huber thermostat. A sample spectra of P4T ($1.0 \cdot 10^{-4}$ M) at different pH values in 40 wt % ($w = 0.4$) ethanol/water is shown in Figure 1a.

Scheme 2. Proposed Tautomerization Equilibria (a) and Ionization Path (b) for 1,2,4-Triazole Derivatives Used in This Study (e.g., P2T)



The principal component analysis of all absorption data matrices obtained in the pH range 1.2 to 12.0 shows two significant factors for equilibria involving P2T and P3T and three significant factors for P4T. These factors may be attributed to the one dissociation equilibrium of a monoprotic acid such as P2T and P3T and two dissociation equilibria of a diprotic acid such as P4T. The proposed dissociation path for these derivatives is shown in Scheme 2. Because of the similarity in the structures of P2T, P3T, and P4T, it is expected that P2T, P3T, and P4T have the same number of protonation equilibria. Different packages showed two protonation equilibria for these compounds at the regular pH range. Therefore, we assigned the pK_{a1} and pK_{a2} to explain their equilibria. However, the first protonation equilibria of P2T and P3T take place at pH < 1 which is hard to establish because of the contribution of acidity error in the pH electrode at high acidity medium especially in mixed organic/water solvent mixtures.

The pK_a values of P2T, P3T, and P4T were investigated in four different ethanol/water binary mixtures by evaluation of their corresponding spectral absorption−pH data by using the EQUISPEC program. From inspection of the experimental spectra, it is difficult to guess even the number of protolytic species involved. The two and three calculated most significant projection vectors with clear spectral features (as compared to noise) evidence the presence of two and three spectroscopically distinguishable components for P2T and P3T and three for P4T, respectively. The pK_a values and their standard deviations, concentration distribution diagrams, and the pure spectrum of each suggested species can be pulled out from the EQUISPEC program. The reliability of estimates of parameters (pK_a and molar absorptivities) can be evaluated using the following diagnostics.[17]

The first diagnostic indicates whether all of the estimated parameters, pK_a and molar absorptivities, have physical meaning. As the standard deviations (log K_a) of pK_a are significantly smaller than their corresponding estimates (Table 1 and Table 2), all the variously protonated species are statistically significant at a significance level of $p < 0.05$. Moreover, the non-negativity at molar absorption (spectra profiles) reveals that the estimated molar absorptivities on wavelength are realistic. Figure 2 shows the spectra profile (pure spectra) for different species of P2T, P3T, and P4T at 20 wt % of ethanol.

The second diagnostic tests whether all of the calculated free concentrations of the variously protonated species on the distribution diagram have physical meaning. A distribution diagram makes it easier to judge the contributions of individual species to the total concentration. Figure 3 shows a sample distribution diagram for P4T as a function of pH at different ethanol/water solvent mixtures. As seen at any pH, the overall concentration of various species is equal to the total concentration of P4T.

The third diagnostic concerns the goodness-of-fit. For achieved goodness-of-fit, the differences between the experimental and calculated values of absorbance are easily examined. Examination of the spectra and the graph of the predicted absorbance response surface (Figure 1b) through all the experimental points should reveal whether the results calculated are consistent and whether any systematic errors exist in the recorded spectra. One of the most important statistics calculated is the standard deviation of absorbance (σ_Y), calculated from a set of refined parameters at the termination of the minimization.[33] The calculated values of σ_Y for each set of titrations are listed in Table 1 and Table 2. Regarding the number of data points in each set of titrations, the calculated values of σ_Y seem to be reasonable.

Effect of Solvent Composition. The calculated pK_a values at different ethanol/water mixtures are listed in Table 1 and Table 2. Due to the low solubility of the used compounds in water, the pK_a values at 0 wt % of ethanol (i.e., pure water) were calculated theoretically by using MARVIN, SPARC, and ACD−Laboratory software, which use the structural information of the compounds for estimation of protonation constants. The predicted pK_a values by ACD software are related to a tautomer with more abundance (Scheme 2a). However, the MARVIN and SPARC software have the ability to calculate pK_a values for different tautomers separately, and therefore both predicted pK_a values for major and minor tautomers are listed in Table 1 and Table 2. (First bolded values are assigned to the major tautomers.)

Comparison between the predicted values (pure water) and empirically calculated pK_a values at 20 wt % ethanol/water reveals that the predicted pK_{a2} values are more in agreement with the empirically derived acidity constants. The exact comparison of the ability of these packages for predication of pK_a values of P2T, P3T, and P4T is impossible. This is due to the absence of empirical values of pK_a at water (0 wt % ethanol) for these compounds and the variation in the tautomer abundances at different pH values. It seems that among the packages which were used for prediction of acidity constant the MARVIN package can predict pK_{a1} values of P4T more accurately than other packages, while the ACD is likely to be more acceptable for pK_{a2} predictions.

As can be seen from Table 1 and Table 2, by increasing the percentage of ethanol the values of pK_{a2} of P2T, P3T, and P4T increase, and the pK_{a1} of P4T decreases, respectively. The data clearly illustrate the important influence of the nature of the solvent on the dissociation reaction. It has been shown that the solvating ability[34] (as expressed by the Guttmann donicity scale) and dielectric constant of the solvent play a fundamental role in dissociation reactions. Water is a solvent with high solvating ability (i.e., donor number DN = 33.0 and dielectric constant ε = 78) which could dissociate the acid and stabilize the produced anion and hydrogen ion. Thus, it is expected that addition of ethanol with lower donor number and dielectric constant (DN = 30.0, ε = 24.3) to water decreases the dielectric constant and donor number of the resulting solvent mixture. If the solvating ability of the mixture of solvent is decreased, more energy will be required to separate the anion and cation, and consequently, the extent of dissociation of the acid (LH) would be decreased (increases pK_{a2}, Figure 4). Moreover, the species LH_2^+ is more stable in water, so addition of ethanol to water increases the dissociation constant (decreases pK_{a1}, Figure 5).

Table 1. Acidity Constants of P2T and P3T at Different Mass Fractions of Ethanol at 25 °C and Constant Ionic Strength (0.1 M KCl)

	P2T			P3T		
mass fraction of ethanol	pK_{a1}	pK_{a2}	σ_Y (d)	pK_{a1}	pK_{a2}	σ_Y (d)
[a]0.0	1.61 (a)	6.83 (a)	-	3.37 (a)	7.06 (a)	-
	2.46, 1.12 (b)	**9.01**, 7.88 (b)	-	**4.07**, 3.96 (b)	**9.30**, 7.86 (b)	-
	2.22, 3.11 (c)	**14.11**, 2.42 (c)	-	**2.42**, 3.07 (c)	**15.29**, 3.29 (c)	-
0.2	-	7.22 ± 0.01	0.02	-	7.94 ± 0.01	0.03
0.3	-	7.94 ± 0.01	0.03	-	8.25 ± 0.01	0.03
0.4	-	8.11 ± 0.01	0.03	-	8.47 ± 0.02	0.03
0.6	-	8.45 ± 0.01	0.02	-	8.77 ± 0.02	0.02

[a] pK_a values at pure water are predicted by (a) ACD−Laboratory/pK_a, (b) MARVIN, and (c) SPARC softwares. Bolded values are related to the major tautomer. (d) Average of the standard deviation of individual absorbance readings of the different titrations (see ref 33).

Table 2. Acidity Constants of P4T at Different Mass Fractions of Ethanol at 25 °C and Constant Ionic Strength (0.1 M KCl)

mass fraction of ethanol	pK_{a1}	pK_{a2}	σ_Y (d)
[a]0.0	4.01 (a)	6.79 (a)	-
	3.42, 3.86 (b)	**9.37**, 7.85 (b)	-
	4.84, 5.41 (c)	**14.21**, 2.46 (c)	-
0.2	2.43 ± 0.01	6.95 ± 0.01	0.03
0.3	2.18 ± 0.01	7.12 ± 0.01	0.03
0.4	1.94 ± 0.02	7.21 ± 0.01	0.03
0.6	1.62 ± 0.04	7.40 ± 0.01	0.03

[a] pK_a values at pure water are predicted by (a) ACD−Laboratory/pK_a, (b) MARVIN, and (c) SPARC softwares. Bolded values are related to the major tautomer. (d) Average of the standard deviation of individual absorbance readings of the different titrations (see ref 31).

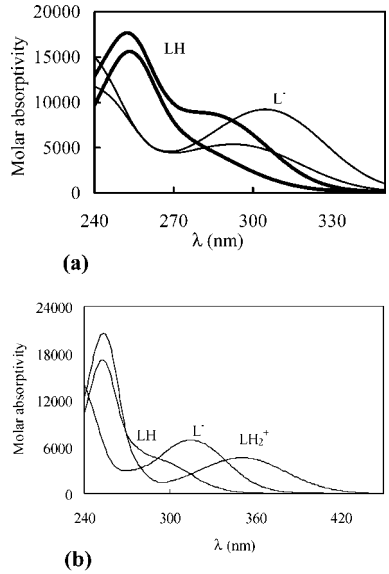


Figure 2. Pure spectra of different species in 20 wt % ethanol to water solvent mixture. (a) P2T (thin curves) and P3T (thick curves). (b) P4T.

Conclusions

In this study, we distinguished the behavior of acidity constants of three 1,2,4-triazole derivatives in water−ethanol binary solvent mixtures at 25 °C and for ionic strength of 0.1 M which has been studied by a multiwavelength spectrophotometric method. Results show that the pK_a values of 1,2,4-triazole derivatives are influenced by varying the percentages of ethanol at the solvent mixture. By using EQUISPEC, we obtained the concentration profiles and pure spectra from the experimental data. The effect of solvent properties on acid−base behavior is discussed. The obtained results show that increasing the weight percent of ethanol has different effects on the protonation equilibria of the 1,2,4-triazole derivatives concerned in this study. The effect of solvent can be interpreted by regarding the properties of mixed solvents and the type of different species involved in the dissociation equilibria.

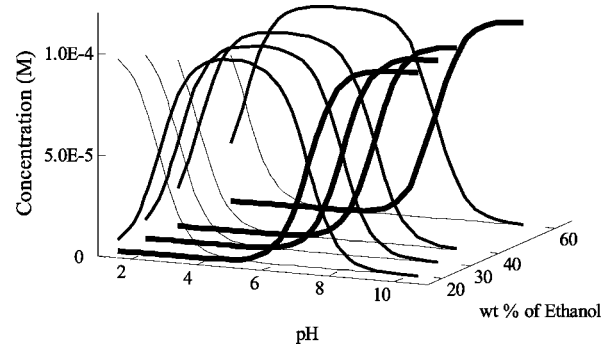


Figure 3. Distribution diagram of major species of P4T as a function of pH in different water−ethanol solvent mixtures. Total concentration of P4T in mixtures is $1 \cdot 10^{-4}$ M. (The corresponding curves from thin to thick are referred to as LH_2^+, LH, and L^-, respectively).

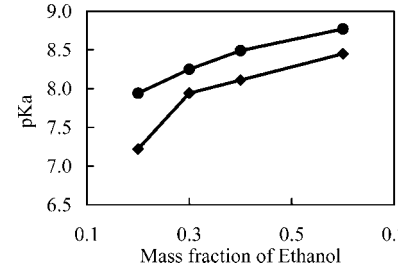


Figure 4. Plot of pK_{a2} values of ●, P2T and ◆, P3T against mass fraction of ethanol in the ethanol/water binary mixed solvents.

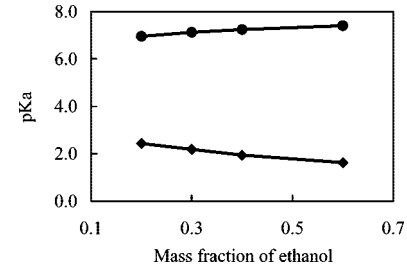


Figure 5. Plot of pK_a values of P4T against mass fraction of ethanol in the ethanol/water binary mixed solvents. ◆, pK_{a1} and ●, pK_{a2}.

Acknowledgment

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