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A study of behavior of some 5-substituted-4-phenyl-1,2,4-triazoline-3-thiones in sulfuric acid solution using characteristic vector analysis

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The protonation process of 1,2,4-triazoline-3-thiones (**T3T**) was studied in aqueous sulfuric acid using the UV method. Characteristic vector analysis (CVA) was used to reconstruct the experimentally obtained UV spectra, to overcome difficulties in calculating the protonation constants of **T3T**, and to separate the effect of protonation from the generalized medium effect. pK_{TH+} values were calculated using the Hammett acidity function method, the Bunnett and Olsen method, and the excess acidity function method. The obtained values for m , ϕ and m^* were close to those characteristic for similar thio compounds, indicating that the protonation site in the molecule of **T3T** is the S atom.

Key Words: 1,2,4-Triazoline-3-thiones, UV, protonation, CVA method

Introduction

The applications of pKa values are extremely diverse, ranging from the most fundamental, e.g. calculating the distribution functions in a given pH interval, to exploring the transportation of substances through the cell membrane. Therefore, knowledge of the pKa value of a substance is essential for various investigations in pharmacology and physiology; in structural, environmental, preparative, and analytical studies; and for industrial purposes.¹

It is well known that 1,2,4-triazole and its derivatives represent one of the most biologically active classes of compounds, possessing a wide spectrum of activities. The 1,2,4-triazole nucleus is associated with diverse pharmacological activities, such as antibacterial, antifungal, hypoglycemic, antihypertensive, and

analgesic properties.²⁻⁸ During the last 10 years, some new 1,2,4-triazole derivatives were synthesized and investigated.⁹⁻¹³

Triazole derivatives act as weak bases in acidic media.¹⁴ Knowledge of the acidity constants of weakly basic substrates is of central importance to the study of the reaction mechanisms taking place in acidic media.^{14,15} It is also known that the dissociation constant pK_a depends on the polarity of the molecule and, directly or indirectly, on the intra- and intermolecular forces.¹⁶ It was implicit that for maximum activity, the 1,2,4-triazoles should have a pK_a that gives the proper equilibrium between the inherent activity and penetration, and the half-dissociated status appears to present the best compromise between transport and activity.

The parameters accounting for the intra- and intermolecular forces and the steric effect definitely play a significant role in the modeling of dissociation constant pK_a . The characteristics of 1,2,4-triazole compounds depend on the acid-base processes in the defined media. For this reason, the acid-base equilibrium of 1,2,4-triazole and its derivatives in different media has been studied by many authors.^{17,18} Katritzky and Rees¹⁷ determined the values of the ionization constants of 1,2,4-triazole as a base ($pK_B = 2.19$) and as an acid ($pK_{BH^+} = 10.26$).

In contrast to the widely studied acid-base properties of the carbonyl group, analogous thiocarbonyl compounds have received little attention. While this is in part due to the lack of stability of many such derivatives, there are nonetheless some which exhibit sufficient stability toward acids. An acidity function (H_T) for thiocarbonyl bases has been constructed, and protonation data for a wide variety of thiocarbonyl compounds have been reported.^{19,20}

According to the literature data, pK data obtained by the Hammett acidity function and the Bunnett-Olsen and Marziano-Cimino-Passerini methods have been compared^{19,20} and found to be rather similar. Moreover, the values of the ϕ parameter¹⁹ indicated that the solvation requirements of those compounds are low and little dependent on structure.

The pK values have been determined for some 1,2,4-triazoline-3-thiones in sulfuric acid and sodium hydroxide media using the ultraviolet spectroscopy.¹⁸ The scope of this work was the study of the behavior of some 5-substituted-4-phenyl-1,2,4-triazoline-3-thiones (**T3T**) in sulfuric acid solution using characteristic vector analysis.

Using the previous experience in this field, it was expected that 1,2,4-triazoline-3-thiones in strong mineral acid media would behave as weak bases and the protonation process should take place. The aim of this work was to analyze the behavior of the selected **T3T** in strong mineral acid media and to determine the ionization constants, data which are not available in the literature. For the calculation of pK_{BH^+} values, several methods known from the literature were applied. For the reaction of protonation of a weak base (such as 1,2,4-triazole) in strong mineral acid, the following equation can be written:



The equilibrium constant according to Hammett²¹ can be defined with the following equation:

$$pK_{BH^+} = H_0 + \log[c(BH^+)/c(B)] \quad (2)$$

H_0 is the Hammett acidity function, which represents the acidity scale of the highly acidic media and is

used for the determination of a weak base strength,²² $c(\text{BH}^+)$ is the concentration of the protonated form of the compound, and $c(\text{B})$ is the concentration of the unprotonated form of the compound.

The original Hammett acidity function has been upgraded by some authors. Their purpose was to reach more reliable pK_{TH^+} values of the investigated compounds. Above all, it was found that the H_0 acidity function depends on the class of the investigated compounds. Sometimes there are differences for closely related compounds, even with the same protonation center. Different values of H_0 are defined as H_X , which describes most accurately the protonation process of different groups of compounds. For thiocarbonyl compounds, H_X is noticed as H_T and its values are established for 10%-90% sulfuric acid aqueous solutions.¹⁹

Accordingly, pK_{TH^+} (pK_{BH^+} for thio compounds) values can be defined by the equation:

$$pK_{\text{TH}^+} = \log I + mH_T \quad (3)$$

where I is the ratio between the concentration of the protonated and unprotonated form of the thio base, $c(\text{TH}^+)/c(\text{T})$, also known as an ionization ratio. A plot of $\log I$ versus $-H_T$ gives a straight line with slope m , whose value is about 1.

For the determination of pK_{TH^+} values, Bunnett and Olsen²³ suggested an equation based on the linear free energy relationship:

$$pK_{\text{TH}^+} = H_0 - \phi[H_0 + \log c_{\text{H}^+}] + \log I \quad (4)$$

The value of the H_T acidity function can be defined by the equation:

$$H_T = H_0 - \phi[H_0 + \log c_{\text{H}^+}] \quad (5)$$

The final equation for determination of pK_{TH^+} values, obtained from Eqs. (4) and (5), is:

$$pK_{\text{TH}^+} = H_T + \log I \quad (6)$$

In Eq. (4), ϕ is a parameter that characterizes the changing activity coefficient behavior of bases with changing acidity. According to this method, it is found that the parameter ϕ has a negative value for the large polarizable molecules. These molecules have a smaller extent of solvation than the protonated indicators. The value of ϕ for thiocarbonyl compounds is -0.4.¹⁹

The method of Cox and Yates,²⁴ also known as the excess acidity function method, is often used in the literature for the calculation of pK_{TH^+} values. According to this method, pK_{TH^+} values can be calculated using the equation:

$$pK_{\text{TH}^+} = [\log I - \log c_{\text{H}^+}] - mr^*X \quad (7)$$

where m^* expresses the hydrogen-bonding solvation of the protonated base. Its value when determined for thiocarbonyl compounds is in the range of 1.39 ± 0.14 . X is a function that represents the difference between the observed acidity and that which the system would have if it were ideal ("excess acidity"). The values of this function were determined depending on sulfuric acid concentration, and they can be found in the literature.²⁴

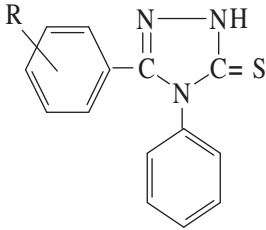
Protonation of complex systems, such as 1,2,4-triazoline-3-thione, causes a more complex change in the UV spectra. For the separation of the medium effect from those spectral changes, the method of characteristic vector analysis (CVA), which has been described by Simonds,²⁵ was used.

Experimental

Materials

In this work, the following compounds were investigated:

Table 1. 5-Substituted-4-phenyl-1,2,4-triazoline-3-thiones (**T1-T4**) used in the present study.

R		Mol. formula, mol. wt.	Elem. analysis cal./found [%]			
			C	H	N	
T1	2-OH	C ₁₄ H ₁₁ N ₃ OS	62.43	4.08	15.60	
		269.318	62.80	4.50	15.24	
T2	3-OH	C ₁₄ H ₁₁ N ₃ OS	62.43	4.08	15.60	
		269.318	62.27	4.05	15.86	
T3	2-OH, 5-Cl	C ₁₄ H ₁₀ N ₃ OCIS	55.35	3.29	13.83	
		303.763	55.50	3.15	13.74	
T4	3,4,5-(OCH ₃) ₃	C ₁₇ H ₁₇ N ₃ O ₃ S	59.46	4.99	12.23	
		343.39	59.05	5.40	11.84	

The newly synthesized compounds, reported elsewhere,^{9,10} were characterized by their melting points, IR, and NMR spectra, as well by elemental analysis.

Spectral measurements

Stock solutions of compounds **T1-T4** (2×10^{-3} mol dm⁻³) were prepared by dissolving the required amounts of the substances in absolute ethanol (Alkaloid, Skopje). These solutions were stable for a long time; for 2 months, there were no changes in their absorption spectra.

The concentration of the test solutions of the investigated compounds was 2×10^{-5} mol dm⁻³, and they were prepared by adding the appropriate volume of the stock solution to a H₂SO₄ solution of known concentration immediately before the recording of the spectra. The solutions of H₂SO₄ were prepared by diluting concentrated H₂SO₄ (Merck), and their concentrations were determined by density measurements. The H_T^{19} and X^{24} acidity functions were used for the characterization of the concentrated H₂SO₄ solutions. Triply distilled water was used for the preparation of the aqueous solutions. The blanks had the same concentration as the test solutions and the same volume of ethanol was added instead of stock solutions of the investigated compounds. The ethanol content in all of the test solutions and the blanks was 1%.

The spectrophotometric measurements were carried out on a Varian Cary 219 spectrophotometer in 1 cm quartz cells at 25 °C.

Method of characteristic vector analysis

CVA is a method of separating independent factors for sets of multivariate response data.²⁵ The method can be used empirically for estimating the number of independent factors contributing to the total variation observed in a family of UV spectra. If p independent factors are involved in generating the absorbance curve, the sample responses at each wavelength for a given concentration will be given by:

$$\begin{aligned} A_1 &= A_1 + c_1 v_{11} + c_2 v_{21} + \cdots + c_p v_{p1} \\ A_2 &= A_2 + c_1 v_{12} + c_2 v_{22} + \cdots + c_p v_{p2} \\ A_r &= A_r + c_1 v_{1r} + c_2 v_{2r} + \cdots + c_p v_{pr} \end{aligned} \quad (8)$$

where the choice of A is arbitrary and the mean values of the absorbance seem to be a convenient choice, while v is characteristic vectors, and c is weighting coefficients.

Result and discussion

UV spectra of T3T in neutral media

The protonation of 1,2,4-triazolin-3-thiones was investigated by the spectrophotometric method, following the changes in the electronic absorption spectra of the investigated compounds in an aqueous solution of H_2SO_4 . The characteristic spectral data for neutral form of compounds **T1-T4** are given in Table 2. Since the structures of the investigated compounds were similar, the absorption spectra of **T1-T4** were also similar. The electronic absorption spectra of the investigated compounds in neutral media (water) were characterized by the presence of absorption maxima at about 255-266 and 279-292 nm.

Table 2. UV spectra in neutral aqueous solutions of compounds **T1-T4**.

Compound	λ [nm]
T1	256 266
T2	257 283
T3	255 292
T4	258 279

As in the case of other heterocyclic thiones,²⁶ it is possible to consider that the 1,2,4-triazoline-3-thions may exist in **thion-thiol** tautomeric forms, but chemical and spectral investigations showed that the thion structure dominates. The absorption maxima or shoulders at around 250-260 and 280-290 nm indicated that some of these compounds exist predominantly in the thion form in ethanolic solution. The absorption at 280-290 nm indicated the presence of a chromophoric C=S group.²⁷

Experimental UV spectra of T3T in sulfuric acid media

The UV spectra of the aqueous sulfuric acid solution of the investigated triazoline-3-thiones (**T1-T4**), recorded in the wavelength region between 190 and 400 nm, are shown in Figures 1a-1d. It can be noticed that isobestic

points around 260 and 290 nm in the experimental UV spectra of compound **T1** and around 260, 280, and 305 nm in the spectra of compound **T3** are not strictly defined. This indicates the probable presence of several forms able to absorb UV radiation in the system. Increasing the mineral acid concentration ($-H_T$) from -2.25 to -15.25 (compound **T1**) and from -2.25 to -13.05 (compound **T3**), the absorption maximum at 255 nm exhibits bathochromic effects, i.e. it shifts toward longer wavelengths, to 265-270 nm, and a new band at 320-330 nm was formed.

In Figure 1b (compound **T2**), the hypochromic effect of the absorption maximum at 255 nm can be noticed, as a result of the increasing of the sulfuric acid concentration from -4.55 to -14.1. Protonation of compound **T4** was followed by bathochromic and hypochromic effects of the maximum at 265-295 nm, with the presence of a nondefined isobestic point at around 290 nm.

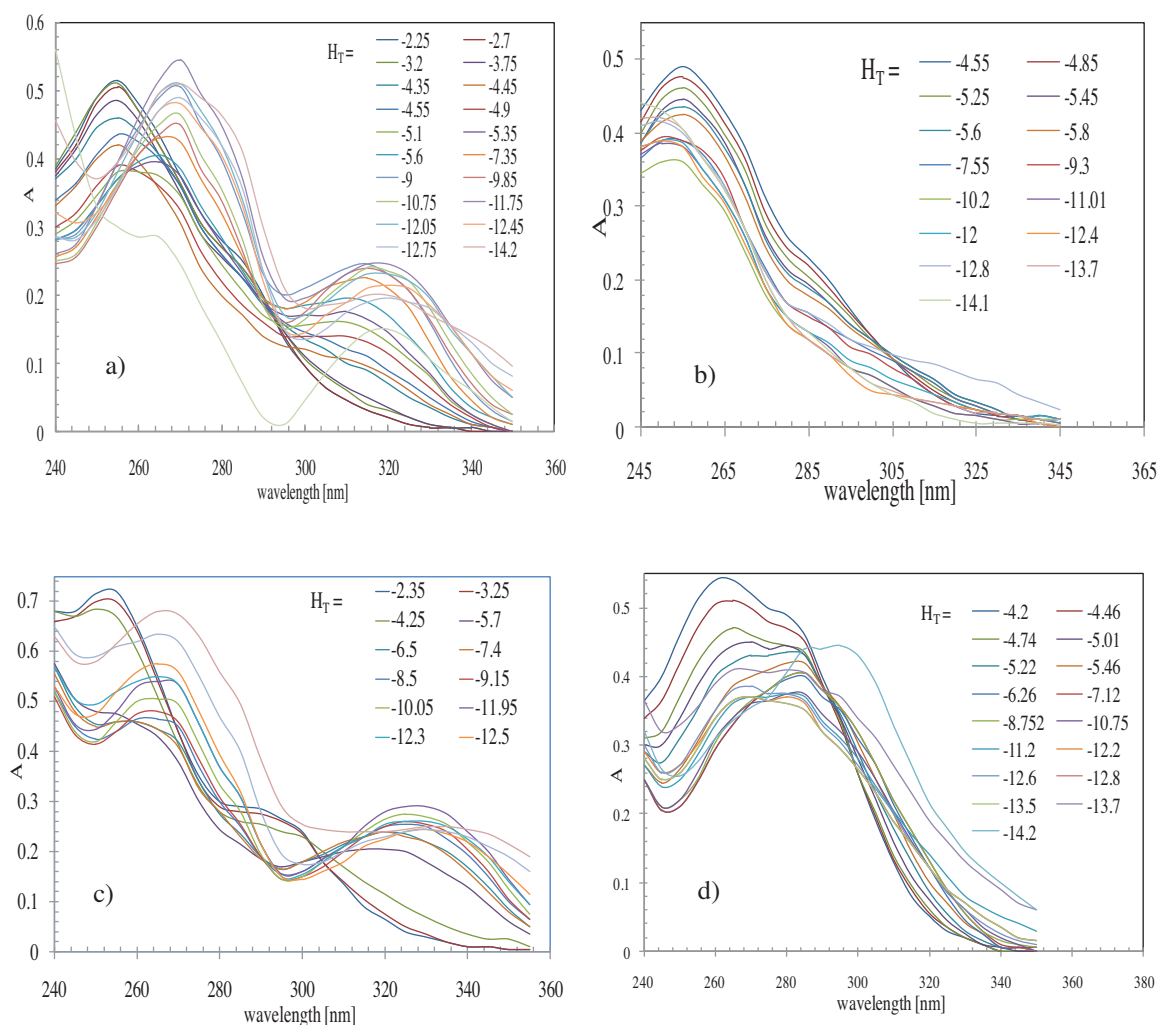


Figure 1. Experimental UV spectra of a) **T1**, b) **T2**, c) **T3**, and d) **T4** in sulfuric acid media ($C = 2 \times 10^{-5}$ mol dm^{-3}).

Reconstructed UV spectra of T3T in sulfuric acid media

As a result of the “medium effect,” which prevented the family of spectral UV curves from passing through a clear point, none of the experimental UV spectra showed precisely defined isobestic points. For this reason, an attempt was made to separate the undesired medium effect from the large change due to the protonation, by analysis of the experimental curves using the method of CVA.²⁵ The results are shown in Figures 2a-2d.

In the reconstructed spectra of compounds **T1** and **T4**, clear isobestic points can be noticed at 260 and 295 nm, respectively. The spectra of compound **T3**, reconstructed using CVA, intersects at 3 isobestic points ($\lambda_{IP} = 265, 285, \text{ and } 305 \text{ nm}$).

Another observation is that in the reconstructed spectra, the same changes happened as in the experimental UV curves: bathochromic and hypochromic effects of the maximum at around 255 nm, followed by the formation of a new band above 300 nm.

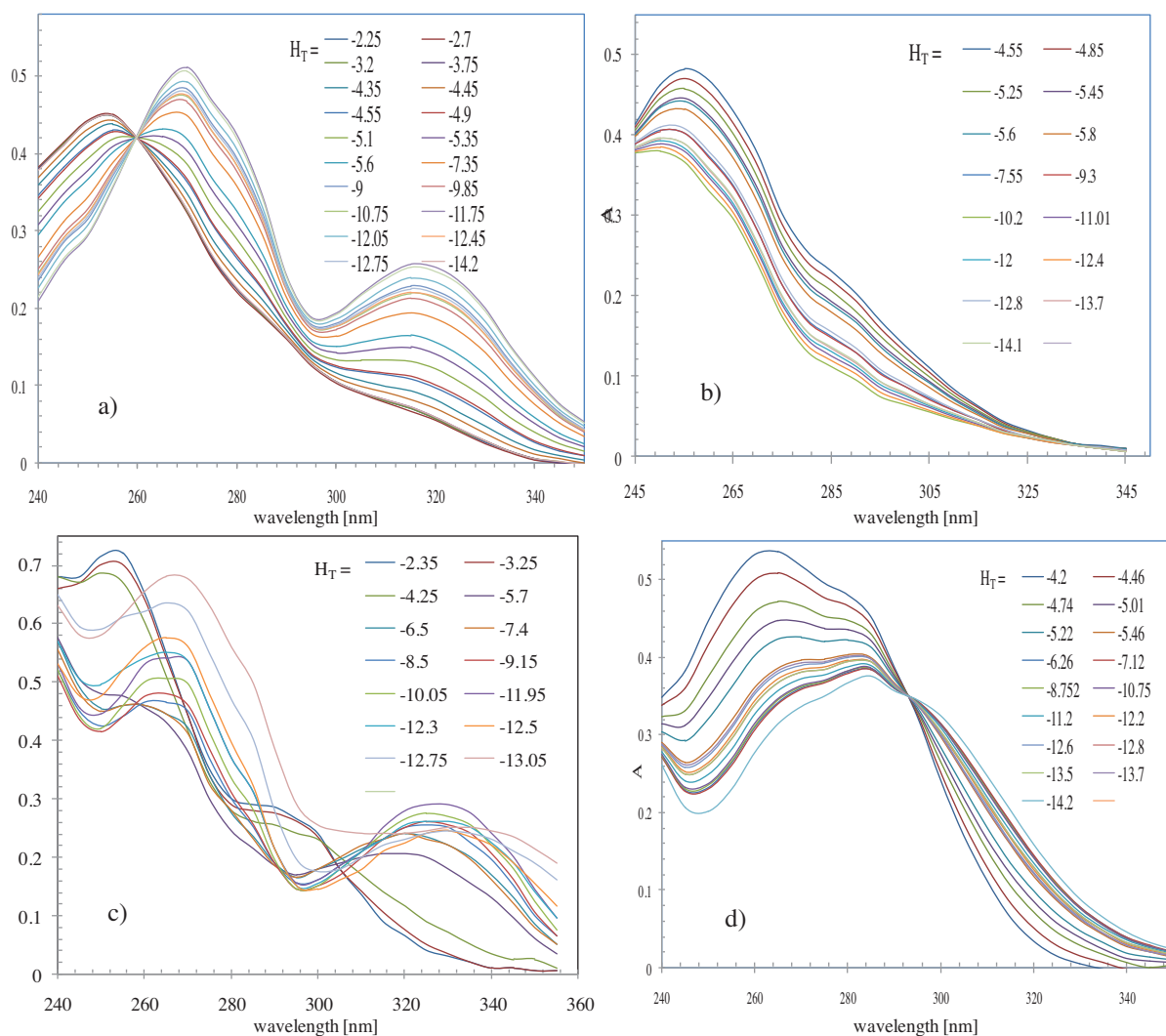


Figure 2. Reconstructed UV spectra of a) **T1**, b) **T2**, c) **T3**, and d) **T4** in sulfuric acid media ($C = 2 \times 10^{-5} \text{ mol dm}^{-3}$).

According to previous investigation on similar compounds, in solutions with high concentrations of H_2SO_4 (more than 15 mol dm^{-3}) the investigated 1,2,4-triazole-3-thiones were hydrolyzed with the formation of new molecular species: the starting thiosemicarbazides.¹⁸

The protonation constants of T3T in sulfuric acid media

Using the absorption spectra recorded in solutions of different concentrations of H_2SO_4 , the protonation processes of triazole derivatives were investigated. As can be seen from the UV spectra, the value of the absorbance depends on the concentration of sulfuric acid, which can be better seen from the plot of absorbance at λ_{max} versus the sulfuric ratio.

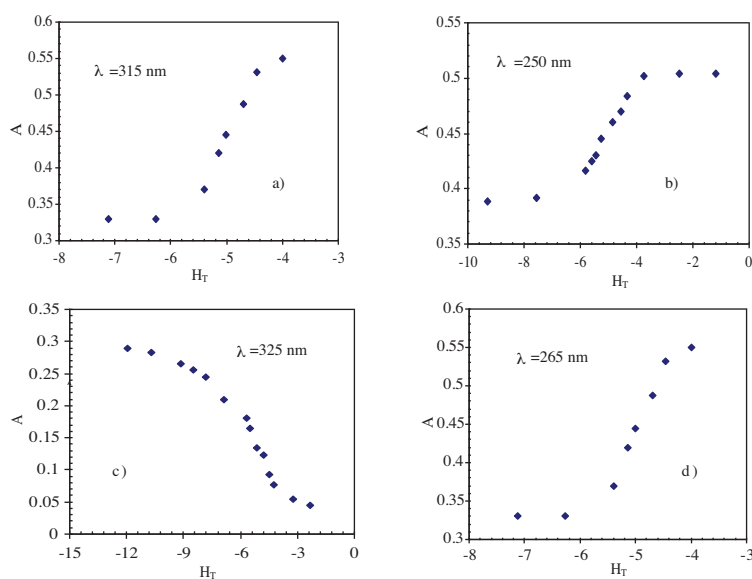


Figure 3. The absorbance changes as a function of H_T : a) **T1**, b) **T2**, c) **T3**, and d) **T4**.

The plots of absorbance on the chosen wavelengths against acidity functions and pH give sigmoidal curves (“S” curves) (Figure 3). The existence of only one plateau on the *S* curve confirms one protonation process for compounds **T1-T4**. This dependence is presented in Figure 3 for the compounds: a) **T1** at 315 nm, b) **T2** at 250 nm, c) **T3** at 325 nm, and d) **T4** at 256 nm.

The equilibrium constants of a weak organic base (pK_{TH^+}) such as 1,2,4-triazole (T) can be defined as the acid dissociation constants of the protonation form (TH^+) using Eq. (3), where the ionization ratio (*I*) can be calculated on the basis of spectral changes under different acidity, from the equation:

$$I = \frac{A - A_T}{A_{TH^+} - A} \quad (9)$$

where A_T is the absorbance of the unprotonated form, A_{TH^+} is the absorbance of the protonated form, and *A* is the absorbance of the solution at a given acidity at the same wavelength λ .

In this work, the pK_{TH^+} values were calculated using the following methods:

1. **Hammett acidity function method (HAFM)**, using Eq. (3), on the condition that the slope parameter m is approximately equal to 1, where the H_T^{13} acidity function was used for H_X ;

2. **Bunnett and Olsen method (BOM)**, using Eq. (4); and

3. **Excess acidity function method (EAFM)**, using Eq. (7), where c_{H^+} is the proton concentration and X the excess acidity, values which are available for an aqueous sulfuric acid system as a function of weight percent composition.²⁴ The slope parameter m^* expresses the hydrogen bonding solvation of the protonated base.

The mean pK_{TH^+} values calculated using Eqs. (3), (4), and (7) are presented in Table 3. The calculations were carried out using Excel.

The values of the dissociation constants of protonated forms of the compounds were also determined graphically using the method of Davis and Geissman.²⁸ Namely, when the concentrations of the protonated and unprotonated forms are equal, $\log I = 0$ and $pK_{BH^+} = H_T$. The dependence of $\log I$ on $-H_T$ is linear with a different slope depending on the method of calculation, and with an intercept equal to the graphic value of pK_{BH^+} for a given compound.

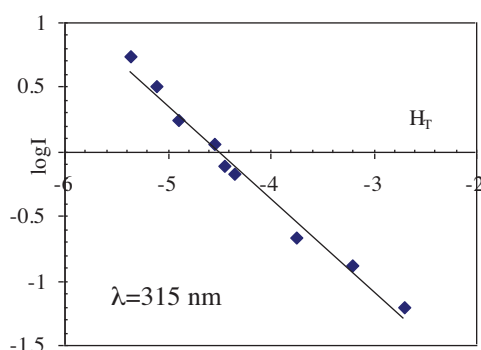


Figure 4. Dependence of $\log I$ on $-H_T$ for **T1** in sulfuric acid media, according to the Hammett method.

From Eq. (4), it is evident that a plot of $(H_0 + \log I)$ against $(H_0 + \log c_{H^+})$, for any thiocarbonyl base, should give a straight line with the hydration parameter ϕ as the slope and the ionization constant pK_{BH^+} as the intercept. In the excess acidity function method (the Cox-Yates method), the dependence of $\log I - \log c_{H^+}$ versus X is linear.

For illustration, the method used for determination of pK_{BH^+} values graphically in sulfuric acid media for **T1**, using the Hammett acidity function method, is presented in Figure 4. From Figure 4, it can be seen that the pK_{TH^+} value obtained graphically for **T1** according to the Hammett method was around -4.41. The obtained results for the numerically and graphically calculated values of pK_{TH^+} using 3 methods from the absorbance data are shown in Table 3 for the experimental spectra.

Similarly, calculations were made using the data from the reconstructed spectra where the solvent influence was eliminated. The obtained pK_{TH^+} values are also shown in Table 3. In Table 3, values are also given for the standard deviations (SD), correlation coefficients (R), and the values of the slope: m for HAFM, ϕ for BOM, and m^* for EAFM.

Compound **T1** had the lowest pK_{TH^+} values, obtained experimentally and after separating the medium effect. This means that this compound is a stronger base compared with the other thiones (**T2-T4**). This

is probably a result of the steric influence of the OH group, placed in the ortho position in phenyl moiety. According to the reconstructed pK_{TH+} values, it can be concluded that they are not much different from the data calculated using the experimental spectra. This indicates that the pK_{TH+} values obtained from the reconstructed spectra are in good agreement with the pK_{TH+} values obtained from the experimental spectra, meaning that there was little influence of the solvent on the appearance of the spectra.

The same agreement is obvious for the numerically and graphically obtained pK_{TH+} values. The standard deviation and correlation coefficients using all 3 methods are statistically good, indicating good reliability for those calculations.

Determination of the protonation place in 1,2,4-triazolin-3-thione moiety

The protonation process probably occurs on the thiourea fragment of the 1,2,4-triazoline-3-thione ring. This was confirmed by the value of the slopes, m , ϕ and m^* , which indicates that the protonation process was in agreement with the H_T acidity function determined for similar types of compounds. Namely, values for m , ϕ and m^* according to the literature data for the same thiocarbonyl compounds are: m approximately equal to 1, ϕ around -0.4, and m^* in the range of 1.39 ± 0.14 , which are characteristic for the protonation of sulfur.²⁴

Table 3. pK_{TH+} values for compounds **T1-T4** in sulfuric acid media (experimental and reconstructed spectra).

			HAFM ^a	HAFM ^b	BOM ^a	BOM ^b	EAFM ^a	EAFM ^b
T1	pK_{TH+}	numerically	-4.3078	-4.6529	-4.4719	-5.0329	-4.0334	-4.5051
		graphically	-4.4191	-4.9016	-4.4720	-5.0328	-4.0333	-4.5053
		slope ^c	-1.0270	-1.0603	-0.3645	-0.4491	1.3005	1.3561
		SD	0.1469	0.2409	0.5902	0.7637	0.1331	0.2316
		R	0.9861	0.966	0.8696	-0.7053	0.9537	0.9564
T2	pK_{TH+}	numerically	-5.1690	-5.0773	-5.3172	-5.2267	-5.3348	-5.2007
		graphically	-5.3160	-5.1509	-5.3174	-5.2270	-5.3353	-5.2019
		slope ^c	-1.0290	1.0144	-0.4217	-0.3946	1.5309	1.5170
		SD	0.0645	0.0630	0.6112	0.5687	0.0925	0.0913
		R	0.9940	0.9931	-0.8678	-0.8971	0.9852	0.9851
T3	pK_{TH+}	numerically	-5.6066	-5.6528	-5.2965	-5.4171	-5.3641	-5.5002
		graphically	-5.7345	-5.1910	-5.2956	-5.4173	-5.3635	-5.5000
		slope ^c	-1.0216	-0.9775	-0.3888	-0.3889	1.5177	1.5259
		SD	0.6037	0.3491	0.0761	0.9876	0.7058	0.5688
		R	0.9574	0.9840	-0.7709	-0.8487	0.9311	0.9031
T4	pK_{TH+}	numerically	-5.3876	-5.6369	-5.2144	-5.4749	-4.0889	-5.7812
		graphically	-5.3390	-5.6129	-5.2135	-5.4755	-4.0893	-5.7825
		slope ^c	-0.9910	0.9956	-0.3900	-0.4004	1.5222	1.5308
		SD	0.3967	0.6266	0.4813	0.5405	0.3862	0.6446
		R	0.9190	0.8298	-0.8095	0.9290	0.8932	0.8000

^a experimentally obtained values; ^b reconstructed values; ^c slope: m for HAFM; ϕ for BOM, and m^* for EAFM; R: correlation coefficient; SD: standard deviation

From the data presented in Table 3, for a group of chosen 1,2,4-triazoline-3-thiones, m values were around 1, ϕ values ranged from -0.36 to -0.45, and m^* values ranged from 1.30 to 1.53. Because the slope of $\log I = pK_{TH+} - mH_T$ was equal to 1, the base (triazoline-3-thiones) does seem to “follow” the acidity function H_T for thiocarbonyl bases. The obtained values for the solvation parameter, m^* , were close to those characteristic for similar thio compounds, indicating that the protonation site in the molecule of **T1-T4** is the S atom. Furthermore, the obtained ϕ values were negative, as expected for that kind of thio compound. Summarizing all of these facts, the protonation process of **T1-T4** can probably be illustrated by the following equation:

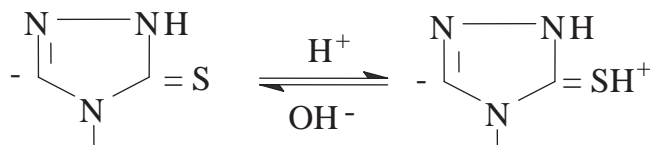


Figure 5. Protonation equilibrium of 5-substituted-4-phenyl-1,2,4-triazoline-3-thiones (**T3T**).

Conclusions

CVA was employed for the purpose of separating the protonation effects from the medium effects in the UV spectra of chosen 5-substituted-4-phenyl-1,2,4-triazoline-3-thiones (**T3T**). Using data from experimental and reconstructed UV spectra, the values of the dissociation constants of protonated forms of **T3T** were determined numerically and graphically, applying 3 methods: HAFM, BOM, and EAFM. There was a little influence of the solvent on the appearance of the spectra, according to the obtained data for pK_{TH+} values. The obtained values for m , ϕ and m^* were in good agreement with literature reported values for similar thio compounds, indicating that the protonation site in the molecule of the investigated 5-substituted-4-phenyl-1,2,4-triazoline-3-thiones is the S atom.

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