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# UV SPECTROPHOTOMETRIC DETERMINATION OF *pK'*s OF 1,2,4-TRIAZOLINE-3-THIONES IN SODIUM HYDROXIDE SOLUTIONS

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## ABSTRACT

Using UV spectroscopy the dissociation constants of previously synthesized 4,5-disupstituted-1,2,4-triazoline-3-thiones are determinate. The UV spectra were recorded in aqueous sodium hydroxide solutions at different pH values in the range from 190 to 360 nm, immediately after preparation of the suspension. The p$K_{TH}$ values were obtained experimentally, graphically and by the Characteristic Vector Analysis method. Results show that the investigated compounds have two dissociation processes. The first process belongs to the dissociation of SH proton, while the second dissociation process is dissociation of OH proton.

**Keywords:** Dissociation constants; CVA method; 1,2,4-triazoline-3-thiones; UV;

## INTRODUCTION

Over the past years, the significant focus was placed on the biological active compounds which posses 5-membered heterocyclic rings in their structure, such as 1,2,4-triazole ring. The 1,2,4-triazole core is related with diverse biological activities such as: antimicrobial, hypoglycemic, anti-inflammatory, anticancer, fungicidal, anticonvulsant, diuretics and antibacterial properties[1-3]. Furthermore, 1,2,4-triazole derivatives have found broad use in the agriculture and chemical industry because of their herbicidal, insecticidal and fungicidal characteristics[2,4]. Some of the triazole derivatives can be used as industrial dyes, agrochemicals, photographic materials emulgators and corrosion inhibitor for copper[5-8].

It is known that the thione form of 1,2,4-triazole-3-thiones were important for their antifungal activity.[2] Motivated by the significant importance and colossal interest for these types of compounds, during the last ten years, we synthesize and investigate a number of new 1,2,4-triazole derivatives[9-11].

Triazole derivatives act as weak bases in acidic media[12]. Knowledge of the acidity constants of weakly basic substrates is in main focus of reaction mechanisms in acidic media[12,13]. The dissociation constant depends upon the polarity of molecule and directly or indirectly on the intra and intermolecular forces[14]. The maximum activity of the 1,2,4-triazoles was unknown, as *pKa* gives the appropriate balance between the penetration and inherent activity. The half-dissociated status of *pKa* was accepted as best compromise between activity and transport. Furthermore, in the modelling of dissociation constants, in consideration were taken the role of the steric effect and molecular forces. Many authors have studied the acid–base equilibrium of 1,2,4-triazole compounds in different media[12,15].

The values of the ionization constants of 1,2,4-triazole as a base ($pK_T$=2.19) and as an acid ($pK_{TH}$+=10.26) were determined by Katritzky and Rees[4]. Starting with the most fundamental ones (calculation of the distribution functions in a given pH-interval), to the most complex (transportation of substances through cell membrane), the applications of *pKa* values are extremely different. For that reason, knowledge of the substance *pKa* value is essential for investigations in physiology, pharmacology, preparative and analytical studies, environmental and for other industrial purposes.

Contrary to the extensively studied acid–base reaction of the carbonyl group, corresponding thio-carbonyl compounds have received modest attention, as a result of the lack of stability of many such derivatives. On the other hand, there are some thio-derivatives which demonstrate sufficient stability towards acids. Protonation constants values for a large number of the thio-carbonyl compounds have been reported and constructed based on the acidity function ($H_T$) for thio-carbonyl[16,17].

The scope of this study was to examine the behaviour of 4,5-disupstituted-2,4-dihydro-1,2,4-triazoline-3-thiones (**T3T**) in sodium hydroxide solution and to find out the value of ionization constants not available in the literature yet.

It is known that the dissociation of 1,2,4-triazole derivatives (weak organic acids), in base media, can be presented with the equation 1:

$$\mathbf{TH} \rightleftarrows \mathbf{T^- + H^+} \qquad (1)$$

The equilibrium constant ($pK_{TH}$) for this dissociation reaction can be calculated with the equation 2:

$$pK_{TH} = \log I + m \cdot pH \qquad (2)$$

Ionization ratio (I) is the ratio between the concentration of the neutral form (TH) and the dissociated form ($T^-$) of **T3T.** The ionization ratio (I) values were determinate spectrophotometricaly from the absorbance values (experimental/reconstructed) using the equation 3.

$$I = \frac{A - A_{T^-}}{A_{TH} - A} \qquad (3)$$

where $A_{TH}$ is the absorbance of the neutral form; $A_{T}$- is the absorbance of the dissociated form; A is absorbance of the solution at given pH at same wavelength λ. Since the concentrations of the investigated compound were very small, it is not necessary to correct the activity coefficients. The ionic strength of the solution was 0.10 moldm$^{-3}$ and was adjusted with addition of $NaClO_4$.

According the literature data, the p*K* values of some substituted 1,2,4-triazoline-3-thiones in different media were determined by use of the UV spectroscopy[15].

Optimized geometry of investigated compounds: 4-phenyl-5-(2-hydroxyphenyl)-2,4-dihydro-1,2,4-triazoline-3-thione **(T3T-1),** 4-phenyl-5-(3-hydroxyphenyl)-2,4-dihydro-1,2,4-triazoline-3-thione **(T3T-2)**, 4-*n*-butyl-5-(2-hydroxyphenyl)-2,4-dihydro-1,2,4-triazoline-3-thione **(T3T-3)** and 4-*n*-butyl-5-(3-hydroxyphenyl)-2,4-dihydro-1,2,4-triazoline-3-thione **(T3T-4)** using AM1 semi empirical SCF-MO method in the HYPERCHEM program are presented in Figure 1. The quantum chemical investigations of thion and thiol tautomers of 4-phenyl-5-(4-hydroxyphenyl)-2,4-dihydro-3H-1,2,4-triazoline-3-thione was previously investigated and discussed[11].

The protonation or deprotonation of complex structures, such as 1,2,4-triazoline-3-thione moiety, causes a more complex UV spectra changes. The method of Characteristic Vector Analysis (CVA), described by *Simonds*[18] was used for separation of the medium effect from these spectral changes.

## EXPERIMENTAL

### Materials

The complete synthesis of the triazoline-3-thione derivatives (**T3T**) are reported earlier[19.] The structure of the novel thiones were confirmed by: IR, $^1H$ and $^{13}C$ NMR, UV and quantitative elemental analyses.

### Spectral measurements

The stock solutions of compounds **T3T** (2×10$^{-3}$ moldm$^{-3}$) were prepared by dissolving the required amounts of the active substances in absolute ethanol (Alkaloid, Skopje). These solutions were stable for a long time and during period of 2 months there were no changes in their absorption spectra.

Alkaline solutions were prepared by dilution of a standard NaOH solution (0.1 moldm$^{-3}$ Titrival, Kemika). The pH values of the diluted aqueous solutions,

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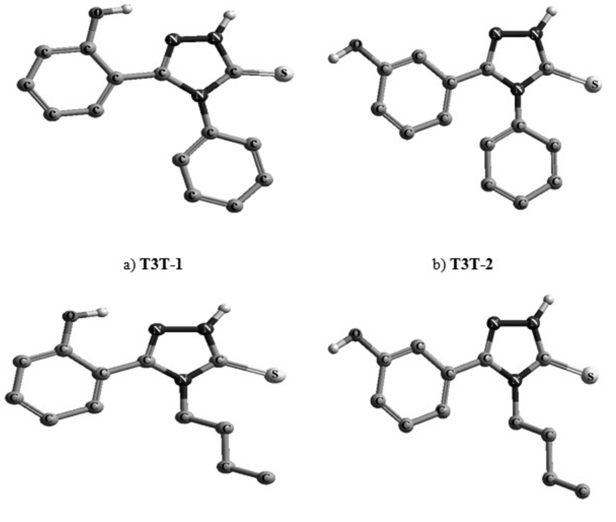
after recording the spectra were measured on an Iskra MA-5,704 pH meter.

The ionic strength (I=0.1 moldm$^{-3}$) of the diluted aqueous solutions was adjusted with $NaClO_4$ (Kemika). All chemicals were analytically graded (p.a.). Triply distilled water was used for the preparation of the aqueous solutions.

Test solutions (with concentration about $2.0\times10^{-4}$ moldm$^{-3}$ for **T3T-1** and **T3T-2**; $2.0\times10^{-5}$ moldm$^{-3}$ for **T3T-3** and **T3T-4**) were prepared. Parallely, the blanks were prepared. The ethanol content in the test solutions and in the blanks was 1%.

The spectrophotometric measurements were performed on a Varian Cary 50 spectrophotometer in the wavelength region from 190 to 360 nm, immediately after preparation of the solutions, at room temperature. For these measurements the quartz cell with 1 cm length was used. The maximum scan rate is 24 000 nm/min and resolution is 1.5 nm.

UV spectra were obtained with computer program Grams Version 4.10, while Excel program was applied for calculation of the dissociation constants of protonated form.



a) T3T-1

b) T3T-2

c) T3T-3

d) T3T-4

**Fig. 1:** Optimized geometry of 4,5-disupstituted-2,4-dihydro-1,2,4-triazoline-3-thiones (**T3T**)

### Method of the Characteristic Vector Analysis

Characteristic Vector Analysis is a method of separating independent factors for sets of multivariate response data[18] and can be used for empirically estimation of number of independent factors contribute to the total variation observes in a family of UV spectra. If *p* independent factors are involved in forming the absorbance curve, the sample responses at each wavelength for a given concentration can be calculated as follows:

$$A_1 = A_1 + c_1v_{11} + c_2v_{21} + .......... + c_pv_{p1}$$
$$A_2 = A_2 + c_1v_{12} + c_2v_{22} + .......... + c_pv_{p2} \qquad (4)$$
$$A_r = A_r + c_1v_{1r} + c_2v_{2r} + .......... + c_pv_{pr}$$

The choice of A is arbitrary; 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 absorption spectra of T3T-1, T3T-2, T3T-3 and T3T-4 in neutral media

The dissociation of 4,5-disupstituted-2,4-dihydro-1,2,4-triazoline-3-thiones (**T3T**) was investigated by spectrophotometric method, monitoring the changes in the electronic absorption spectra of aqueous solution of these compounds in NaOH.

The UV spectral data for neutral form of **T3T** in neutral media (water) are characterized by the presence of absorption maxima at about 255-258 nm and 276-292 nm (Tab. 1), characteristic maxima for the electron transition in the 1,2,4-triazoline-3-thione ring[9].

**Table 1.** UV spectra in neutral form of aqueous solutions of compounds T3T.

| Compound | $\lambda_{max}$ | $e_{max} \times 10^{-3}$ |
|---|---|---|
| **T3T-1** | 256; 276 | 15.48; 17.88 |
| **T3T-2** | 257; 283 | 17.68; 11.46 |
| **T3T-3** | 255; 292 | 16.16; 11.46 |
| **T3T-4** | 258; 279 | 23.92; 20.64 |

As in the case of other heterocyclic thiones[9,20] it is possible to consider that the 1,2,4-triazoline-3-thions may exist in ***thiol-thion*** tautomeric forms. The results from our investigations indicated that the thione form is the predominant tautomer. The absorption maxima (shoulders) between 250-260 nm and 280–290 nm indicated that some of these compounds predominantly exist in thion form in ethanolic solution. According the literature data[9,21] the absorption at 280-290 nm indicate the presence of a chromophoric C=S group.

### Experimental UV spectra of T3T-1, T3T-2, T3T-3 and T3T-4 in sodium hydroxide media

The dissociation processes of compounds **T3T** were investigated by using the absorption spectra recorded in solutions of different pH (NaOH solutions). The experimental UV spectra of the investigated compounds in sodium hydroxide medium are presented in Figure 2 (a-d).

It can be noticed that isobestic points (**IP**) at around 250 and 295 nm in the experimental UV spectra of compound **T3T-1** and around 245 and 285 nm in the spectra of compound **T3T-2** are not strictly defined (Fig. 2a - b). This indicates the probable presence of several forms able to absorb UV radiation in the system.

Increasing the pH of solution from 6.23 to 11.35 (compound **T3T-3**) and from 6.32 to 13 (compound **T3T-4**), the absorption maximum at around 250 nm exhibits hypsochromic effect, i.e. it shifts towards lower wavelengths, at around 240 nm and a new band at 305 nm was formed. Not clearly defined IP forming can be noticed at around 245 and 285 nm in **T3T-3** and at around 240 and 280 nm in **T3T-4** (Fig. 2c - d).

### Reconstructed UV spectra of T3T-1, T3T-2, T3T-3 and T3T-4 in sodium hydroxide media

None of the experimental UV spectra showed precisely defined isobestic points, as a result of "*medium effect*", which prevented the group of spectral UV curves to pass through clear point. For the reason, in order to exclude the undesired protonation medium effect, attempt to reconstruct UV spectra was made by analysis of the experimental curves using the Characteristic Vector Analysis method[18]. The results are shown in the Figure 3a-d.

The reconstructed spectra of **T3T** show clear isobestic points at: 257 and 297 nm (**T3T-1**); 257 and 287 nm (**T3T-2**); 246 and 286 nm (**T3T-3**) and 257 and 282 nm (**T3T-4**). Additionally, it can be noticed that the reconstructed spectra and experimental UV curves demonstrate identical changes.

### Protonation constants of T3T-1, T3T-2, T3T-3 and T3T-4 in sodium hydroxide media

The changes in the experimental and reconstructed UV spectra of **T3T** could be efficiently observed from the absorbance plot at given wavelength against the pH value of the solution. The absorbance values are measured at four selected wavelengths vary in the range of absorption maximum. For the **T3T-1** maximum was at 287 nm (Fig. 4a); **T3T-2** at 227 nm; **T3T-3** at 238 nm (Fig. 4b) and for the **T3T-4** at 241 nm.

It can be noticed that this dependence is a sigmoid ("*S"*) curve and has two plateaus, which confirms that the dissociation process occurs in two steps. When the pH value increases, there is no significant changes in the absorbance value, which noticed from the initial part of the *"S"* curve (Fig. 4; lower *"S"* curve plateaus).

At lower pH range, almost certainly is domination of neutral form of the compounds. At the higher pH values, the similar plateau of the *"S"* curve could be noticed (upper *"S"* curve plateaus), where in the solution exist only the dissociated form of **T3T**. The dissociation process in pH interval can be determined from the steep part of the *"S"* curve.

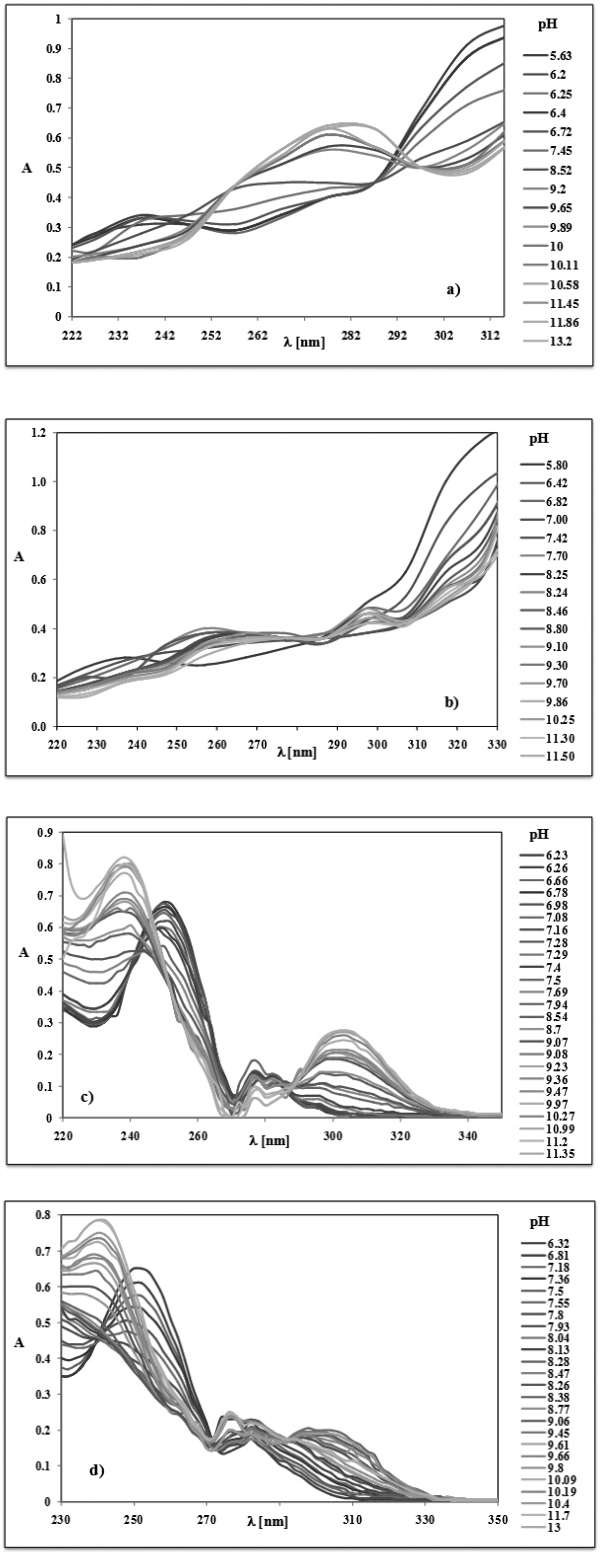


**Fig. 2:** Experimental UV spectra of: a) **T3T-1**, b) **T3T-2**, c) **T3T-3** and d) **T3T-4** in sodium hydroxide medium

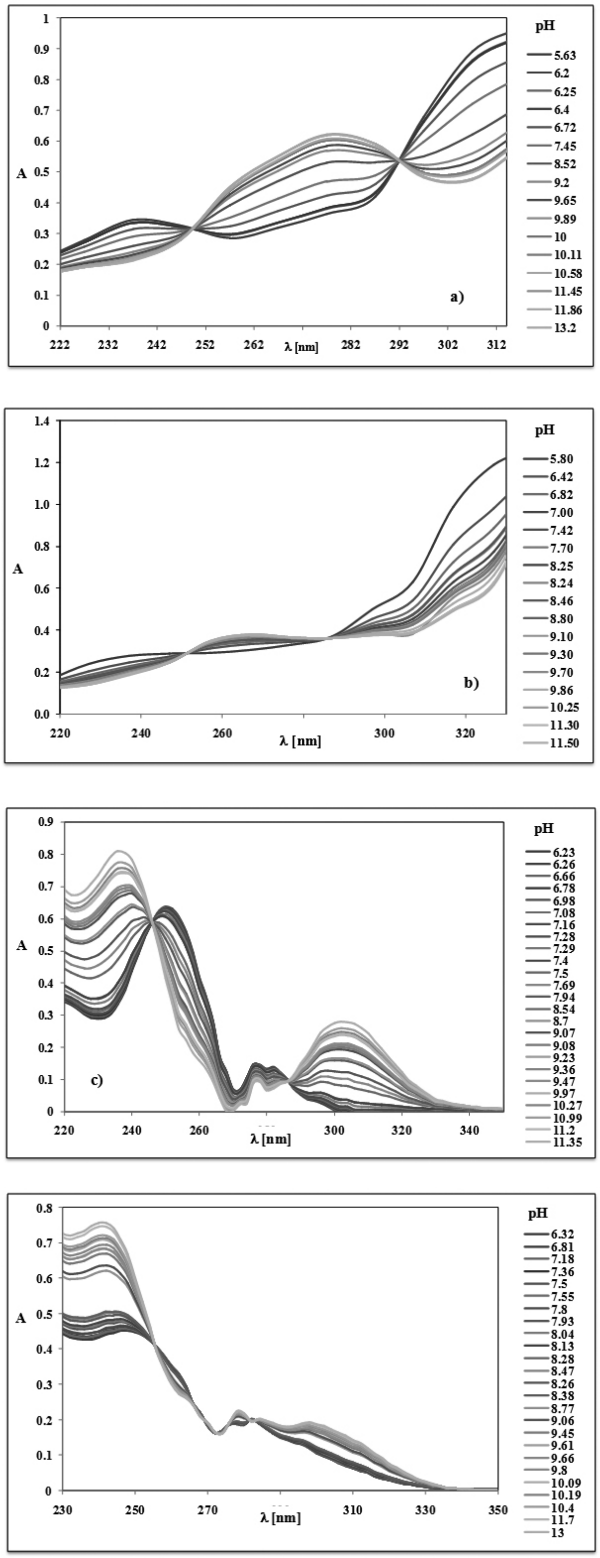


**Fig. 3:** Reconstructed UV spectra of: a) **T3T-1**, b) **T3T-2**, c) **T3T-3** and d) **T3T-4**

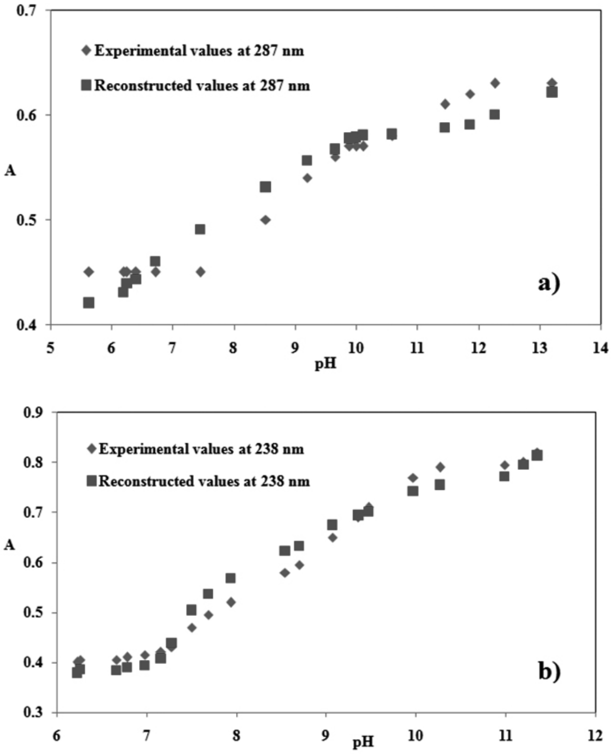


**Fig. 4:** "*S*" curves for: a) **T3T-1** and b) **T3T-3**

The **T3T** dissociation constants in sodium hydroxide media were calculated using the equation (2). Furthermore, the dissociation constants were determined graphically as an intercept of the plot of log I *vs*. pH values (Fig. 5).

In the Table 2, the average experimental p$K_{TH}$ values obtained numerically and graphically from the three measurements series are presented. In addition, the values of the slope (*m*), correlation coefficient ($R^2$) and standard deviation (SD) were determined (Tab 2).

Analogous, reconstructed p$K_{TH}$ values obtained numerically and graphically, the values of the slope (*m*), correlation coefficient ($R^2$) and standard deviation (SD) are presented in the Table 3.

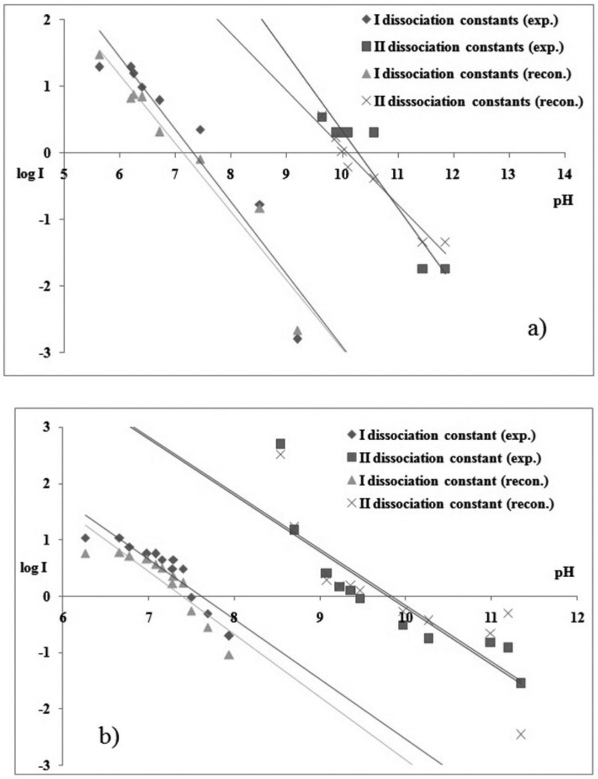


**Fig. 5:** Dependence of log I from pH value for: a) **T3T-1** and b) **T3T-3**

The ionisation equilibrium of 4,5-disupstituted-2,4-dihydro-1,2,4-triazoline-3-thiones are presented in Figure 6:

$TH_2 \rightleftharpoons TH^- + H^+$ (*p$K_{TH2}$* or *pKa(SH)*)

$TH^- \rightleftharpoons T^{2-} + H^+$ (*p$K_{TH^-}$* or *pKa(OH)*)

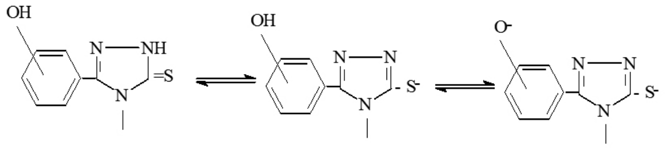


**Fig. 6:** Dissociation reaction of the neutral form of the **T3T**

**Table 2.** Experimental *p$K_{TH2}$* (dissociation of SH group) and *p$K_{TH^-}$* (dissociation of OH group) values for **T3T**; $R^2$ - correlation coefficient; SD - standard deviation.

| **Comp.** | ***p$K_{TH2}$*** | | | | ***p$K_{TH^-}$*** | | | |
|---|---|---|---|---|---|---|---|---|
| | **numerically** | **graphically** | **slope (*m*)** | **$R^2$ (SD)** | **numerically** | **graphically** | **slope (*m*)** | **$R^2$ (SD)** |
| **T3T-1** | **7.750** | **7.306** | -0.936 | -0.748<br>(0.974) | **10.357** | **10.363** | -1.000 | -0.899<br>(0.561) |
| **T3T-2** | **6.813** | **6.856** | -1.006 | -0.777<br>(0.571) | **9.147** | **9.099** | -0.995 | -0.844<br>(0.332) |
| **T3T-3** | **7.629** | **7.674** | -1.006 | -0.812<br>(0.632) | **9.794** | **9.791** | -1.000 | -0.873<br>(0.545) |
| **T3T-4** | **7.321** | **7.390** | -1.009 | -0.839<br>(0.596) | **9.599** | **9.546** | -0.994 | -0.849<br>(0.330) |

**Table 3.** Reconstructed $pK_{TH2}$ (dissociation of SH group) and $pK_{TH}^-$ (dissociation of OH group) values for **T3T**; $R^2$ - correlation coefficient; SD - standard deviation

| **Comp.** | **$pK_{TH2}$*** | | | | **$pK_{TH}^-$*** | | | |
|---|---|---|---|---|---|---|---|---|
| | **numerically** | **graphically** | **slope (*m*)** | **$R^2$ (SD)** | **numerically** | **graphically** | **slope (*m*)** | **$R^2$ (SD)** |
| **T3T-1** | **7.140** | **7.153** | -1.001 | -0.991<br>(0.152) | **10.156** | **10.156** | -1.039 | -0.915<br>(0.348) |
| **T3T-2** | **7.273** | **7.295** | -1.003 | -0.985<br>(0.255) | **8.964** | **8.981** | -1.002 | -0.960<br>(0.251) |
| **T3T-3** | **7.388** | **7.358** | -1.000 | -0.982<br>(0.273) | **9.828** | **9.812** | -0.998 | -0.938<br>(0.235) |
| **T3T-4** | **7.632** | **7.421** | -0.971 | -0.910<br>(0.235) | **9.180** | **9.259** | -1.008 | -0.964<br>(0.223) |

* Calculated by CVA method[18]

## CONCLUSIONS

As we already point out, 1,2,4-triazoline-3-thione unit can exist in the thiol form, i.e. it can behave as an acid as is case with other types of heterocyclic thiones**.** Therefore, the first dissociation equilibrium corresponds to the dissociation of SH groups. The values of 6.8 to 7.8 are in accordance with the data presented in literature for the $pK_{TH}$ values of the ***thiol-thion*** form of the pyrimidine-thione[20]. The second dissociation equilibrium of 4,5-disupstituted-2,4-dihydro-1,2,4-triazoline-3-thiones is in rage of 8.9 to 10.4, which corresponds to $pK_{TH}^-$ of the phenolic OH group[23].

Most of the $pK_{TH}$ values determined using experimental spectra data differ than results determined with the reconstructed spectra. The influence of the solvent can be spotted as main reason for differences in the experimental and reconstructed $pK_{TH}$ values.

The values of the correlation coefficient ($R^2$) and standard deviation (Sd) for $pK_{TH2}$ and $pK_{TH}^-$ determined from reconstructed spectra data (Tab. 3) are higher/lower, respectively, than the ones determined from the experimental spectra data (Tab. 2). As it was expected, this suggests that the values obtained from reconstructed UV spectra are more precise. Result show that compared to the compounds **T3T-3** and **T3T-4,** compound **T3T-1** and **T3T-2** has lower values of the first dissociation constant (Tab. 3). This is probably resulting of the impact of the phenyl group in position 4 in triazole moiety of investigated **T3T**. Hence, compound **T3T-1** is stronger acid than the other compounds, maybe as a result of influence of *orto*- substituted OH group in phenyl moiety.

Second dissociation constant, which is a result of OH dissociation, depends of position of the OH group. According the calculated constants, *meta* substituted OH group in **T3T-2** and **T3T-4** has higher impact on compound acidity increase when this group is in *orto*-position and the values of the slope *m* were around one (Table 3).

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