








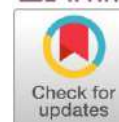


# Determination of $pK_a$ of triazolo[5,1-c][1,2,4]triazines in non-aqueous media by potentiometric titration

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## Abstract

In this work, for the first time, an approach was suggested for determining the  $pK_a$  of triazolo[5,1-c][1,2,4]triazine compounds in N,N-dimethylformamide using potentiometric titration. It was noted that change in system potential in the titration curves of triazolo[5,1-c][1,2,4]triazine are observed for compounds containing either  $H^+$  located at the nitrogen atom in position 1 or an  $-NH_2$  group. The calculated  $pK_a$  values of the studied molecules correspond to the  $pK_a$  values of moderately strong acids and are in the range of 2–8. The relationship was established between the acidity of the heterocyclic fragment and the presence of electron donor substituents. The obtained  $pK_a$  values of the compounds correlate with the calculated possible deprotonation centers of the molecule. It was found that determining the acidity constants of substances is useful for clarifying the mechanisms of their transformations. The possibility of establishing a relationship between the structure of the compound,  $pK_a$ , and probable biological activity was shown.

## Keywords

azoloazines  
triazolo[5,1-c][1,2,4]triazines  
potentiometric titration in non-aqueous media  
the acidity constant  
 $pK_a$

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## Key findings

- The  $pK_a$  of triazolo[5,1-c][1,2,4]triazine compounds was determined in N,N-dimethylformamide using potentiometric titration.
- The obtained  $pK_a$  values of the compounds correlate with the calculated possible deprotonation centers of the molecule.
- The possibility of finding a relationship between the structure of the compound,  $pK_a$ , and probable biological activity was shown.

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## 1. Introduction

One of the most important tasks facing modern organic chemistry is the targeted search for compounds with useful biological properties in order to create new original and effective drugs [1]. The most interesting cluster of compounds exhibiting broad biological activity are azaheterocyclic compounds. These include both natural alkaloids, some amino acids, nucleotides, etc., and synthetic compounds based on the azaheterocycle. Promising synthetic heterocyclic compounds include nitroazolo[5,1-c][1,2,4]triazines [2]. These compounds are of great interest due to their structural similarity to known antiviral drugs used in medical practice (Triazavirin®, Acyclovir®, Ganciclovir®,

Ziagen®, Baraclude®, etc.), purine antimetabolites (Purinethol®, Fludara®, Arranon®, Alimta®) and other compounds included in the list of essential drugs. Despite the successful introduction of substances of the nitroazolo[5,1-c][1,2,4]triazine class into therapeutic practice, the reasons for their high biological activity and wide spectrum of action are still not fully understood.

It is known that nitroheterocyclic substances can undergo transformations in the human body under the influence of nitroreductases localized in the microsomal and soluble fractions of cells [3]. Reduction of substrates by enzymes can occur either through intermediates such as nitroso compounds and hydroxylamines [3], or through anionic radicals [4]. Some authors suggest that one of the

reasons determining the biological properties of these compounds may be the formation of free radicals resulting from the reduction of the nitro group [5–7]. The differences in the formation of one or another type of intermediate, its quantity and, probably, the greater biological activity may be associated with the direct participation of protons [8].

According to the authors [9], “acidic” hydrogen, which is characteristic of proton-donor acids, can fully correspond to “active hydrogen” in organic chemistry with the only limitation that acidity reflects the mobility of hydrogen. Many compounds, which are known as neutral substances in proton-donating solvents, may exhibit acidic or basic properties in non-aqueous media [10]. The literature reports [9, 10] that methyl, hydroxy, carboxy, and amino groups can act as acids in aprotic solvents. Previously [11–13], when studying the redox transformations of nitroazolo[5,1-c][1,2,4]triazines, we hypothesized that in aprotic media, the molecule itself can act as a donor. The ability of a substance to act as a “supplier” of protons and, as a consequence, to undergo a self-protonation reaction can be indicated by the acidity constant ( $pK_a$ ) of this compound.

Previously, we briefly reported on the possibility of determining the  $pK_a$  of some 7-oxo-4,7-dihydro-1,2,4-triazolo[5,1-c][1,2,4]triazines by titrating their sodium salts in aqueous solutions [14]. Since the transport of drugs in the patient’s body occurs mainly in mixed (intracellular environment, blood, lymph) and non-aqueous environments (lipid cell membranes) [15], it is useful to consider the acid-base properties in aprotic solvents. This is necessary for a more detailed clarification of the possible transformation of the drug. Currently, the literature does not describe methods for determining the  $pK_a$  of compounds for triazolo[5,1-c][1,2,4]triazines in aprotic media.

Spectrophotometry and potentiometric titration are most widely used to find the values of the acidity or basicity constants of substances, since they have high sensitivity and accuracy, which makes it possible to determine  $pK_a$  with minimal errors. Spectrophotometric determination of the acidity constants of nitrotriazolo[5,1-c][1,2,4]triazines in aprotic solvents is difficult due to overlap of wavelengths at which maximum absorption of the test substance and decomposition of the solvent under the influence of UV rays are observed. Therefore, potentiometric titration is the most effective method for determining the  $pK_a$  of nitrotriazolo[5,1-c][1,2,4]triazines in non-aqueous media.

The aim of this work is to develop a method for determining the  $pK_a$  of compounds from the series of triazolo[5,1-c][1,2,4]triazines in non-aqueous media by potentiometric titration.

## 2. Experimental

### 2.1. Reagents and apparatus

N,N-dimethylformamide (DMF) “special purity grade” (USA, PanReac) was used to carry out potentiometric

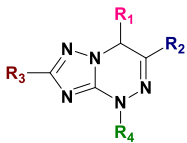
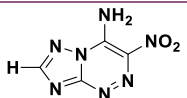
titration in an aprotic solvent. No additional purification was performed for the solvents. Analytical grade potassium hydroxide and reagent grade methanol were used for the preparation of titrant. The indicator for standardizing potassium hydroxide with succinic acid was a 1% alcohol solution of analytical grade thymol blue (Russia, REAHIM). Lithium perchlorate ( $LiClO_4$ ) 0.1 M, prepared from a special grade sample (USA, Sigma-Aldric), was used as an electrolyte for potentiometric titration in an aprotic medium. Hydrochloric acid prepared by diluting concentrated reagent grade acid was used to introduce a correction during standardization. In order to eliminate fluctuations in the diffusion potential during potentiometric titration in an aprotic medium [9], 3.0 M solution of lithium chloride ( $LiCl$ ) in DMF, prepared from a special purity grade anhydrous  $LiCl$  (USA, Sigma-Aldric), was used as an electrolyte solution in the combined electrode. Preparation and standardization of alkali were carried out according to the methods described in [9].

Weighing of the samples was carried out on a class I Shimadzu AUX220 analytical balance (Shimadzu, Japan). Potentiometric titration was achieved in a two-electrode cell. The potential was recorded on a pH meter “Expert-pH” (LLC “Econics-expert”, Russia). The combined glass electrode-ESK-10603 (LLC “Izmeritelnaya Tekhnika”, Russia) was used as a working electrode. The calibration was performed using three aqueous buffer solutions prepared in accordance with TU 2642-072-56278322-2009.

### 2.2. Objects of research

The compounds from the series of triazolo[5,1-c][1,2,4]triazines (compounds I–X) presented in Table 1 were synthesized at the Department of Organic and Biomolecular Chemistry of the Ural Federal University. The structure of these compounds was determined using NMR spectroscopy, IR spectroscopy, HPLC and elemental analysis [16–22].

**Table 1** Objects of research.

Structure formula	Substituents	Name
	$R_1$ -NO <sub>2</sub> ; $R_2$ =O; $R_3$ -S-CH <sub>3</sub> ; $R_4$ -Na <sup>+</sup>	I
	$R_1$ -Br; $R_2$ =O; $R_3$ -H; $R_4$ -Na <sup>+</sup>	II
	$R_1$ -NO <sub>2</sub> ; $R_2$ =O; $R_3$ -S-CH <sub>2</sub> -CH <sub>3</sub> ; $R_4$ -Na <sup>+</sup>	III
	$R_1$ -NO <sub>2</sub> ; $R_2$ -OH; $R_3$ -S-CH <sub>3</sub> ; $R_4$ -Na <sup>+</sup>	IV
	$R_1$ -Br; $R_2$ =O; $R_3$ -H; $R_4$ -H <sup>+</sup>	V
	$R_1$ -NO <sub>2</sub> ; $R_2$ =O; $R_3$ -S-CH <sub>3</sub> ; $R_4$ -H <sup>+</sup>	VI
	$R_1$ -NO <sub>2</sub> ; $R_2$ -OH; $R_3$ -S-CH <sub>3</sub> ; $R_4$ -H <sup>+</sup>	VII
	$R_1$ -NO <sub>2</sub> ; $R_2$ -OH; $R_3$ -S-CH <sub>2</sub> -CH <sub>3</sub> ; $R_4$ -H <sup>+</sup>	VIII
	$R_1$ -NO <sub>2</sub> ; $R_2$ -OH; $R_3$ -S-CH <sub>2</sub> -C≡CH; $R_4$ -H <sup>+</sup>	IX
		X

### 2.3. Calibration of potentiometric titrator for aprotic media

Calibration in an aqueous environment was carried out using three aqueous buffer solutions with pH 9.18, 6.86 and 1.65. Currently, there are no specialized buffer solutions for aprotic media that are commercially available, which made measurements on a device calibrated for aqueous solutions impossible. To take this feature into account, an amendment was introduced according to article [23]. In article [23], the galvanic cell was calibrated by introducing a correction through determining the specific constant  $E_a^{o'}$  of the cell in the acidic medium.  $E_a^{o'}$  includes standard glass electrode potential, reference electrode potential, diffusion potential and activity coefficients. The determination of  $E_a^{o'}$  (mV) was based on the complete dissociation of hydrochloric acid (HCl) in dimethylformamide. In the presence of  $E_a^{o'}$ , the pH value in the cell can be determined by Equation (1):

$$\text{pH} \cong \text{p}C_{\text{HCl}} = \frac{E_a^{o'} - E}{59.16}, \quad (1)$$

where  $\text{p}C_{\text{HCl}}$  is the proton concentration index and  $E$  is the potential at equivalence point, mV.

### 2.4. Quantum chemical calculations

Quantum chemical calculations on the ORCA 5.0 platform [24–26] were performed by the Head-Gordon's method with a disperse correction function – wB97X-D3BJ [27]. The def2-TZVP basis set [28] supplemented with the Weigend def2/J universal Coulomb basis [29] was used. Solvent effects were taken into account in the CPCM model [30]. Full geometric optimization TightOpt was used to find stationary minimum points of the electron energy surface. The TightSCF option was used in calculations with self-consistent field. Numerical calculations of vibrational frequencies were performed to verify that the found stationary points were local minima.

The calculation of the distributions of electric state potentials was performed in the Multiwfn 3.8 package [31], visualization – in GaussView 6.0 [32]. This is an example of a second-order subsection.

## 3. Results and Discussion

One of the most convenient solvents for titrating organic acids and their analogues is N,N-dimethylformamide. Since it is the main differentiating solvent, its sensitivity to carbon dioxide is so low that it can be neglected. In addition, it has a high solubilizing ability for many organic compounds [9, 10]. When titrating substances in non-aqueous media, it is also necessary to take into account the fact that solvated ions in aprotic solutions (unlike aqueous solutions) are capable of association with the formation of ion pairs and large aggregates, especially in solvents with low dielectric constant [9]. DMF is characterized by a fairly large acidity scale ( $\text{p}K_s = 18$ ) and a high dielectric constant ( $\epsilon = 36.71$ ) [33]. In addition, DMF is a small amphiphilic

molecule with a hydrophilic aldehyde group and two hydrophobic methyl groups. Its amphiphilic nature appears to be an important defining characteristic for its action on membranes: DMF is widely employed in cell biology to induce cell fusion and cell differentiation. It is also an effective penetration promoter [15, 34]. Therefore, DMF was chosen as a solvent for the titration of triazolo[5,1-c][1,2,4]triazines in non-aqueous media. Electrolyte, 0.1 M  $\text{LiClO}_4$ , was added to maintain a constant ionic strength of the solution of the analytes.

Methanol solutions of potassium or sodium hydroxide are widely used as standard titrant solutions for the titration of compounds with acidic properties in non-aqueous media. They provide stability and accuracy in titrations due to their high methanol solubility and strong basic nature, allowing effective neutralization of organic acids [9]. These alkalis are not stable during storage, so they require setting titers for succinic or benzoic acid [9]. Low cost, commercial availability, and high basic strength of KOH are the undeniable advantages of using potassium hydroxide as a titrant.

The use of methanol as a solvent for alkali is justified, first, by the good solubility of KOH in it, and second, by the proximity of the values of dielectric constant with DMF ( $\epsilon = 32.6$ ) and acidity scale ( $\text{p}K_s = 15.5$ ). This allows one to avoid the negative impact on the dissociation of the analyte. In addition, methanol is a differentiating solvent, which allows one to enhance the alkaline properties of strong bases. [9,10].

The tendency of a molecule to self-protonate is usually observed in organic substances that are weak acids [35, 36]. According to the theory of aprotic acids [9, 10], triazolo[5,1-c][1,2,4]triazines, in both acidic and ionic (salt) forms, can be weak acids in non-aqueous media. Therefore, the calculation of the acidity constant of these substances during potentiometric titration with a strong base was carried out using the Henderson Equation (2) [37]:

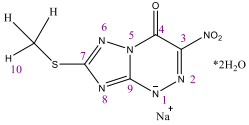
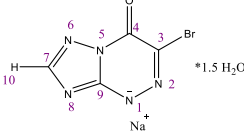
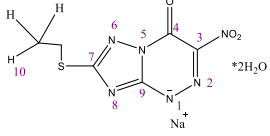
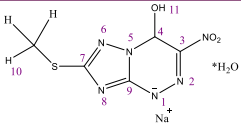
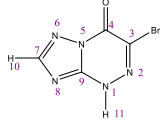
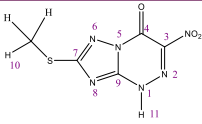
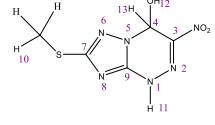
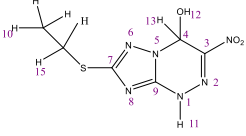
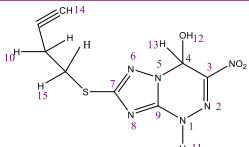
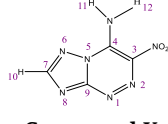
$$\text{pH} = \text{p}K_a + \lg \frac{[\text{A}^-]}{[\text{HA}]} \quad (2)$$

where  $K_a$  is the dissociation constant of the weak acid,  $\text{p}K_a = \log_{10}K_a$ ,  $[\text{HA}]$  and  $[\text{A}^-]$  are the molarities of the weak acid and its conjugate base.

According to Equation (2), the  $\text{p}K_a$  value is equal to the pH at the point of half-neutralization of the substance (half-neutralization method). The pH value was calculated using Equation (1), where  $E$  is the potential of the system with a degree of neutralization of the substance equal to 50%.

The results of determining the  $\text{p}K_a$  of triazolo[5,1-c][1,2,4]triazine compounds in aprotic media by potentiometric titration in DMF are given in Table 2. The concentration of the initial analytes was chosen to be 0.1 M. When using lower concentrations, no pronounced changes in system potential in the titration curves were observed. The calculated  $\text{p}K_a$  values of the studied molecules correspond to the  $\text{p}K_a$  values of moderately strong acids and are in the range of 2–8 [9].

**Table 2** Experimental values of  $pK_a$  and calculated thermodynamics parameters for compounds I-X.

Name and structural formula	$pK_a^a$	$\Delta G_H^b/eV$	$\Delta G_{Na^+}^c/eV$	$l^d/\text{\AA}$
 <b>Compound I</b>	-	H <sub>10</sub> 14.42	0.80	2.37
 <b>Compound II</b>	-	H <sub>10</sub> 14.22	0.89	2.35
 <b>Compound III</b>	-	H <sub>10</sub> 13.04	0.91	2.38
 <b>Compound IV</b>	-	H <sub>10</sub> 14.46 H <sub>11</sub> 13.11	0.93	2.34
 <b>Compound V</b>	5.24±0.16	H <sub>10</sub> 14.09 H <sub>11</sub> 12.17	-	-
 <b>Compound VI</b>	2.72±0.21	H <sub>10</sub> 14.42 H <sub>11</sub> 11.81	-	-
 <b>Compound VII</b>	7.89±0.14	H <sub>10</sub> 14.48 H <sub>11</sub> 12.35 H <sub>12</sub> 12.84 H <sub>13</sub> 16.05	-	-
 <b>Compound VIII</b>	7.55±0.21	H <sub>10</sub> 13.19 H <sub>11</sub> 12.35 H <sub>12</sub> 12.84 H <sub>13</sub> 15.85 H <sub>15</sub> 13.29	-	-
 <b>Compound IX</b>	7.67±0.13	H <sub>10</sub> 13.65 H <sub>11</sub> 12.33 H <sub>12</sub> 12.82 H <sub>13</sub> 15.03 H <sub>14</sub> 13.20 H <sub>15</sub> 13.35	-	-
 <b>Compound X</b>	8.35±0.10	H <sub>10</sub> 14.05 H <sub>11</sub> 12.60 H <sub>12</sub> 13.00	-	-

<sup>a</sup> Decimal logarithm of the acidity constant calculated using Equation (2).

<sup>b, c</sup> Changes in the Gibbs energies, characterizing the probability of separation of a proton or sodium cation, respectively, from a molecule. They were calculated as the difference between the energy of the molecule in the stationary state and the deprotonated/ionized form.

<sup>d</sup> Bond length between the nitrogen atom in position 1 and the sodium cation.

Sodium salts of triazolo[5,1-c][1,2,4]triazines (**I–IV**) are crystalline hydrates. Therefore, one should not assume that their transformations occur in an absolutely dry environment. The work [38] reported that it is typical for molecules of this series to be in both ionic (salts) and protonated forms, depending on pH of the aqueous medium. This is also evidenced by the  $pK_a$  values obtained by potentiometric and optical methods for acid and ionic forms in aqueous media [14]. That work showed that the acidity constants of both protonated and salt forms of azoheterocycles are close to each other. It is possible that the acid-base balance of compounds, which is characteristic of this class of substances, can affect the path of their electrotransformations. It was initially assumed that the amount of water introduced into the solution along with the substance would be sufficient to create the acidic form of the molecule in solutions depleted in  $H^+$ . However, in studies [12, 13] of the electroreduction of compounds **I** and **IV** as well as their acidic forms, compounds **VI** and **VII**, in DMF using cyclic voltammetry it was shown that in aprotic media the salts of nitrotriazolo[5,1-c][1,2,4]triazines undergo transformations in ionic form. The addition of excess proton donors of varying strengths to solutions of compounds **I** and **IV** in DMF led to a change in the shape of cyclic voltammograms (CVs). Their shape became similar to CVs of **I** and **IV** obtained in aqueous solutions and to CVs of **VI** and **VII** in DMF. These data indicate that **I** and **IV** in aprotic media undergo transformations in ionic form, and the amount of water added to the solution with the test substance is not enough to protonate the molecule itself.

Therefore, to clarify this assumption, sodium salts (compounds **I–IV**) as well as their protonated forms (compounds **V–VII**) were initially taken as objects of study. A sharp change in the system potential was observed in titration under the selected conditions only for triazolo[5,1-c][1,2,4]triazine acids (Figure 1).

According to [39], the analytical signal can be interfered with by oxygen in solution during titration in non-aqueous media. Also, according to [9], it is necessary to take into account the influence of dissolved carbon dioxide on the completeness of the reaction during titration using basic solvents. Therefore, all 7 systems were purged with argon during titration.

In the systems with sodium salt forms there was still no change in the system potential seen in the curves. If we make the assumption that in aprotic media the sodium cation is firmly bound to nitrogen in the 1st position, then this may not allow hydrogen from the water introduced with the crystalline hydrate to protonate triazolo[5,1-c][1,2,4]triazine at the same nitrogen. However, according to the optimization of the structures of molecules **I–IV** in DMF using quantum chemical calculations, the distance between  $Na^+$  and nitrogen in the 1st position is greater than  $\sim 2.3 \text{ \AA}$  (Table 2). This is sufficient for the formation of a proton bond with this nitrogen. It is also worth considering the competing solvation reaction of the molecule in DMF [9]. All the

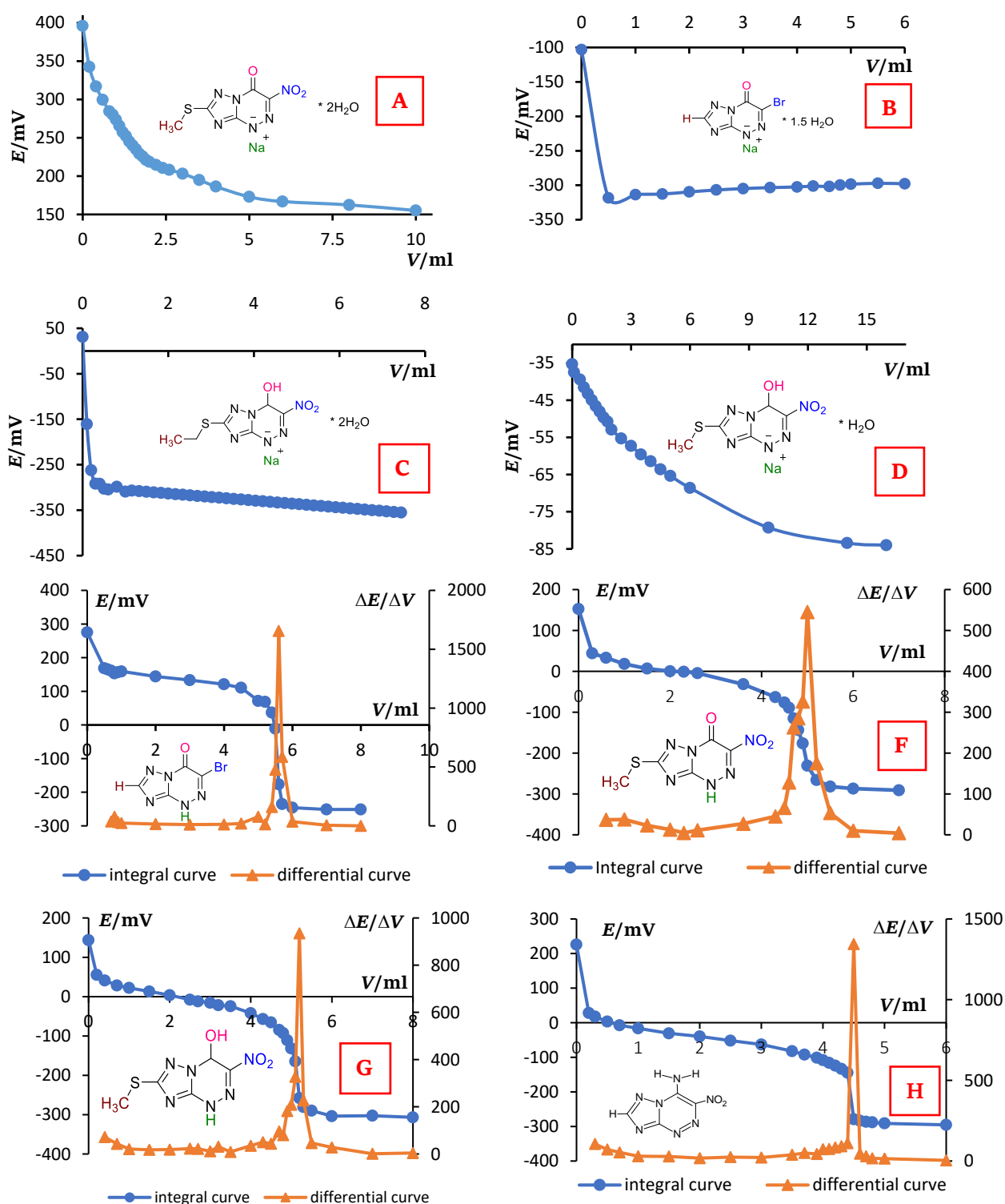
above data confirm the hypothesis that the sodium salts of triazolo[5,1-c][1,2,4]triazines undergo transformations in ionic form in aprotic media. At the same time, the amount of water introduced into the solution with the test substance is not enough to protonate the molecule itself. Consequently, the sodium salts of triazolo[5,1-c][1,2,4]triazines in aprotic media are not capable of self-protonation.

In [40] it was shown that in aqueous media the transfer of the first electron to the nitro group occurs simultaneously with or after its protonation. Therefore, generated radicals are short-lived. In  $H^+$  depleted media, hydrogen will join the nitro group after one electron transfer or even after two. This leads to the formation of anion and/or dianion radicals, the latter of which can be detected on an EPR spectrometer without the use of spin traps [41]. The ability of a substance to participate in the self-protonation reaction reduces the possibility of formation of dianion radicals and decrease the lifetime of the radical. Therefore, the inability of compounds **I–IV** to be a source of protons may have a positive effect on the number and lifetime of intermediate particles of a radical nature. This is probably one of the reasons for the more pronounced biological activity of triazolo[5,1-c][1,2,4]triazine salts in a number of structural analogues [2, 11–13, 21].

In solutions with acidic forms of triazolo[5,1-c][1,2,4]triazines change in system potential after purging with an inert gas was less pronounced than without it. There was also a 3-fold increase in the stabilization time of the equilibrium potential compared to conditions without bubbling with gas. Further titration was carried out in the absence of purging.

The acidity constants obtained for compounds **V–IX** (Table 2) gave reason to believe that the acidic properties are provided by hydrogen localized at the nitrogen atom in position 1. This may probably be due to the structure of triazolo[5,1-c][1,2,4]triazines: the electron density of the molecules shifts towards nitro-, bromine- and the oxo groups. As a result, the bond of the nitrogen atom in the first position with any counterion can be considered ionic [8]. The introduction of more electroacceptor substituents into the structure can lead to a more uniform distribution of the electron density [8, 9]. In this regard, the hydrogen atom conjugated with nitrogen in position 1 is likely to become less mobile. This can lead to a decrease in the acidic properties of the molecule and its ability to self-protonate.

According to the analysis of charges on atomic maps, protons with the highest positive charges for each compound were identified. On their basis, the thermodynamic parameters of the Gibbs energy change were calculated (Table 2). They characterize the probability of separation of a proton from a molecule. Based on the values obtained, it follows that the hydrogen atom in position 1 has pronounced acidic properties. A comparison of the thermodynamic parameters of all molecules in a number of analogues confirms that compounds **V**, **IV** have a greater ability to release a proton.



**Figure 1** Integral and differential potentiometric titration curves of 0.1 M compounds I (A), II (B), III (C), IV (D), V (E), VI (F), VII (G), X (H) in DMF with 0.1 M alcohol solution of KOH.

With the introduction of the hydroxogroup into the molecule's structure, a redistribution of the electron density in the six-membered ring of the heterocycle was observed. It led to a decrease in the mobility of  $H^+$  in the 1<sup>st</sup> position. This probably cause an increase in the values of the  $pK_a$  of compounds VII–IX with respect to V, VI. The elongation of the thiol bond at position 7 does not significantly affect both the electron density and the  $pK_a$  value, and, as previously

shown, the potential for electroreduction of the nitrotriazolo[5,1-c][1,2,4]triazine nitro group [18].

Gyenes reported [9] that amino groups can also act as acids in aprotic solutions. Therefore, compound X was of great interest, where the nitrogen atom in position 1 is not protonated, but there is an amino group at 4 (Figure 1). According to simulation data using quantum chemical calculations (Table 2), the highest probability of the appearance of a mobile hydrogen atom for compound X exists only at

one of the H<sup>+</sup> atoms in the amino group, since the second can be hydrogen bonded to oxygen from the nitro group. In addition, due to electrostatic interaction, it is this hydrogen that prevents the formation of a bond between nitrogen and K<sup>+</sup> in the neutralization reaction with alkali. Comparison of pK<sub>a</sub> values for all compounds and their thermodynamic parameters allows us to make the assumption that **X** is the weakest acid in the series of presented analogs of triazolo[5,1-*c*][1,2,4]triazines and may have a lower ability to self-protonate. By analogy with the salt forms of triazolo[5,1-*c*][1,2,4]triazines, it is likely that compound **X** will have more stable intermediates of a radical nature. Thus, a the biological activity of compound **X** is likely higher compared to compounds **V–IX**, but lower than that for compounds **I–IV**.

Summarizing all of the above, it can be noted that determining the acidity constants of substances is useful for clarifying their transformation pathways. It is possible to connect the dots between the compound's structure, pK<sub>a</sub> and possible biological activity. The absence of acidic properties of the nitrotriazolo[5,1-*c*][1,2,4]triazine molecule or their decrease in a number of analogues can lead to a greater formation of more stable radicals during redox transformations of the nitro group.

#### 4. Limitation

The study encountered limitations related to the lack of standard commercial solutions for calibrating the combined electrode in conjunction with pH meters in non-aqueous media. This limitation may be overcome by developing a calibration technique for the instrumentation, including the preparation of solutions based on an organic solvent, such as DMF/DMSO/acetonitrile, with the addition of an electrolyte, such as LiClO<sub>4</sub>.

#### 5. Conclusions

The approach was suggested for determining the pK<sub>a</sub> of triazolo[5,1-*c*][1,2,4]triazine compounds using potentiometric titration. Conditions were selected to achieve stable and reproducible results for the determination of pK<sub>a</sub> of compounds from the series of triazolo[5,1-*c*][1,2,4]triazines using potentiometric titration methods. The titrant was an alcohol solution of KOH (0.1 M). The solvent was dried DMF with the addition of 0.1 M LiClO<sub>4</sub>. The concentration of the analyte was 0.1 M. The working electrode was a combined glass electrode with a non-standard internal electrolyte – a saturated solution of lithium chloride in anhydrous DMF.

The acidity constant of triazolo[5,1-*c*][1,2,4]triazines in anhydrous dimethylformamide was determined for the first time by potentiometric titration with a combined glass electrode. It was noted that pronounced change in system potential in the titration curves of triazolo[5,1-*c*][1,2,4]triazines are observed for compounds containing either H<sup>+</sup> located at the nitrogen atom in position 1 or an –NH<sub>2</sub> group.

The calculated pK<sub>a</sub> values of the studied molecules correspond to the pK<sub>a</sub> values of moderately strong acids and are in the range of 2–8. The relationship was established between the acidity of the heterocyclic fragment and the presence of electron donor substituents. The obtained pK<sub>a</sub> values of the compounds correlate with the calculated possible deprotonation centers of the molecule.

The measurements of the acidity constants of molecules in aprotic media will make it possible to clarify the mechanisms of their probable transformations. Information about them can form the basis of a unified approach to studying the relationship between the structure, biological activity and physicochemical properties of new original potential medicinal substances.

#### • Supplementary materials

No supplementary materials are available.

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#### • Conflict of interest

The authors declare no conflict of interest.

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