Current Pharmaceutical Analysis, 2021, *17*, 812-821

RESEARCH ARTICLE

Spectroscopic Determination of Dissociation Constants of Some 4-nitrobenzaldehyde-4-substituted phenyl-1-carbonylhydrazones in Sodium Hydroxide Media

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Abstract: ***Purpose*:** Hydrazones are a class of azomethines with a wide spectrum of pharmacological properties that are influenced by the pH of the media. The purpose of this study was to investigate acid-base properties of five 4-nitrobenzaldehyde-4-substitutedphenyl-1-carbonylhydrazones in sodium hydroxide media (14>pH>7).

***Methods*:** The dissociation process was followed by UV-Vis spectroscopy, in ethanol-water (*V*/*V*, 1:1) solutions, at room temperature. Semiempirical methods AM1 and PM3 were applied for determination of the deprotonation enthalpies.

***Results*:** The changes in the UV-Vis spectra, as well as the deprotonation enthalpies, suggested that the dissociation process for four investigated hydrazones with an amide group took place in one step. The exception with two dissociation steps was hydrazone with amide and hydroxyl group. The pH region of dissociation was from pH 10.8 to pH 11.6 for the first step and between pH 11.7 and pH 12.1 for the second step of dissociation. The influence of the ethanol on the UV-Vis spectra was eliminated by the method of Characteristic Vector Analyses (CVA). The stoichiometric dissociation constants were determined numerically ($pK_{HA} = n \cdot pH + \log I$) and graphically (intercept of the dependence of $\log I$ on pH) from the absorbance data using experimental and reconstructed UV-Vis spectra, at three different ionic strengths. Thermodynamic dissociation constants were estimated graphically as an intercept of dependence of dissociation constant on the square root of the ionic strength.

***Conclusion*:** The obtained results demonstrated that the influence of the substituents on pK_{HA} values was not significant, except for hydrazone with amide and hydroxyl group. Namely, the dissociation of the amide group of this hydrazone was retarded due to the influence of the phenolic group.

ARTICLE HISTORY

Received: November 15, 2019
Revised: March 14, 2020
Accepted: April 07, 2020

DOI:
10.2174/1573412916999200502025457

Keywords: Hydrazones, dissociation, dissociation constants, deprotonation enthalpy, UV-Vis spectroscopy, semi-empirical methods.

1. INTRODUCTION

Hydrazones constitute a wide and important class of organic compounds that possess an azomethine group (–NHN=CH–) in their structure. Therefore, many researchers have synthesized these compounds as target structures and evaluated their biological activities. It is well known that hydrazones and their derivatives are characterized by a variety of biological activities such as: antimicrobial, anticonvulsant, antidepressant, anti-inflammatory, analgesic, anticancer, antifungal, antitubercular, antiviral *etc.* [1-6]. These diverse biological activities of hydrazones are probably due to the presence of the azomethine nitrogen atom, which has a lone pair of electrons in a sp^2 hybridized orbitals and is capable of forming stable chelates with metal ions having a biological activity [7-10].

Due to the ability to react with electrophilic and nucleophilic reagents, hydrazones are widely used in organic synthesis, especially in the synthesis of heterocyclic compounds [11]. The introduction of functional groups in the hydrazone molecules expands the scope of their application in organic synthesis. In this way, compounds with unique physical and chemical properties can be obtained. Hence, there is a growing interest in the structural studies of hydrazones as they show a broad spectrum of applications in the pharmaceutical and industrial fields [12]. Furthermore,

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1875-676X/21 $65.00+.00

they possess herbicides, insecticides, nematocides and rodenticides effects as well as plant growth regulators activity [13]. Many hydrazone derivatives possess a broad spectrum of insecticidal activities and they are used as active ingredients for controlling agricultural and horticultural pests [14, 15].

The ionic form in which they exist in the solution determines the physicochemical properties of hydrazones. Since they have acidic and/or basic functional groups, their ionization state depends on the value of the pH of the media and their dissociation constant (pK_a) values. The pK_a of a compound is a physicochemical parameter that significantly affects its pharmacokinetic behavior [16]. Hence, knowledge of the pK_a values of organic compounds is important to perform tests of biopharmaceutical characterization, and in developing new pharmaceutical drugs or improving the available ones [17]. Furthermore, dissociation constants have high significance in the optimization of analytical procedures such as acid-base titration, solvent extraction and complex formation. Taking all these ideas into consideration, the accurate determination of pK_a values is often required in many chemical and biochemical areas [18, 19]. The determination of pK_a can be performed by measuring a physical property of the investigated compound as a function of the pH of the solution. For that purpose, several different methods can be employed [19]. In recent years, frequently used methods are potentiometry, UV-Vis spectroscopy, Liquid Chromatography (LC), Capillary Electrophoresis (CE), reversed-phase HPLC and so on [20-26]. Sometimes, a combination of two different techniques can be employed [27, 28]. UV-Vis spectroscopy has some advantages over the other methods such as: simplicity, availability, low cost, the possibility of analyzing compounds with low solubility, accuracy, reproducibility *etc.* [29-32]. The most important thing is that spectroscopy is a highly sensitive and suitable method for the determination of pK_a values in very dilute aqueous solutions since it requires relatively simple equipment and low compound concentration (about 10^{-6} mol·dm^{-3}) [33]. Therefore it is one of the most employed techniques for the determination of pK_a values [27-33]. Determination of the dissociation constants by employing the UV-Vis spectroscopy includes recording the UV-Vis spectra at different pHs of the solution. The position, shape and the intensity of the peaks in the absorption spectra depend on the concentration of neutral and ionized forms present in the solution. The most significant changes in the absorbance value are observed at the pH, which corresponds to the pK_a values. In order to use UV-Vis spectrometry for pK_a determination, the presence of a chromophore close to the ionization site of the compound is required. Where this is a case, the spectrum of the dissociated and ionized forms of the molecule is different [34-36].

The objective of the present work was to investigate the dissociation process of five aromatic hydrazones and establish a pH region in which the determined ionic form is predominant. Analyses were performed in sodium hydroxide media (14>pH>7) by means of UV-Vis spectroscopy. Moreover, the aim of the study was to determine the stoichiometric and thermodynamic dissociation constants as important parameters for applications of hydrazones in different areas. In order to eliminate the influence of ethanol on the UV-Vis spectra, the CVA method was applied [37, 38]. Furthermore, an attempt was made in order to predict the dissociation site of investigated hydrazones using AM1 and PM3 semiempirical methods.

2. MATERIALS AND METHODS

2.1. Materials

The investigated hydrazones before use were twice purified by recrystallization from 96% ethanol. Their purity was tested by measuring the melting point, as well as by elemental analysis. Sodium hydroxide, sodium perchlorate and ethanol were of analytical grade p.a. (Merck) and they were used without further purification. The aqueous solution of sodium hydroxide was prepared with a concentration of 0.5 mol/dm^3. The diluted solutions of sodium hydroxide (0.05, 0.005 and 0.005 mol/dm^3) were used after setting the pH values of the investigated solutions (14>pH>7). The ionic strength of solutions 0.1, 0.25 and 0.5 mol/dm^3 was adjusted using sodium perchlorate with concentration 1, 2.5 and 5 mol/dm^3, respectively.

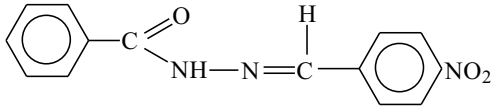
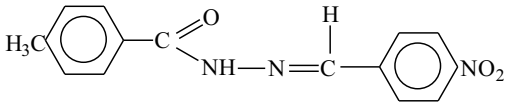
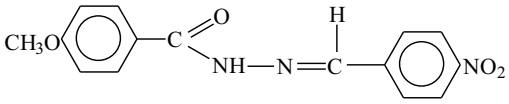
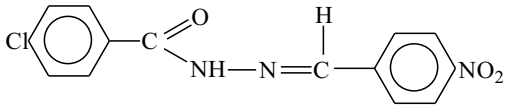
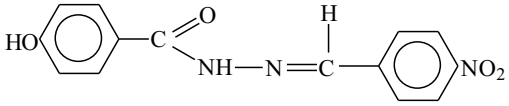
2.2. Instrumentation

A digital pH meter with a glass electrode was used for measurements of the pH values (pH range from 1 to 14). The UV-Vis measurements were carried out on a Varian Cary 50 spectrophotometer controlled by a computer and equipped with a 1 cm path length quartz cell, in the wavelength region ranging from 190 nm to 400 nm. The maximum scan rate was 24 000 nm/min and the resolution was 1.5 nm. An Excel program was applied for calculation of the dissociation constants, while the UV spectra were obtained with computer program Grams Version 4.10.

2.3. Preparation and Stability of Stock and Test Solutions

The stock solutions were prepared in 96% ethanol with a concentration of investigated hydrazones around $1{\cdot}10^{-3}$ mol/dm^3. The stability of these solutions was confirmed by measuring the absorbance values in two months' time. The obtained results showed that there were no changes in the intensity and position of the absorption bands in the UV spectra. The test solutions were prepared with a concentration of investigated hydrazones around $3{\cdot}10^{-5}$ mol/dm^3. Preparation of these solutions was as follows: a known volume of the stock solution was transferred in volumetric flasks of 25 cm^3 in which sodium perchlorate and sodium hydroxide were added shortly before recording the UV spectra. The test solutions were prepared in redistilled water and ethanol with a volume ratio 1:1 (*V*/*V*) because the solubility of the investigated hydrazones in aqueous solutions is low. The recorded UV spectra showed that the stability of these solutions was satisfactory for only 24 hours. After that period of time, changes in the intensity of the absorption bands were noticed. For this reason, the UV spectra were recorded immediately after the preparation of the test solutions at room temperature. At the same time, the blanks were prepared with the same composition as the test solutions, but without investigated hydrazone. The pH of each test solution was measured after recording the UV spectra. All measurements were performed in triplicate using three series of test solutions for each investigated hydrazone.

Table 1. The structure of investigated 4-nitrobenzaldehyde-4-substitutedphenyl-1-carbonylhydrazone.

No.	Structural Formula	Molecular Formula	Melting Point [°C]
H_1	 4-nitrobenzaldehydephenyl-1-carbonylhydrazone	$C_{14}H_{11}O_3N_3$	236 - 239
H_2	 4-nitrobenzaldehyde-4-methylphenyl-1-carbonylhydrazone	$C_{15}H_{13}O_3N_3$	246 - 248.5
H_3	 4-nitrobenzaldehyde-4-metoxyphenyl-1-carbonylhydrazone	$C_{15}H_{13}O_4N_3$	243 - 245.5
H_4	 4-nitrobenzaldehyde-4-chlorophenyl-1-carbonylhydrazone	$C_{14}H_{10}O_3N_3Cl$	253 - 255
H_5	 4-nitrobenzaldehyde-4-hydroxyphenyl-1-carbonylhydrazone	$C_{14}H_{11}O_4N_3$	328.5 - 330

2.4. Semiempirical Calculations

Semiempirical methods can be used to predict proton transfer or the exact place where the molecule can lose a proton at a given pH for different types of organic molecules [39]. This prediction is especially important when the dissociation process takes place in more than one step. AM1 (Austin Model 1) and PM3 (Parametric Method 3) semiempirical methods were used for optimization of the geometry of investigated hydrazones [40, 41]. Theoretical calculations were carried out at the restricted Hartree-Fock level (RHF) using the HYPERCHEM program. All structures were optimized to a gradient norm of < 0.1. The enthalpy of deprotonation (DPE) was calculated according to equation (1) by using the enthalpy of formation of dissociated form $\Delta H_f(A^-)$ and enthalpy of formation of neutral form $\Delta H_f(HA)$.

$$DPE(HA) = \Delta H_f(H^+) + \Delta H_f(A^-) - \Delta H_f(HA) \quad (1)$$

$\Delta H_f(H^+)$ value is 367.15 kcal/mol [40]. The calculated DPE values were further used to predict the place where the molecule losses proton (deprotonation site).

3. RESULTS AND DISCUSSION

3.1. Structure of Investigated Hydrazones

In this study, we used five hydrazones with different substituents [42, 43]. The structural and molecular formulae of investigated hydrazones as well as their melting points are given in Table **1**.

The investigated hydrazones were structurally characterized by UV-Vis spectroscopy, infrared spectroscopy (IR), nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR), as well as, by elemental analysis [42].

3.2. UV-Vis Spectra of Investigated 4-nitrobenzaldehyde-4-substituted phenyl-1-carbonylhydrazones in Basic Media

The experimental UV-Vis spectra of investigated hydrazones were recorded at three different ionic strengths (0.1, 0.25 and 0.5 mol/dm^3) in the pH region from 7 to 14. The changes in the UV-Vis spectra of hydrazones H_1 and H_5 at an ionic strength of 0.1 mol/dm^3 are presented in Figs. (**1** and **2**), respectively.

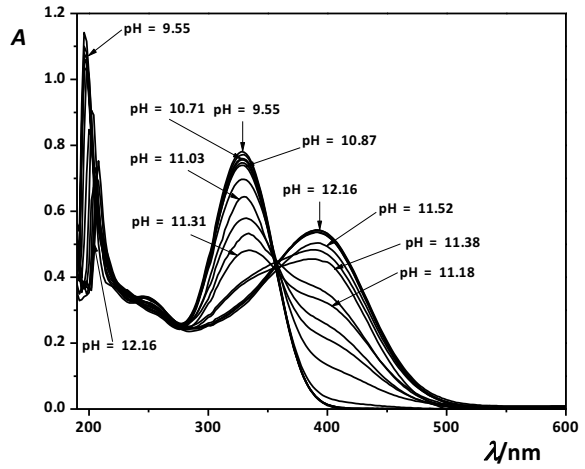


Fig. (1). UV-Vis spectra of 4-nitrobenzaldehydephenyl-1-carbonylhydrazone ($c(H_1)$ = $3.05 \cdot 10^{-5}$ mol/dm^3) in pH region from 9.55 to 12.16, μ = 0,1 mol/dm^3.

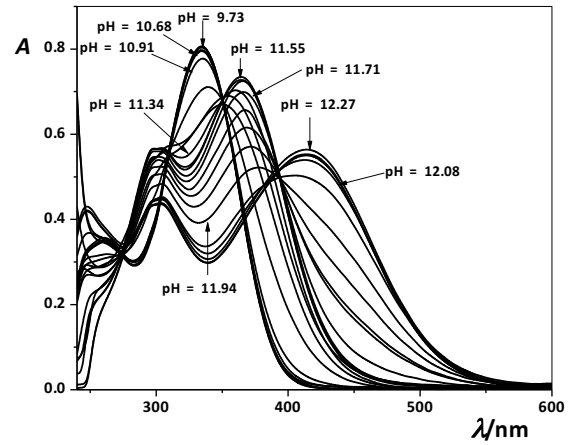


Fig. (2). UV-Vis spectra of 4-nitrobenzaldehyde-4-hydroxyphenyl-1-carbonylhydrazone ($c(H_5)$ = $3.11 \cdot 10^{-5}$ mol/dm^3) in pH region from 9.73 to 12.27, μ = 0,1 mol/dm^3.

Two absorption bands were observed in the UV-Vis spectrum of hydrazone H_1 (Fig. **1**) at a pH of 9.5. The first one has an absorption maximum at 196 nm wavelength, while the absorption maximum of the second band is located at 328 nm. The appearance of the absorption band at around 195-200 nm is due to a $\pi \rightarrow \pi^*$ electronic transition in the benzene ring, while the absorption band at around 290-300 nm is as a result of $n \rightarrow \pi^*$ electron transition in the azomethine group [44].

There were no changes in the position of the first absorption band with increasing the basicity of the solution; only an insignificant decline in its intensity was observed. The changes that occurred in the second absorption band with increasing the basicity of the solution were more significant (Fig. **1**). For our further investigation, we followed the changes in the second absorption band. Namely, increasing the pH of the solution up to 10.7, there were no changes in the position and intensity of the second absorption band. When the pH of the solution was higher than 10.9, the intensity of the band started to decrease, and at pH of 11.2, the band shifted batochromic about 10 nm. At a pH value of 11.4, this absorption band disappeared and a new band appeared at 390 nm. By increasing the basicity of the solution, the intensity of this band increased, while at pH values higher than 11.5, there were no further changes in the position and intensity of this absorption band. Similar changes were observed in the UV spectra of hydrazones H_2, H_3 and H_4.

In the UV-Vis spectra of hydrazone H_5 in the wavelength region from 240 to 600 nm at a pH of 9.7, the absorption band at 336 nm appeared (Fig. **2**). There were no changes in the position and intensity of this band at pH values up to 10.2, while with further changes in the basicity of the solution, the absorption band shifted towards higher wavelengths and its intensity decreased. When the pH of the solution was 11.5, the absorption maximum of the band was located at 262 nm and a new absorption band with a position at 300 nm and low intensity appeared. At pH values higher than 11.7, the intensity of the band at 262 nm decreased and its maximum shifted at a wavelength of 414 nm. With further increase in the basicity of the solution (pH>12.1), there were no additional changes in the UV-Vis spectra of hydrazone H_5 (Fig. **2**).

The changes in the intensity of the absorbance band that appeared at around 390 nm (H_1-H_4) and 362 nm (H_5) with the basicity of the solution (from pH 9.5 to pH 12.1) can be better seen from the curves obtained by plotting the absorbance *vs*. pH of the solution. These curves for all investigated hydrazones have a sigmoidal shape (Fig. **3**).

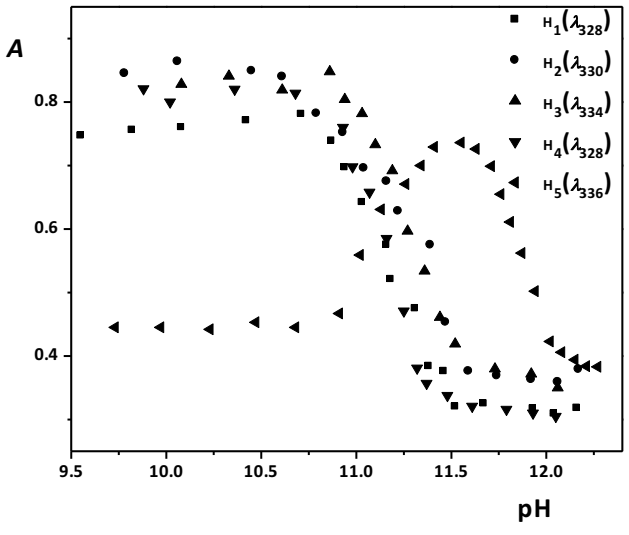


Fig. (3). The dependence of absorbance on pH of the solution of hydrazones H_1-H_5, μ = 0.1 mol/dm^3.

The changes in the UV-Vis spectra (Figs. **1** and **2**) as well as the sigmoidal curves (Fig. **3**) of hydrazones H_1-H_4 suggested that probably the dissociation process occurred. The possible site of deprotonation is the amide group *i.e.*, the

Table 2. Absorption maxima wavelengths for investigated hydrazones (H_1-H_5) in neutral and basic media and pH region of dissociation.

Hydrazone	Neutral Form			Dissociated Form			pH Region of Dissociation
	pH	λ_{1max}	λ_{2max}	pH	λ_{1max}	λ_{2max}	
H_1	9.5	196	328	11.67	198	390	10.8 - 11.5
H_2	9.4	198	330	11.74	198	392	10.8 - 11.6
H_3	9.6	198	334	12.06	198	394	10.8 - 11.5
H_4	9.8	198	328	12.05	198	392	10.9 - 11.6
H_5	9.7	198	336	11.55	198	362	10.9 - 11.3
				12.15	198	414	11.7 - 12.1

Table 3. E_{tot}, ΔH_f and DPE values of investigated hydrazones (H_1-H_5) obtained using AM1 and PM3 semiempirical methods.

AM1					
Hydrazone	E_{tot} (HA)	E_{tot} (A^-)	ΔH_f(HA)	ΔH_f(A^-)	DPE
H_1	-81187.3	-80917.5	64.66	19.52	322.01
H_2	-84782.1	-84514.2	56.69	9.716	320.17
H_3	-92161.9	-91894.1	25.96	-21.09	320.09
H_4	-89491.0	-89226.1	58.24	8.259	317.17
H_5	-88581.2	^{1}-88314.0	19.85	^{1}-27.84	319.46
		^{2}-87983.9		^{2}-12.63	334.67
PM3					
H_1	-73585.7	-73282.7	46.57	-3.975	316.60
H_2	-77039.6	-76740.2	35.79	-18.38	312.97
H_3	-83800.6	-83501.4	6.880	-47.42	312.85
H_4	-80536.1	-80240.1	40.32	-17.29	309.54
H_5	-80363.5	^{1}-80065.5	0.8205	^{1}-54.74	311.58
		^{2}-79701.9		^{2}-43.84	322.48

dissociation process takes place in one step. On the other hand, the dissociation process of hydrazone H_5 (Fig. **3**) takes place in two steps because of the presence of the phenolic group in its molecule (Table **1**).

The position of the absorption maxima of neutral and dissociated form, as well as the pH region of dissociation of investigated hydrazones H_1-H_5 is presented in Table **2**.

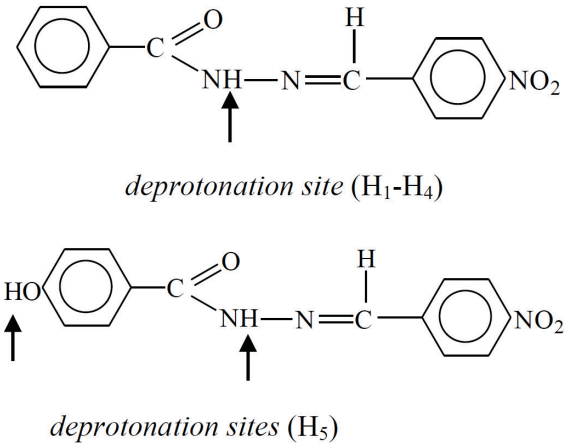
3.3. Deprotonation enthalpy values (DPE)

DPE values were calculated according to Eq. (1) in order to predict the place where the molecule losses proton. The obtained data for the total energy of neutral (E_{tot}(HA)) and dissociated ($E_{tot}(A^-)$) form, enthalpy of formation for neutral (ΔH_f(HA)) and dissociated ($\Delta H_f(A^-)$), and DPE are listed in Table **3**.

Hydrazone H_5 has two dissociable groups, amide group (–NH–) and hydroxyl group (–OH). Comparing the DPE values, it can be seen (Table **3**) that they are lower for the –OH group. This suggests that the first dissociation step is due to the dissociation of the hydroxyl group, while the second dissociation step is as a result of dissociation of amide group. E_{tot} of neutral form is a little lower in comparison with E_{tot} of the dissociated form, indicating the higher stability of neutral form. In accordance with the results obtained by DPE data, as well as the shape of the sigmoidal curves (Fig. **3**), the dissociation site should be presented by Scheme **1**.

3.4. Dissociation Constants (pK_{HA}/pK_{H2A})

The changes in the absorbance values, changing the basicity of the investigated solutions, were used for the determination of dissociation constants. The absorbance data at



Scheme 1. Dissociation site of hydrazones H_1-H_5.

four wavelengths around the absorption maximum were used for the calculation of stoichiometric dissociation constants. The selected wavelengths used for pK_{HA}/pK_{H2A} determination were as follows: H_1 and H_2 (326, 334, 390, and 398 nm), H_3 (258, 266, 330, and 338 nm), H_4 (242, 250, 332, and 324 nm) and H_5 (332, 360, 368, and 400 nm). In order to determine the molar absorption coefficients, UV spectra were recorded at three different concentrations of investigated hydrazones ($2.40 \cdot 10^{-5}$, $3.00 \cdot 10^{-5}$ and $3.60 \cdot 10^{-5}$ mol/dm^3) at ionic strengths 0.1 mol/dm^3, 0.25 mol/dm^3 and 0.5 mol/dm^3. The measurements were performed from the absorbance data measured when the hydrazones H_1-H_5 existed in neutral and dissociated forms. These data were further used for the determination of the concentrations of neutral and dissociated forms of investigated hydrazones. The overdetermined system of four equations (four absorbance values) with two unknown parameters (concentration of neutral and dissociated forms) in accordance with Beer's law was obtained. The concentrations of neutral and dissociated forms were applied for the calculation of the ionization ratio (I). Finally, the pK_{HA} values of investigated hydrazones (H_1-H_5) were calculated using the Henderson – Hasselbach equation (Eq. 2) [45]. In the literature, these constants are referred to as stoichiometric dissociation constants.

$$pK_{HA} = n \cdot pH + \log I \quad (2)$$

where, pK_{HA} is the dissociation constant, I is the ionization ratio ($I = (c(HA)/c(A^-))$, and n is the number of transferred protons.

Parallel to this, the pK_{HA} values of investigated hydrazones were determined graphically, as the intercept of the dependence of log$I$ on pH. When c(HA) = c(B), log$I$ = 0, the graphically determined pK_{HA} value is equal to the pH value of the solution [46]. The organic solvent composition has an influence on the dissociation constant values [47]. In order to eliminate the influence of the solvent from the experimental spectra, the method of Characteristic Vector Analyses (CVA) was applied [48]. The absorbance data from the reconstructed spectra were further used for calculation of dissociation constant values. The thermodynamic pK_{HA} values were evaluated as an intercept with extrapolation of the curve $pK_{HA} = f(\sqrt{\mu})$ to zero ionic strength [49].

Stoichiometric pK_{HA} values of investigated hydrazones (H_1–H_5) calculated from the absorbance values of experimental and reconstructed spectra at ionic strength of 0.1, 0.25 and 0.5 mol/dm^3 are given in Tables **4** and **5**, respectively. Statistical data (Standard Deviation (SD), relative standard deviation (RSD), coefficient of determination (R^2)) and the range in which the obtained results are placed with a confidence level of 0.05 (95%) are also given in Tables **4** and **5**.

By comparison of numerically calculated pK_{HA} values with those evaluated graphically, it can be seen that they are almost similar. This is also confirmed statistically with the t-test. Namely, the obtained value of the t parameter was -0.107, which is obviously lower than the critical value of this parameter (2.306). Similar pK_{HA} values were obtained at different ionic strengths (Tables **4** and **5**). The pK_{HA} values obtained graphically showed a satisfactory correlation of dependence log$I$ on pH. Furthermore, the obtained pK_{HA} values from the absorbance data of reconstructed spectra (CVA method) were higher compared to those obtained from experimental spectra. This was not the case for hydrazones with a similar structure in which dissociation constants were already determined [50]. Actually, in this investigation, the ratio of ethanol in the investigated solutions was higher (50% V/V), which probably is the reason for observed differences in pK_{HA} values. The obtained results from the t-test showed that there were no statistically significant differences *i.e.* the obtained value for t parameter was −0.316, which is lower than the critical value. By comparison of thermodynamic pK_{HA} values of investigated hydrazones H_1-H_4 and pK_{HA^-} value of hydrazone H_5, the following order was obtained:

Hydrazone:	**H_2**	**H_4**	**H_3**	**H_1**	**H_5**
pK_{HA}	10.98	11.06	11.10	11.14	12.07

→ The strength of the acid decreased

Hydrazones H_2, H_3 and H_4 (–CH_3, –OCH_3 и –Cl) have lower pK_{HA} values compared with hydrazone H_1 (–H) which means that they are stronger acids, but the differences are not significant. Hydrazone H_5 has the highest pK_{HA} value, which refers to the dissociation of amide group, probably as a result

Table 4. Stoichiometric dissociation constants (graphically and numerically), thermodynamic dissociation constants, and statistical data (standard deviation (SD), relative standard deviation (RSD), coefficient of determination (R^2)) of investigated hydrazones H_1-H_5, experimental spectra.

No.	μ	n	Numerically			Graphically	
	[mol/dm^3]		pK_{HA}	SD	RSD	pK_{HA}	R^2
H_1	0.1	9	11.19(±0.02)	0.03	0.29	11.22	0.980
	0.25	9	11.25(±0.03)	0.05	0.41	11.23	0.956
	0.5	9	11.26(±0.03)	0.04	0.39	11.24	0.966
	*pK_{HA} (R^2)		11.14 (0.862)			11.21 (0.946)	
H_2	0.1	8	11.06(±0.02)	0.03	0.25	11.08	0.990
	0.25	8	11.12(±0.02)	0.02	0.23	11.13	0.988
	0.5	8	11.16(±0.03)	0.04	0.36	11.17	0.962
	*pK_{HA} (R^2)		10.98 (0.993)			11.02 (0.987)	
H_3	0.1	9	11.15(±0.03)	0.05	0.41	11.20	0.960
	0.25	9	11.19(±0.03)	0.05	0.49	11.21	0.946
	0.5	9	11.22(±0.03)	0.05	0.47	11.25	0.958
	*pK_{HA} (R^2)		11.10 (0.944)			11.17 (0.951)	
H_4	0.1	9	11.09(±0.02)	0.04	0.38	11.11	0.966
	0.25	9	11.10(±0.04)	0.06	0.52	11.14	0.941
	0.5	9	11.12(±0.02)	0.04	0.33	11.18	0.976
	*pK_{HA} (R^2)		11.06 (0.999)			11.05 (0.999)	
-	-	-	pK_{H2A}	SD	RSD	pK_{H2A}	R^2
H_5	0.1	5	11.03(±0.06)	0.06	0.60	11.06	0.872
	0.25	5	11.10(±0.03)	0.04	0.35	11.10	0.948
	0.5	5	11.12(±0.01)	0.01	0.07	11.13	0.996
	^{1*}pK_{H2A} (R^2)		10.96 (0.883)			11.00 (0.994)	
-	-	-	pK_{HA^-}	SD	RSD	pK_{HA^-}	R^2
H_5	0.1	7	12.05(±0.02)	0.02	0.17	12.13	0.976
	0.25	7	12.09(±0.01)	0.02	0.13	12.15	0.980
	0.5	7	12.11(±0.03)	0.04	0.34	12.20	0.896
	^{2*}pK_{HA^-} (R^2)		12.07 (0.986)			12.01 (0.955)	

pK_{HA} - dissociation constant ^{1}pK_{H2A} - first dissociation constant, ^{2}pK_{HA^-} - second dissociation constant; *pK - thermodynamic dissociation constants, μ - ionic strength, n - number of measurements.

Table 5. Stoichiometric dissociation constants (graphically and numerically), thermodynamic dissociation constants, and statistical data (standard deviation (SD), relative standard deviation (RSD), coefficient of determination (R^2)) of investigated hydrazones H_1-H_5, reconstructed spectra.

No.	μ	n	Numerically			Graphically	
	[mol/dm^3]		pK_{HA}	SD	RSD	pK_{HA}	R^2
H_1	0.1	9	11.24(±0.03)	0.05	0.04	11.25	0.958
	0.25	9	11.27(±0.03)	0.04	0.35	11.29	0.970
	0.5	9	11.31(±0.04)	0.06	0.51	11.41	0.944
	*pK_{HA} (R^2)		11.18 (0.996)			11.11 (0.936)	
H_2	0.1	8	11.19(±0.02)	0.03	0.28	11.21	0.986
	0.25	8	11.23(±0.02)	0.03	0.29	11.26	0.978
	0.5	8	11.23(±0.03)	0.04	0.35	11.29	0.954
	*pK_{HA} (R^2)		11.17 (0.888)			11.15 (0.977)	
H_3	0.1	9	11.23(±0.01)	0.02	0.19	11.26	0.990
	0.25	9	11.27(±0.01)	0.02	0.20	11.30	0.990
	0.5	9	11.32(±0.03)	0.04	0.35	11.38	0.976
	*pK_{HA} (R^2)		11.16 (0.998)			11.16 (0.980)	
H_4	0.1	9	11.18(±0.02)	0.03	0.39	11.16	0.952
	0.25	9	11.26(±0.02)	0.04	0.34	11.18	0.972
	0.5	9	11.28(±0.02)	0.03	0.32	11.21	0.974
	*pK_{HA} (R^2)		11.11 (0.902)			11.12 (0.993)	
			pK_{H2A}	SD	RSD	pK_{H2A}	R^2
H_5	0.1	5	11.13(±0.04)	0.04	0.37	11.15	0.946
	0.25	5	11.15(±0.04)	0.05	0.45	11.24	0.919
	0.5	5	11.17(±0.04)	0.05	0.45	11.29	0.913
	^{1*}pK_{H2A} (R^2)		11.10 (0.989)			11.04 (0.953)	
			pK_{HA^-}	SD	RSD	pK_{HA^-}	R^2
H_5	0.1	7	12.06(±0.02)	0.03	0.26	12.14	0.946
	0.25	7	12.07(±0.02)	0.02	0.18	12.17	0.968
	0.5	7	12.11(±0.02)	0.03	0.26	12.22	0.938
	^{2*}pK_{HA^-} (R^2)		12.02 (0.834)			12.07 (0.983)	

pK_{HA} - dissociation constant ^{1}pK_{H2A} - first dissociation constant, ^{2}pK_{HA^-} - second dissociation constant; *pK - thermodynamic dissociation constants, μ - ionic strength, n - number of measurements.

of the influence of the phenolic group present in its molecule. Namely, the phenolic group is stronger acid compared to amide group. It means that the phenolic group dissociated first, causing delayed dissociation of the amide group at higher pH values. It is known from the literature that deprotonation of the negative ion is more difficult compared to the deprotonation of the neutral molecule. Hence, hydrazone H_5 is the weakest acid compared to other investigated hydrazones (H_1-H_4) in relation to the second step of dissociation. The obtained pK_{HA} values (stoichiometric and thermodynamic) of hydrazones H_1-H_4 suggest that the substituents present in their molecules have no significant influence on the behavior of these hydrazones in basic media. The situation is different for hydrazone H_5 with a hydroxyl group in its molecule, which caused dissociation in two steps. The first step is due to the dissociation of phenolic group, while the second one is as a result of the dissociation of amide group. The difference in the first (pK_{H2A}) and second (pK_{HA^-}) dissociation constant is about 1, indicating that the two dissociation steps are not completely separated. This suggests that the obtained pK_{H2A} and pK_{HA^-} for hydrazone H_5 have to be accepted with some changes. In order to obtain results that are

more precise, another method should be used, for example, differential UV-Vis spectroscopy.

CONCLUSION

The dissociation process of five 4-nitrobenzaldehyde-4-substituted phenyl-1-carbonylhydrazone (–H, $-CH_3$, $-OCH_3$, –Cl and –OH) was followed in sodium hydroxide media (14>pH>7) by UV-Vis spectroscopy. Analysing the changes in the UV-Vis spectra, the pH region of dissociation was determined. When the dissociation process took place in one step, that was due to the dissociation of amide group (H_1-H_4). The deprotonation enthalpy values obtained by AM1 and PM3 semiempirical methods showed that when the dissociation process took in two steps, the first deprotonation site was due to the dissociation of phenolic group, while the second one was a result of the dissociation of the amide group (H_5). In order to eliminate the influence of the solvent (50% ethanol), the UV-Vis spectra were reconstructed by the method of Characteristic Vector Analysis (CVA). The stoichiometric pK_{HA} values were determined numerically and graphically using the absorbance data from the UV-Vis spectra (experimental and reconstructed) recorded at three different ionic strengths (0.10, 0.25, and 0.50 mol/dm^3). The thermodynamic pK_{HA} values were evaluated with extrapolation of the dependence $pK_{HA} = f(\sqrt{\mu})$ to zero ionic strength. The significant influence of the substituents on the pK_{HA} values was noticed only for hydrazone H_5 with a hydroxyl group present in its molecule.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

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