INVESTIGATION OF ACID-BASE PROPERTIES OF AROMATIC HYDRAZONES IN BASIC MEDIA AT CONSTANT IONIC STRENGTH

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Abstract. UV spectroscopic methods were used in order to determine dissociation constants of some aromatic hydrazones. The acid-base properties of investigated hydrazones were followed in sodium hydroxide media at constant ionic strength of 0.5 mol/dm³ adjusted with sodium perchlorate. Absorption band with the maximum of 330 nm was noticed in neutral media. A bathochromic shift of this band was observed in basic media, probably due to the dissociation process. The dissociation process took place in one step for four investigated hydrazones and in two steps for the hydrazone with a phenol group in its molecule. The absorbance data from the UV spectra were used for the calculation of dissociation constants. The obtained pK_{HA} values were between 2.11 and 2.62 which suggested that the influence of the substituents is not significant. At the same time, pK_{HA} values were determined graphically from the intercept of the dependence of logI on pH. There are no important differences between calculated and graphically determined dissociation constant values.

Key words: Aromatic hydrazones, UV spectroscopy, dissociation, dissociation constants

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1. INTRODUCTION

The hydrazones are a well-known class of organic compounds with a wide spectrum of application, which is a result of having an azomethine proton (-NH-N=CH-) [1]. A number of researchers synthesized and evaluated the biological activities of hydrazones. These compounds possess diverse biological and pharmacological properties such as antimicrobial, anti-inflammatory, analgesic, antifungal, anti-tubercular, antiviral, anticancer, antiplatelet, antimalarial, anticonvulsant, cardio protective, antihelminthic, antiprotozoal, anti-trypanosomal, antischistosomiasis and so on [2-6]. On the other hand, hydrazones are useful spectrophotometric reagents because of their selectivity for metal ions [7]. Hydrazones and their metal complexes exhibit a wide spectrum of physiological and pharmacological activities. Due to their physiological activity, they are used as herbicides, insecticides, and plant growth stimulants [8]. Furthermore, hydrazones are also used in industry as plasticizers, polymer stabilizers, antioxidants, polymerization initiators [9]. The dissociation constants of organic compounds like hydrazones play a fundamental role in many analytical procedures such as acid-base titration, solvent extraction, complex formation and ion transport [10]. Moreover, the

biological activity of hydrazones depends on the ionic forms in which they exist in the solution. Therefore, determination of dissociation constants in defined media is very important. In the literature, there are different methods for the determination of dissociation constant values such as potentiometric titration, NMR spectroscopy, capillary electrophoresis (CE), liquid chromatography (LC), UV spectrophotometry and so on [11-15]. The great advantage of UV-Vis spectrophotometry is that this method can handle compounds with lower solubility and lower sample concentrations [15]. It is obvious that the usage of hydrazones is mostly as a result of biological activity which depends on the pH values of the media. For this reason, the objective of this study was to investigate the acid-base behavior of some aromatic hydrazones in basic media and to determine the dissociation constant values. The UV-Vis spectroscopic method was applied in order to observe changes in the UV spectra varying the pH of the media.

2. EXPERIMENTAL

2.1. Chemicals and instrumentation

The investigated hydrazones were purified by twice recrystallization from ethanol or diluted ethanol. Purity was tested by measuring the melting point as well as by

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elemental analysis. The other chemicals (NaOH, NaClO₄ and ethanol) were of analytical grade p.a. (Alkaloid) and were used without further purification. A digital pH meter with glass electrode (pH range from 7 to 14) was used for measurements of the pH values of the solutions. The spectral 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 from 190 nm to 400 nm. The maximum scan rate was 24 000 nm/min and resolution was 1.5 nm. An Excel program was applied for the calculation of the dissociation constants, while the UV spectra were obtained with the computer program Grams Version 4.10.Cl.

2.2. Preparation of solutions

A stock solution of the hydrazones was prepared by dissolving about 60 mg of the investigated compounds in 96% ethanol in a volumetric flask of 250 cm³. The volume of 0.75 cm³ of this solution was transferred into 25 cm³ volumetric flask, and after adding an appropriate volume of NaOH (*c* = 0.5 mol dm⁻³) and NaClO₄ (*c* = 1 mol dm⁻³) the flask was diluted up to the mark with deionized water. The degree of dilution of the stock solutions was chosen to obtain the concentration of hydrazones in the test solution of about 3·10⁻⁵ mol dm⁻³ i.e. the absorbance to have a value between 0.1 and 1 at the studied wavelengths. The pH of the test solutions was adjusted with NaOH, while the ionic strength was maintained constant (0.5 mol dm⁻³) using the solution of NaClO₄. The UV spectra were taken immediately after preparation of the test solutions, at room temperature. After that the pH of each test solution was measured. The solution which did not contain the investigated hydrazone, but had the same composition as the tested one was used as a blank. The stock solutions were stable for a long period of time under ordinary conditions, while the stability of the working solution was satisfactory for only 24 hours.

3. RESULTS AND DISCUSSION

3.1. Structure of investigated hydrazones

The aromatic hydrazones investigated in this study share the general structural formula presented in Table 1. The investigated hydrazones were synthesized in our laboratory and structurally characterized by UV spectroscopy, infrared spectroscopy (IR), nuclear magnetic resonance (¹H NMR and ¹³C NMR), as well as by the elemental analysis [16].

Table 1. Structural formula of investigated hydrazones (H₁-H₅)

Compound	R	R'
H ₁	-H	-NO ₂
H ₂	-CH ₃	
H ₃	-OCH ₃	
H ₄	-Cl	
H ₅	-OH	

IUPAC names of investigated compounds are: H₁: N-*p*-nitrobenzaldehydebenzoylhydrazone, H₂: N-*p*-nitrobenzaldehyde-*p*-methylbenzoylhydrazone, H₃: N-*p*-nitrobenzaldehyde-*p*-methoxybenzoylhydrazone, H₄: N-*p*-nitrobenzaldehyde-*p*-chlorobenzoylhydrazone and H₅: N-*p*-nitrobenzaldehyde-*p*-hydroxybenzoylhydrazone.

3.2. UV spectra

UV spectrophotometric measurements for investigated hydrazones H₁-H₅ were performed in the pH region between 7 and 14. The UV spectra were recorded in aqueous solutions containing 3% ethanol (*v/v*) at constant ionic strength of 0.5 mol/dm³ (Figures 1 and 2).

Figure 1. UV spectra of N-*p*-nitrobenzaldehydebenzoylhydrazone (H₁) (*c* = 3.05·10⁻⁵ mol/dm³) in pH region from 9.5 to 12.1 and ionic strength of 0.5 mol/dm³

Figure 2. UV spectra of N-*p*-nitrobenzaldehyde-*p*-hydroxybenzoylhydrazone (H₅) (*c* = 3.11·10⁻⁵ mol/dm³) in pH region from 9.7 to 12.3 and ionic strength of 0.5 mol/dm³

Two absorption bands with maximum at around 195 and 330 nm were observed in the UV spectra of investigated hydrazones (Figs. 1 and 2). The appearance of the absorption band at around 195-200 nm is due to a $\pi \rightarrow \pi^*$ electronic transition in benzene ring, while the absorption band at around 330 nm is as a result of $n \rightarrow \pi^*$ electron transition in the azomethine group [17]. For further investigation we followed the

changes in the absorption band that appeared at wavelength around 330 nm.

From Fig 1 it can be noticed that the intensity of this band decreased when the basicity of the solution increased. In basic media, a bathochromic shift was observed probably as a result of the influence of basicity of the solution. Similar changes were observed in the UV spectra of hydrazones H₂-H₄. Difference in behavior was noticed in the UV spectra of hydrazone H₅ (Fig. 2). The intensity of the absorption band decreased when the basicity of the solution increased and, at the same time, the bathochromic shift was observed. The absorption maximum reaches the position at 328 nm wavelength at a pH value of 9.8 and further changes were not observed until a pH of 11.1. At a higher pH value, the intensity of the absorption band again decreased and another bathochromic shift of about 6 nm was observed. This behavior of hydrazone H₅ was expected because of the presence of a phenolic group in its molecule.

The position of the absorption maxima of the investigated aromatic hydrazones in basic media at ionic strength of 0.5 mol/dm³, as well as the pH region of dissociation are listed in Table 2.

Table 2. Position of absorption maxima and pH region of dissociation of investigated hydrazones

Comp.	Electroneutral form			Dissociated form		
	pH	$\lambda_{1\max}$	$\lambda_{2\max}$	pH	$\lambda_{1\max}$	$\lambda_{2\max}$
H ₁	9.5	196	328	11.67	198	390
H ₂	9.4	198	330	11.74	198	392
H ₃	9.6	198	334	12.06	198	394
H ₄	9.8	198	328	12.05	198	392
H ₅	9.7	198	336	11.55	198	362
				12.15	198	414
pH region of dissociation						
H ₁	10.8-11.5					
H ₂	10.8-11.6					
H ₃	10.8-11.5					
H ₄	10.9-11.6					
H ₅	10.9-11.3					
	11.7-12.1					

3.4. Determination of pK_{HA} values

The determination of pK_{HA} values was made graphically and numerically. The calculations were made from the absorbance data at constant ionic strengths [18]. The dissociation constants were determined numerically according to following equation:

$$pK_{HA} = n \cdot pH + \log I$$

where, pK_{HA} is the dissociation constant, *I* is the ionization ratio ($I = (c(\text{HA neutral form})/c(\text{A-dissociated form}))$), and *n* is number of transferred protons

When $c(\text{HA}) = c(\text{A}^-)$, the ionization ratio is zero ($\log I = 0$), then the dissociation constant is obtained as an intercept of linear dependence on $\log I$ vs. pH (Table 3).

Statistical data (standard deviation (SD), relative standard deviation (RSD), coefficient of determination (*R*²)), as well as the range in which the obtained results

are placed with confidence level of 0.05 (95%) were calculated, too (Table 3).

Table 3. pK_{HA} values of investigated hydrazones and statistical data (SD, RSD, *R*²)

Comp.	pK _{HA} numerically		
	pK _{HA}	SD	RSD
H ₁	11.26±0.03	0.04	0.39
H ₂	11.16±0.03	0.04	0.36
H ₃	11.22±0.03	0.05	0.47
H ₄	11.12±0.02	0.04	0.33
H ₅	¹ 11.12±0.01	0.01	0.07
	² 12.11±0.03	0.04	0.34
pK _{HA} graphically			<i>R</i> ²
H ₁	11.24		0.966
H ₂	11.17		0.962
H ₃	11.25		0.958
H ₄	11.18		0.976
H ₅	11.13		0.996
	12.20		0.896

¹dissociation of phenolic group

²dissociation of amide group

The pK_{HA} values obtained numerically have identical values to those estimated graphically (Table 3). The dependence of $\log I$ vs. pH is linear with the coefficient of determination ≈ 1 suggesting satisfactory precision in the determination of dissociation constants graphically. This implies that the pK_{HA} values can be successfully determined in both ways. From the results presented in Table 3 it can be seen that there are no differences in pK_{HA} values of hydrazones H₁-H₄ which referred to the dissociation of amide group. This result probably is a result of a similar structure of investigated hydrazones suggesting that the influence of substituents (–CH₃, –OCH₃, –Cl and –OH) is not significant. The dissociation process of hydrazone H₅, as it was mentioned before, takes place in two steps. The first step is due to the dissociation of the phenolic group which is a stronger acid, while the second step is as a result of dissociation of the amide group. For this reason, the constant value of dissociation, which referred to the dissociation of the amide group, is higher in comparison with the investigated hydrazones H₁-H₄. These differences are due to the presence of the phenolic group in the molecule of hydrazone H₅, which caused a late dissociation of the amide group [19].

4. CONCLUSION

The dissociation process of some aromatic hydrazones was followed using UV spectroscopy. The observed changes in the UV spectra showed that the reaction of dissociation occurred in one (H₁-H₄) or two steps (H₅) depending of the structure of hydrazones. The observed pH range of the first step of dissociation it was from 10.8 to 11.6, while for the second step was 11.7-12.1. The changes in the UV spectra were used to calculate the pK_{HA} values. The pK_{HA} values were determined graphically and numerically from the absorbance data. The similarity of the pK_{HA} values suggested that the substituents have no important influence on the dissociation process. There was a good agreement between the pK_{HA} values of the investigated

hydrazones and those of similar classes of compounds [20].

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