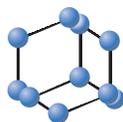


RESEARCH ARTICLE

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Investigation of Dissociation Process of Some 4-Methoxybenzaldehyde Benzoylhydrazones in Sodium Hydroxide Media by UV Spectroscopy



Mirjana S. Jankulovska^{1,*}, Ilinka Spirevska² and Vesna Dimova³

¹Food Institute, Faculty of Agricultural Sciences and Food, Ss. Cyril and Methodius University, Skopje, Republic of Macedonia; ²Institute of Chemistry, Faculty of Natural Sciences and Mathematics, Ss. Cyril and Methodius University, Skopje, Republic of Macedonia; ³Institute of Organic Technology, Faculty of Technology and Metallurgy, Ss. Cyril and Methodius University, Skopje, Republic of Macedonia

Abstract: The importance and wide application of hydrazones are due to a broad spectrum of biological activities that they possess. The biological activity generally depends on the pH values of the media which have influence on the extent of ionization of organic compounds. The dissociation process of five 4-methoxybenzaldehyde benzoylhydrazones in aqueous sodium hydroxide media was determined using UV spectroscopy method, at room temperature. The influence of the substituents in the *p*-position of benzoyl moieties was investigated. Prediction of the deprotonation site in the hydrazone molecule was made by using the deprotonation enthalpy values calculated using the AM1 and PM3 semiempirical methods. The dissociation process took place in one step as a result of the dissociation of the amide group, or in two steps for 4-methoxybenzaldehyde 4-hydroxybenzoylhydrazone. The changes in the UV spectra with the basicity of the media were used for calculation of the dissociation constant values. One dissociation constant (pK_{HA}) was calculated for hydrazones which contain only an amide group. The first and the second dissociation constants (pK_{H_2A} and pK_{HA^-}) were determined as a result of the dissociation of the amide and phenolic group of 4-methoxybenzaldehyde 4-hydroxybenzoylhydrazone which contain these groups. The dissociation of amide group of this hydrazone was retarded due to the influence of phenolic group. In order to obtain thermodynamic pK_{HA} values, the measurements were performed at three different ionic strengths (0.10, 0.25, and 0.50 mol/dm³). The first deprotonation site was due to dissociation of phenolic group, while the second one was a result of dissociation of the amide group. The strength of the acid decreased by following the order: 4-chlorobenzoyl- > benzoyl- > 4-methoxybenzoyl- > 4-methylbenzoyl- > 4-hydroxybenzoyl hydrazone. The obtained results demonstrated that the influence of the substituents was not significant, except for 4-methoxybenzaldehyde 4-hydroxybenzoylhydrazone.

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1. INTRODUCTION

Hydrazones are a class of azomethines with wide application in many scientific fields due to the presence of -NHN=CH- group in their molecule. They contain two connected nitrogen atoms of different nature and a C=N that is conjugated with a lone electron pair on the terminal nitrogen atom. These structural fragments are mainly responsible for the physical and chemical properties of hydrazones. Both

nitrogen atoms of the hydrazone group are nucleophilic, although the amino type nitrogen is more reactive.

The carbon atom of hydrazone group has both electrophilic and nucleophilic character [1]. The introduction of different functional groups in the hydrazone molecules expands their use in organic synthesis. Moreover, the combination of the hydrazono group with other functional groups leads to compounds with unique physical and chemical properties allowing their usage in organic chemistry as substances for new drug development [2]. Hydrazones and their derivatives possess a wide spectrum of pharmacological properties such as antimicrobial, anticonvulsant, analgesic, antiplatelet, antiinflammatory, antitubercular and antitumoral activities [3-7]. Hydrazones are also important compounds in

*Address correspondence to this author at the Department of Food Quality and Safety, Faculty of Agricultural Sciences and Food, Ss. Cyril and Methodius University, P.O. Box: 297, Skopje, Macedonia; Tel/Fax: +389 70 858 045, +389 2 3134 310; E-mails: jankulovska_m@yahoo.com; jankulovska@fznh.ukim.edu.mk

organic, inorganic, analytical, and coordination chemistry. They easily form stable complexes with most of the transition metal ions which can be used as biologically important compounds [8]. They act as multidentate ligands with metals, forming colored chelates which can be used in selective and sensitive determination of the metals. Hence, hydrazones are a very important group of analytical reagents for the determination of various metal ions by using various analytical techniques [9]. Due to their structure, hydrazones are also interesting as ligands in coordination chemistry. The basic coordination sites are carbonyl oxygen and the azomethine nitrogen [10, 11]. Hydrazones also act as herbicides, insecticides, nematocides, rodenticides and plant growth regulators [12]. Many hydrazone derivatives have been reported to possess broad spectrum insecticidal activity and are used as active ingredients for controlling agricultural and horticultural pests [13].

The properties of hydrazones and their derivatives depend on acid-base processes, so their acid-base equilibria are frequently investigated in acidic and basic media. The dissociation constant values of these kinds of compounds are important in a wide range of applications and research areas especially for studying reaction mechanisms which take place in different media. The degree of ionization of organic compounds strongly affects their solubility, permeability, and drug disposition properties. On the other hand, dissociation constants are most valuable parameters used to understand chemical phenomena such as biological activity, absorption and extent of ionization of compound at different pH. The dissociation constant of a compound is the pH at which the compound is 50 % protonated [14-17]. These constants are important in many analytical procedures and research areas such as acid-base titration, solvent extraction, ion transport and complex formation. Hence, their accurate determination is often needed in various chemical and biochemical areas. In particular, knowing the dissociation constant values of organic compounds is important for understanding chemical reactions in which they participate [18, 19]. Furthermore, the biological activities of hydrazones depend on the ionic form (cationic, anionic, or neutral) which exists in the solution. Since hydrazones have acidic and basic functional groups their ionization state is controlled by both pH of the solution and acid dissociation constants. There are various methods for determination of disso-

ciation constant values such as potentiometric titration, NMR spectroscopy, Capillary Electrophoresis (CE), Liquid Chromatography (LC), UV spectrophotometry and so on [20, 21]. The great advantage of UV-Vis spectrophotometry is that this method can handle compounds with low solubility and low sample concentrations [18].

Taking into consideration the wide application of hydrazones and their derivatives and the influence of acidity of the medium, the objective of this study was to investigate the behavior of 4-methoxybenzaldehyde benzoylhydrazones **1-5** in basic media by UV spectroscopy and to determine the thermodynamic dissociation constants. In order to investigate the influence of the substituents, five benzoylhydrazones **1-5** with different structures were studied [22]. To predict the deprotonation site in the hydrazone molecule in basic media, Deprotonation Enthalpy (DPE) values were determined applying AM1 and PM3 semiempirical methods.

2. RESULTS AND DISCUSSION

2.1. Structure of Investigated 4-Methoxybenzaldehyde Benzoylhydrazones 1-5

The structural formulas of the investigated hydrazones used in this study and their melting points are shown in Table 1.

The investigated hydrazones were synthesized and structurally characterized by UV spectroscopy, infrared spectroscopy (IR), nuclear magnetic resonance (^1H and ^{13}C NMR), as well as by elemental analysis [22]. The IR, ^1H and ^{13}C NMR spectral data are presented in Table 2. The positions of the IR absorption bands and the signals obtained from H_2O and DMSO in the ^1H NMR and ^{13}C NMR were in accordance with literature data [23, 24].

2.2. Electronic Behavior of Investigated 4-Methoxybenzaldehyde Benzoylhydrazones 1-5 in Basic Media

In order to investigate the behavior of hydrazones **1-5** in basic media the spectrophotometric measurements were performed in the pH region between 7 and 14. The UV spectra were recorded in aqueous solutions containing 3% ethanol (v/v) at ionic strength of 0.10, 0.25, and 0.50 mol/dm³. The UV spectra at ionic strength of 0.1 mol/dm³ of hydrazones **3**

Table 1. Structural formulas and melting points of investigated 4-methoxybenzaldehyde benzoylhydrazones 1-5 [22].

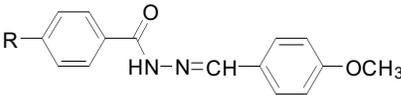
			
Comp.	R	Melting Point (°C)	Name
1	H	163-166	4-methoxybenzaldehyde benzoylhydrazone
2	CH ₃	217-219	4-methoxybenzaldehyde 4-methylbenzoylhydrazone
3	OCH ₃	176-178.5	4-methoxybenzaldehyde 4-methoxybenzoylhydrazone
4	Cl	200-202	4-methoxybenzaldehyde 4-chlorobenzoylhydrazone
5	OH	230-233	4-methoxybenzaldehyde 4-hydroxybenzoylhydrazone

Table 2. IR, ^1H and ^{13}C NMR spectral data of investigated benzoylhydrazones 1-5.

IR (KBr, cm^{-1})	
1	3201 [$\nu(\text{N-H})$], 1639 [$\nu(\text{C=O})$], 1604, [$\nu(\text{C=N})$], 1548 [$\nu(\text{NH-C=O})$], 2935-3068 [$\nu(\text{CH arom})$]
2	3165-3201 [$\nu(\text{N-H})$], 1636 [$\nu(\text{C=O})$], 1601, [$\nu(\text{C=N})$], 1548 [$\nu(\text{NH-C=O})$], 2997-3029 [$\nu(\text{CH arom})$]
3	3178-3211 [$\nu(\text{N-H})$], 1643 [$\nu(\text{C=O})$], 1608, [$\nu(\text{C=N})$], 1548 [$\nu(\text{NH-C=O})$], 3039 [$\nu(\text{CH arom})$]
4	3289 [$\nu(\text{N-H})$], 1659 [$\nu(\text{C=O})$], 1604, [$\nu(\text{C=N})$], 1542 [$\nu(\text{NH-C=O})$], 3026-3074 [$\nu(\text{CH arom})$]
5	3162 [$\nu(\text{N-H})$], 1637 [$\nu(\text{C=O})$], 1591, [$\nu(\text{C=N})$], 1552 [$\nu(\text{NH-C=O})$], 3025-3065 [$\nu(\text{CH arom})$]
^1H NMR(400 MHz, $\text{DMSO-}d_6$, δ ppm) H_2O : 3.33 ppm, DMSO : 2.50 ppm	
1	δ = 3.83 (3H, OCH_3), 7.92 (1H, NH), 8.48 (1H, N=CH), 6.98-7.92 (9H, ArH)
2	δ = 2.38 (3H, CCH_3), 3.82 (3H, OCH_3), 7.86 (1H, NH), 8.41 (1H, N=CH), 7.02-7.84 (8H, ArH)
3	δ = 3.83 (6H, 2 x OCH_3), 7.90 (1H, NH), 8.41 (1H, N=CH), 7.06-7.92 (8H, ArH)
4	δ = 3.83 (3H, OCH_3), 7.95 (1H, NH), 8.40 (1H, N=CH), 6.99-7.95 (8H, ArH)
5	δ = 3.80 (3H, OCH_3), 5.37 (1H, OH), 7.93 (1H, NH), 8.48 (1H, N=CH), 6.79-7.93 (8H, ArH)
^{13}C NMR (100 MHz, $\text{DMSO-}d_6$, δ ppm) DMSO : 39.52 \pm 0.06 ppm	
1	δ = 55.8 (OCH_3), 148.2 (N=CH), 163.4 (C=O), 127.4-134.1 (ArC)
2	δ = 21.3 (CCH_3), 55.9 (OCH_3), 147.0 (N=CH), 163.7 (C=O), 116.2-135.2 (ArC)
3	δ = 55.7 (2 x OCH_3), 147.6 (N=CH), 161.2 (C=O), 114.1-129.9 (ArC)
4	δ = 55.8 (OCH_3), 148.5 (N=CH), 162.4 (C=O), 127.3-136.9 (ArC)
5	δ = 55.8 (OCH_3), 147.2 (N=CH), 162.9 (C=O), 119.2-136.4 (ArC)

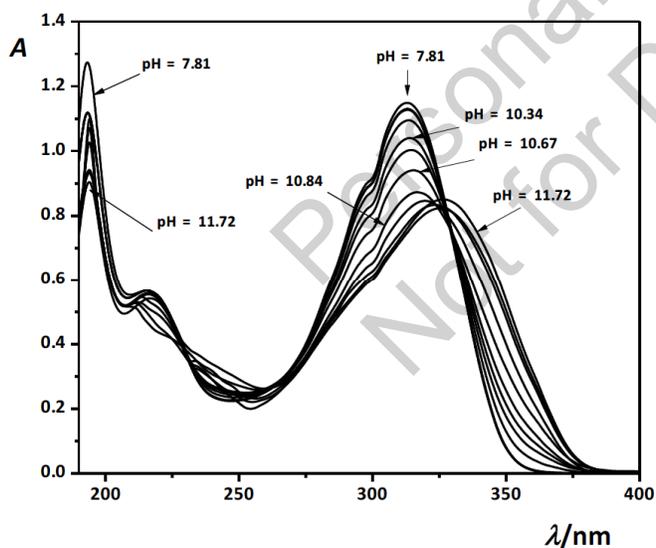


Fig. (1). UV spectra of 4-methoxybenzaldehyde 4-methoxybenzoylhydrazone (3) ($c = 3.21 \times 10^{-5} \text{ mol/dm}^3$) in pH region from 7.8 to 11.7 and ionic strength of 0.1 mol/dm^3 .

and 5 are presented in (Figs. 1 and 2), respectively. Three absorption bands with maximum at around 195, 215-225 and 310 nm were observed in the UV spectra of the investigated hydrazones (Figs. 1 and 2). The first (195 nm) and the third band (310 nm) were more intense, while the second one (215-225 nm) had very low intensity. From the literature it is

known that 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 290-300 nm is as a result of $n \rightarrow \pi^*$ electron transition in the azomethine group [25].

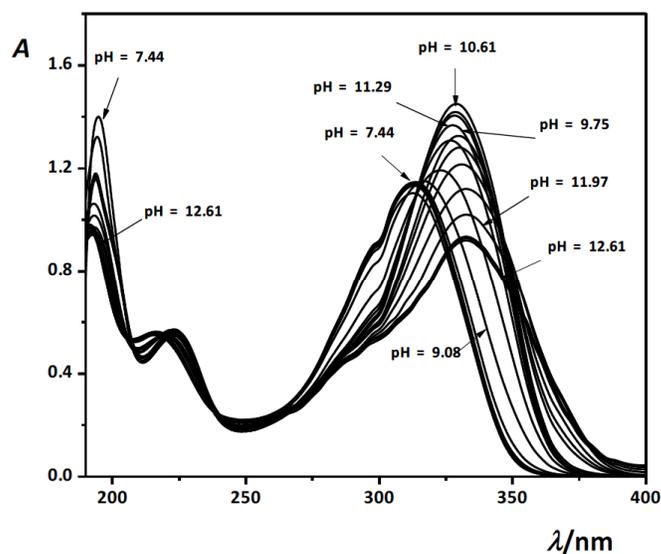


Fig. (2). UV spectra of 4-methoxybenzaldehyde 4-hydroxybenzoylhydrazone (5) ($c = 2.92 \times 10^{-5} \text{ mol/dm}^3$) in pH region from 7.4 to 12.6 and ionic strength of 0.1 mol/dm^3 .

Table 3. pH region of dissociation of hydrazones 1-5.

Comp.	Neutral Form		Dissociated Form		pH Region of Dissociation
	pH	λ_{\max}	pH	λ_{\max}	
1	8.2	312	11.6	322	9.9-11.3
2	7.4	312	11.6	324	10.0-11.5
3	7.8	314	11.3	326	10.0-11.2
4	7.8	312	11.5	326	9.8-10.8
5	7.4	314	10.1	328	9.1-9.7
			12.1	334	11.1-11.9

Noticeable changes were observed in the absorption band that appeared at 310 nm, while there were no significant changes in the position and intensity of the other two bands (195 and 215-225 nm). This behavior was expected and is in accordance with the literature data for compounds with similar structures [26]. For further investigation we followed the changes in the absorption band that appeared at wavelength around 310 nm. From Fig. (1) it can be noticed that there were no changes in this absorption band until pH of 9.9, where its intensity decreased when the basicity of solution increased. At pH value of 10.1 a bathochromic shift of this band was observed probably as a result of the influence of basicity of the solution. The final position of the absorption band was reached at pH value of 11.3 (322 nm). Similar changes were observed in the UV spectra of hydrazones 2-4 and for this reason their UV spectra are not presented here. Different behavior was noticed in the UV spectra of hydrazone 5 in basic media (Fig. 2). The intensity of the absorption band decreased when the basicity of the solution increased (pH = 9.1) and at the same time the bathochromic shift was observed. The absorption maximum reach the position at 328 nm wavelength at a pH value of 9.8 and there were not observed further changes until a pH of 11.1. At higher pH values (pH > 11.3) the intensity of the absorption band again decreased and another bathochromic shift of about 6 nm was noticed. At pH values greater than 12.1 there were no changes in the position and the intensity of this absorption band. The behavior of hydrazone 5 was expected taking into consideration its structure. Namely, this hydrazone contains a phenolic group in *para* position of the benzene ring. Taking into consideration all this, it can be noticed that dissociation process which occurred in basic media caused changes in the UV spectra of investigated hydrazones.

2.3. Absorbance Values vs. pH

The pH values when the investigated hydrazones existed in neutral (undissociated) and dissociated forms, as well as the pH region of dissociation, are given in Table 3.

From the Table 3 it can be seen that the dissociation of hydrazones 1-3 takes place in the pH region from 9.9 to 11.5, while the dissociation process of hydrazone 4 occurred at lower pH values (9.8-10.8). The first dissociation process for hydrazone 5 occurred between 9.1 and 9.7, while the second dissociation process occurred between pH values from 11.1 to 11.9. The changes in the intensity of the absorbance band

that appeared at 310 nm with the basicity of the solution can be better seen from the curves obtained by plotting the absorbance vs. pH of the solution. These curves have a sigmoidal shape (Fig. 3).

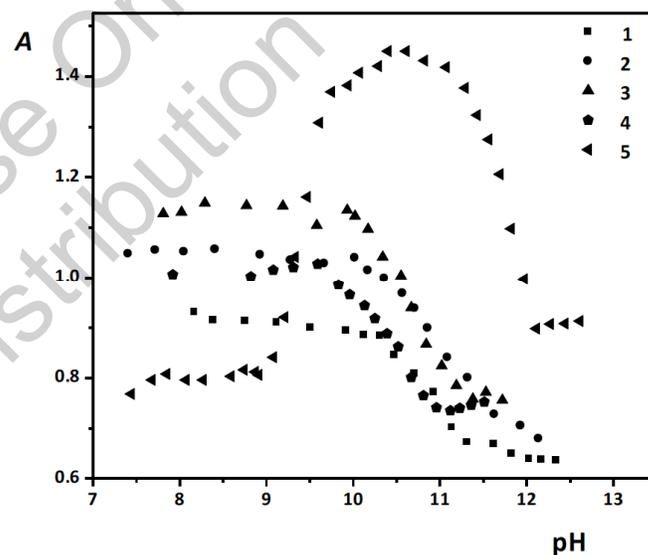


Fig. (3). Dependence of absorbance on pH for the investigated hydrazones 1-4 ($\lambda = 312$ nm) and 5 ($\lambda = 328$ nm), at ionic strength of 0.1 mol/dm^3 .

Sigmoidal curves of hydrazones 1-4 had one step indicating that dissociation process for these hydrazones took place in one step. On the other hand, the presence of two steps on the sigmoidal curve of the hydrazone 5 indicated two dissociation processes (Fig. 3). The first dissociation process was as a result of dissociation of the phenolic group, while the second dissociation process observed for hydrazone 5 was due to dissociation of the amide group present in the hydrazone molecule.

2.4. DPE Determination

As it was mentioned in experimental section the exact place where the molecule losses proton can be predicted using the DPE values. To achieve this goal we made some semiempirical calculations. The obtained data for total energy of neutral ($E_{\text{tot}}(\text{HA})$) and dissociated ($E_{\text{tot}}(\text{A}^-)$) form,

Table 4. $E_{\text{tot}}(\text{HA})$, $E_{\text{tot}}(\text{A}^-)$, $\Delta H_f(\text{A}^-)$, $\Delta H_f(\text{HA})$ and DPE data obtained by AM1 and PM3 semiempirical methods.

AM1					
Hydrazone	$E_{\text{tot}}(\text{HA})$	$E_{\text{tot}}(\text{A}^-)$	$\Delta H_f(\text{HA})$	$\Delta H_f(\text{A}^-)$	DPE
1	-73002.6	-72721.5	21.22	-12.61	333.32
2	-76597.3	-76315.6	13.33	-19.84	333.98
3	-83977.3	-83695.9	-17.46	-51.05	333.56
4	-81306.9	-81028.4	14.27	-22.20	330.68
5	-80396.7	¹ -80115.9	-23.72	-57.87	333.00
		² -79771.5		-28.40	362.47
PM3					
Hydrazone	$E_{\text{tot}}(\text{HA})$	$E_{\text{tot}}(\text{A}^-)$	$\Delta H_f(\text{HA})$	$\Delta H_f(\text{A}^-)$	DPE
1	-66933.5	-66619.8	15.67	-24.25	327.23
2	-70386.1	-70072.2	6.21	-33.43	327.51
3	-77147.4	-76833.4	-22.97	-62.55	327.57
4	-73884.3	-73572.6	9.01	-32.91	325.23
5	-73711.3	¹ -73397.6	-30.06	-69.99	327.22
		² -73017.3		-43.21	354.00

¹dissociation of phenolic group, ²dissociation of amide group ΔH_f , E_{tot} and DPE [kcal/mol]

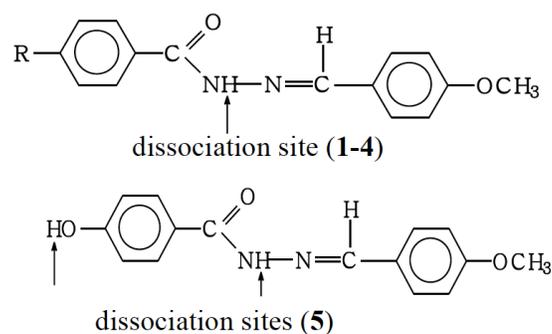
enthalpy of formation for neutral ($\Delta H_f(\text{HA})$) and dissociated ($\Delta H_f(\text{A}^-)$), and DPE are listed in Table 4.

The E_{tot} values of the neutral forms were a little lower compared to those of the dissociated forms suggesting that the acid-base equilibrium between neutral and dissociated forms of hydrazones 1-4 was shifted towards the neutral forms *i.e.* in the solution these forms predominate (Table 4). Furthermore, the E_{tot} values of hydrazone 5 calculated using the AM1 method demonstrated that the neutral form (-80396.7 kcal/mol) was more stable in comparison with the ionic form obtained with dissociation of amide group (-79771.5 kcal/mol) as well as with that obtained by dissociation of both phenolic and amide groups (-80115.9 kcal/mol). The same situation was observed calculating the data obtained with PM3 semi-empirical method.

Comparing the DPE values it can be seen that they were similar for hydrazones 1-3, suggesting that the influence of the substituents in these molecules is insignificant. A lower DPE value was obtained for hydrazone 4 since the dissociation of the amide group in basic media was expected to occur more easily. Hence, this hydrazone is a stronger acid compared to hydrazones 1-3, nonetheless significant differences in the acidity of hydrazones 1-4 did not exist (Table 4).

Hydrazone 5 possesses two dissociable groups in basic media, *i.e.* the amide and phenolic groups. The DPE value of the amide group (362.47 kcal/mol) was higher than DPE value of the phenolic group (333.00 kcal/mol). This result confirmed that the first dissociation step concerned the phenolic group, while the second dissociation step related to amide group. This result was in agreement with the literature data confirming that phenolic group loses a proton easier compared to the amide group [26, 27]. According to the re-

sults obtained by semiempirical calculation, as well as the shape of the sigmoidal curves the dissociation process should be presented as follows in Scheme 1.



Scheme (1). Dissociation process of investigated hydrazones 1-5.

2.5. $pK_{\text{HA}}/pK_{\text{H2A}}$ Determination

As was mentioned before, the dissociation constants are important parameters for assessment of the acidity of weak organic acids and bases. This is important for biological systems because the biological activity of some compounds depends on their ionic form that exists in solution. The determination of pK_{HA} values was made as it was explained in the experimental section. The calculations were made from the absorbance data at three different ionic strengths (0.10, 0.25, and 0.50 mol/dm³). The 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%),

Table 5. Dissociation constant values (numerically, graphically and thermodynamic) and statistical data (SD, RSD, R^2) of benzoylhydrazones 1-5.

	μ	n	Numerically			Graphically	
	[mol/dm ³]		pK_{HA}	SD	RSD	pK_{HA}	R^2
1	0.1	8	10.73±0.04	0.07	0.67	10.66	0.978
	0.25	8	10.86±0.07	0.11	0.98	10.82	0.948
	0.5	8	10.90±0.06	0.09	0.87	10.98	0.951
	[*] $pK_{HA} (R^2)$		10.62 (0.898)			10.41 (0.997)	
2	0.1	8	11.09±0.04	0.06	0.56	11.11	0.986
	0.25	8	11.13±0.04	0.06	0.58	11.14	0.984
	0.5	8	11.14±0.02	0.04	0.36	11.19	0.994
	[*] $pK_{HA} (R^2)$		11.07 (0.853)			11.04 (0.969)	
3	0.1	8	10.82±0.05	0.08	0.77	11.04	0.960
	0.25	8	10.88±0.07	0.11	0.99	11.09	0.931
	0.5	8	10.91±0.04	0.07	0.65	11.24	0.974
	[*] $pK_{HA} (R^2)$		10.76 (0.948)			10.86 (0.962)	
4	0.1	8	10.58±0.02	0.03	0.25	10.49	0.992
	0.25	8	10.63±0.02	0.03	0.31	10.59	0.998
	0.5	8	10.70±0.02	0.02	0.17	10.67	0.996
	[*] $pK_{HA} (R^2)$		10.47 (0.999)			10.35 (0.996)	
			pK_{H_2A}	SD	RSD	pK_{H_2A}	R^2
5	0.1	6	9.57±0.03	0.04	0.43	9.48	0.972
	0.25	6	9.60±0.07	0.08	0.91	9.59	0.885
	0.5	6	9.64±0.03	0.04	0.41	9.67	0.968
	¹ $pK_{H_2A} (R^2)$		9.51 (0.999)			9.35 (0.985)	
			pK_{HA^-}	SD	RSD	pK_{HA^-}	R^2
5	0.1	7	11.59±0.04	0.05	0.47	11.60	0.968
	0.25	7	11.63±0.04	0.05	0.45	11.67	0.970
	0.5	7	11.66±0.02	0.03	0.30	11.76	0.988
	² $pK_{HA^-} (R^2)$		11.54 (0.999)			11.47 (1.00)	

pK_{HA} – thermodynamic dissociation constant, ¹ pK_{H_2A} – first dissociation constant, ² pK_{HA^-} – second dissociation constant, n - number of measurements.

were calculated. The pK_{HA} values (numerically and graphically) at different ionic strengths, thermodynamic pK_{HA} values, and statistical parameters are listed in Table 5.

The calculated results demonstrated that the pK_{HA} values obtained numerically have almost identical values to those estimated graphically (Table 5). The dependence of log I vs. pH was linear with coefficient of determination ≈ 1 , suggesting satisfactory precision in determination of dissociation constants graphically. This implies that the pK_{HA} values can be successfully determined in both ways. The same conclusion can be performed comparing the values of SD and RSD which refers to the numerically and graphically obtained

pK_{HA} values (Table 5). By increasing the ionic strength of the solution, the stoichiometric pK_{HA} values increased, but differences were not significant. Comparing the thermodynamic pK_{HA} values calculated as a result of dissociation of amide group the following order was obtained:

Hydrazone:	4	1	3	2	5
pK_{HA}	10.47	10.62	10.76	11.07	11.54
	the strength of the acid decreased				

From the results presented in Table 5 it can be seen that there were no differences in pK_{HA} values of hydrazones 1-4 which referred to the dissociation of amide group. This result suggested that the influence of substituents (CH_3 , OCH_3 , Cl) was not significant. Hydrazone 4 had the lowest pK_{HA} value which means that this hydrazone is the strongest acid in comparison with other investigated hydrazones due to the presence of chlorine atom in the *para* position of benzene ring [28]. This result was also in agreement with the data obtained with semiempirical methods, *i.e.* the hydrazone 4 had lower DPE value compared to hydrazones 1-3. Higher pK_{HA} values were obtained for hydrazone 2 compared to 1, 3, and 4 probably due to the influence of CH_3 which contributes to decreasing of acidity of this hydrazone through electron donation by the inductive effect. The dissociation process of hydrazone 5 took place in two steps as it was mentioned before. The first step was due to dissociation of phenolic group which is a stronger acid, while the second step was as a result of dissociation of amide group. For this reason the pK_{HA}^- value which referred to the dissociation of amide group in 5 had higher values in comparison with the pK_{HA} values of hydrazones 1-4. These differences were due to the presence of phenolic group in the molecule of hydrazone 5 which influenced the dissociation of the amide group [27].

3. EXPERIMENTAL SECTION

3.1. Materials and Method

The investigated benzoylhydrazones 1-5 were purified by twice recrystallizing from ethanol or diluted ethanol (ethanol/water 1:1 *v/v*). Purity was tested by measuring the melting point, as well as by elemental analysis. The other chemicals (NaOH, $NaClO_4$ 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 1 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 calculation of the dissociation constants, while the UV spectra were obtained with computer program Grams Version 4.10.

3.2. Procedure

A stock solution of the hydrazones 1-5 was prepared by dissolving about 60 mg of the compound in 96% ethanol in a volumetric flask of 250 cm^3 . The volume of 0.75 cm^3 of this solution was transferred into 25 cm^3 volumetric flask, and after adding an appropriate volume of NaOH ($c = 0.5 \text{ mol dm}^{-3}$) and $NaClO_4$ ($c = 1 \text{ mol dm}^{-3}$) the flask was diluted up to the mark with deionized water. The degree of dilution of the stock solutions was chosen to obtain concentration of the hydrazones 1-5 in the test solution of about $3 \times 10^{-5} \text{ mol dm}^{-3}$ *i.e.* the absorbances 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 (0.10, 0.25 and 0.50 mol/dm^3) using the solution of $NaClO_4$. 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 tested one, was used as a blank. The stock solutions were stable a long period of time under ordinary conditions, while the stability of the working solution was satisfactory for only 24 h of time.

3.3. Quantum Chemical Investigation

Semiempirical methods were used for calculation of the different parameters for the prediction of some properties and the reactions of the organic compounds which took place in defined media [29]. In this work, the optimization of the geometry of investigated hydrazone molecules 1-5 was performed applying the AM1 (Austin Model 1) and PM3 (Parametric Method 3) semiempirical methods [30, 31]. Theoretical calculations were carried out at the restricted Hartree-Fock level (RHF) in the HYPERCHEM program. All the structures were optimized to a gradient norm of < 0.1 . The exact place where the molecule can lose a proton can be predicted knowing the DPE values. This prediction is especially important when dissociation process take place in more than one step. By knowing the DPE values, also the order of losing protons in the molecule can be predicted. DPE values were calculated according to the equation (1).

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

where DPE is enthalpy of deprotonation, $\Delta H_f(A^-)$ is enthalpy of formation of dissociated form, $\Delta H_f(HA)$ is enthalpy of formation of neutral form, and $\Delta H_f(H^+)$ is 367.15 kcal/mol [30].

3.4. pK_{HA} Calculations

Determination of dissociation constants was accomplished in four steps. The first step was the choice of the wavelengths of which the absorbance values were going to be used for further calculations. The calculation can be performed from the absorbance value at one wavelength which corresponds to the absorption maximum in the UV spectra. In order to obtain more precise results we used the absorbance values at four wavelengths around the absorption maximum. The selected wavelengths used for pK_{HA}/pK_{H2A} determination were as follows: 1 (302, 308, 314, and 316 nm), 2-4 (302, 308, 316, and 322 nm), and 5 (326, 328, 330, and 332 nm). The second step was the determination of molar absorption coefficients. For this purpose, the UV spectra were recorded at three different concentrations. The measurements were performed from the absorbance data measured when the hydrazones 1-5 existed in neutral and dissociated forms. These data were further used in the third step for determination of the concentrations of neutral and dissociated forms of the hydrazones. These calculations were made through the use of four equations (four absorbance values) with two unknown parameters (concentration of neutral and dissociated forms) in accordance with Beer's law. The concentrations of neutral and dissociated forms were applied for the calculation of the ionization ratio I , $I = (c(HA)/c(A^-))$. Finally, in the fourth step the dissociation constants were calculated according to equation (2).

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

where pK_{HA} is the dissociation constant, I is the ionization ratio, and n is the number of transferred protons.

In the literature these constants are referred to as stoichiometric dissociation constants. Their values were further used for determination of thermodynamic dissociation constants which are independent of the ionic strength of the solution. The thermodynamic dissociation constants were evaluated by extrapolation of the curve which represents the dependence $pK_{HA} = f(\sqrt{\mu})$ to zero ionic strength. Additionally, the dissociation constants were estimated graphically. When the concentrations of neutral and dissociated forms are equal ($c(HA) = c(A^-)$), the ionization ratio is zero ($\log I = 0$) and the dissociation constant values were obtained as an intercept of linear dependence on $\log I$ vs. pH [32].

CONCLUSION

The spectroscopic behavior of five benzoylhydrazones **1-5** (Table 1) was investigated in sodium hydroxide media by UV spectroscopy. The changes in the UV spectra of hydrazones were a result of the reaction of dissociation. The pH region of dissociation of investigated hydrazones can be seen from the sigmoidal curves (Fig. 3). The semiempirical data (AM1 and PM3) showed that the first deprotonation site was due to dissociation of phenolic group, while the second one was a result of dissociation of the amide group. The stoichiometric pK_{HA} values (numerically and graphically) determined at three different ionic strengths (0.10, 0.25, and 0.50 mol/dm³) were used to estimate the thermodynamic dissociation constants. The obtained pK_{HA} values suggested that the influence of the substituents (CH₃, OCH₃, Cl) present in hydrazone molecules **2-4** was not significant, except for hydrazone **5** (OH).

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

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

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