COMPARISON AND QSAR STUDY OF THERMODYNAMIC PK_{BH}^+ VALUES OF BENZOYLHYDRAZONES DETERMINED BY UV-VIS SPECTROSCOPY

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT:
Thermodynamic dissociation constants of fifteen protonated aromatic hydrazones were determined by UV-Vis spectroscopic method, as one of the main methods for determination of pK_{BH}^+ values of a weak acids and bases. The stoichiometric pK_{BH}^+ values of investigated compounds were determined at three different ionic strengths (0.1, 0.25 and 0.5 mol/dm^3) in acidic media ($1 < pH < 7$). The thermodynamic pK_{BH}^+ values were evaluated graphically as an intercept with extrapolation to zero ionic strength of the curve which represents dependence of ionization constants on square root of ionic strength. The obtained results showed that there are no significant differences in the values of thermodynamic dissociation constants of investigated hydrazones with no substituent and metoxy group (–OCH_3) on hydrazide part of the molecule. The differences in the pK_{BH}^+ values were noticed for hydrazones with nitro group (–NO_2) on the benzene ring of benzaldehyde. Nitro hydrazones have lower pK_{BH}^+ values, probably due to the influence of the –NO_2 group present in their molecule. The obtained pK_{BH}^+ values were in accordance with those for similar class of compounds. QSAR study has been made by correlation thermodynamic ionization constants with structure using various physicochemical parameters.

KEYWORDS: UV-Vis spectroscopy, benzoylhydrazones, protonation, thermodynamic dissociation constant, Characteristic Vector Analysis, QSAR, descriptors

INTRODUCTION

Hydrazones are organic compounds with an azomethine group (–NHN=CH–) in their structure. Due to the presence of this group hydrazones and their derivatives possess variety of different biological activities [1] - [5]. The introduction of different functional groups in the hydrazone molecules expands the scope of their application in organic synthesis. Because of that, there is a growing interest in the structural studies and synthesis of hydrazones as they show a broad spectrum of applications in different fields [6]. Various derivatives of hydrazones possess insecticidal activities and they are used as active ingredients for controlling agricultural pests [7]. Many chemical, physical and biological properties of organic compounds depend on the interactions of acidic and basic groups present in their molecule. As hydrazones and their derivatives have acidic and basic functional groups, their biological activity depends on the ionic form in which they exist in solution. The degree of ionization of molecules in solution at different pH values can be predicted knowing the value of the ionization constants (pK_{BH}^+). The pK_{BH}^+ is a physicochemical parameter which is important to know for developing new pharmaceutical drugs or improving the available ones [8]. Ionization constants have significant importance in optimization of analytical procedures such as acid – base titration, solvent extraction and complex formation. Taking into consideration the importance of hydrazones it is of a great interest to determine their pK_{BH}^+ values. For that purpose, various analytical methods can be used. Among them, the UV-Vis spectroscopy is the most common method [9-11]. This technique has some advantages such as: simplicity, availability, low cost, possibility of analyzing compounds with low solubility, accuracy, reproducibility and so on [12]. The most important thing is that spectroscopy is a highly sensitive and suitable method for determination of pK_{BH}^+ values in very dilute aqueous solutions with low compound concentration. Usage of UV-Vis spectrometry for pK_{BH}^+ determination requires presence of a chromophore close to the ionization site of the compound. In that case, the spectra of the dissociated and the ionized forms of the molecule are different [13]. The organic solvent usually has influence on the appearance on the UV-Vis spectra as well as on pK_{BH^+} values. The influence of the solvent can be eliminated by employing method of Characteristic Vector Analysis (CVA), which has been tested and proven to be applicable in the analysis of spectroscopic data [14]. The purpose of this work was examination of the protonation process of fifteen different aromatic hydrazones in perchloric acid media ($1 < pH < 7$) by UV-Vis spectrophotometry. Furthermore, the aim was calculation of the stoichiometric and thermodynamic ionization constants from the absorbance data of the experimental and reconstructed spectra. In order to see how significant statistical differences are between pK_{BH^+} values determined numerically and graphically, and between pK_{BH^+} values calculated from absorbance data obtained from experimental and reconstructed spectra, t-test and Analyses of Variance (ANOVA) test will be performed.

The second part of this work was to investigate QSAR analysis of thermodynamic ionization constants using different physicochemical (electronic, steric and hydrophobic) parameters. It is also known that the ionization constants depend upon the polarity of molecule and directly or indirectly on the intra and intermolecular forces [15,16]. The parameters accounted for the intra and intermolecular forces and steric effect definitely plays a significant role in the modeling of dissociation constants. Physicochemical parameters taken into consideration in this QSAR study are Hammett substituents parameter (σ), hydrophobic parameters (log P and π), field effect as electronic influences (F), Verloop’s STERIMOL parameter L and molar refractivity (MR) as steric parameter [17,18].

EXPERIMENTAL

MATERIALS AND METHODS

The investigated hydrazones were twice purified by recrystallization from 96 % ethanol. The perchloric acid, sodium perchlorate and ethanol were of analytical grade p.a. (Merck). The ionic strength of solutions (0.1, 0.25 and 0.5 mol/dm^3) was adjusted using sodium perchlorate. 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. The UV spectra were recorded immediately after preparation of the test solutions, at room temperature.

INSTRUMENTATION

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 from 190 nm to 400 nm. A digital pH meter with glass electrode was used for measurements of the pH values (pH range from 1 to 7).

STOCK AND TEST SOLUTIONS

The stock solutions were prepared in 96 % ethanol with concentration of investigated hydrazones around $1\cdot10^{-3}$ mol/dm^3. The test solutions were prepared with concentration of investigated hydrazones around $3\cdot10^{-5}$ mol/dm^3. The test solutions for nitrohydrazones (H_{11}-H_{15}) were prepared in redistilled water and ethanol with volume ratio 1:1 (*V*/*V*) because the solubility of investigated hydrazones in aqueous solutions is low. The stability of these solutions was satisfactory for only 24 hours of time.

QSAR ANALYSIS AND SELECTED DESCRIPTORS

The MVA (multi variable analysis) approach in QSAR analysis has been most widely and effectively used for theoretical drug design due to various physicochemical (electronic, steric and hydrophobic) parameters. The assumption can be formulated as:

$$pK_{BH^+} = A_1x + A_2y + A_3z + B$$

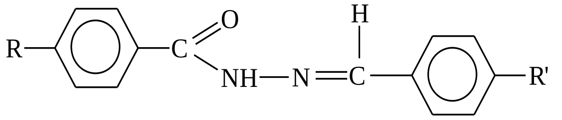
where x, y and z are molecular properties (descriptors). From the values of linear slopes A_1, A_2, A_3 we can see the correlation of the particular molecular properties with the activity of the investigated compounds.

Several descriptors with different characteristics were selected for QSAR analysis: classical Hammett electronic parameter of substituents (σ) [19], hydrophobic parameter (π) [19], molar refractivity (MR) [20], field effect (F) as electronic influences [19], Verloop’s STERIMOL parameter for the steric interactions of the substituents (L) [19]; L is defined as the length of a substituent along the axis of its substitution to the parent skeleton. Hammett electronic parameter of substituents were calculated as sum from individual substituents values in both aromatic rings. For each hydrazone the partition coefficient (log P) has been calculated [21].

RESULTS AND DISCUSSION

STRUCTURE OF INVESTIGATED HYDRAZONES

The subject of this study was fifteen benzoylhydrazones with different substituents on the benzene ring of benzaldehyde [22]. All investigated hydrazones have following substituents on the benzene ring on the hydrazide part of the molecule: –H, $–CH_3$, $–OCH_3$, –Cl, –OH. Five of them (H_1 – H_5) have no substituent on the benzene ring of benzaldehyde, five (H_6 – H_{10}) have metoxy group ($–OCH_3$), and five (H_{11} – H_{15}) have nitro group ($–NO_2$) on the benzaldehyde group of the molecule. The structural formula of investigated hydrazones is presented in Scheme 1.



H_1-H_5: R = –H, $–CH_3$, $–OCH_3$, –Cl, –OH; R' = –H
H_6-H_{10}: R = –H, $–CH_3$, $–OCH_3$, –Cl, –OH; R' = $–OCH_3$
H_{11}-H_{15}: R = –H, $–CH_3$, $–OCH_3$, –Cl, –OH; R' = $–NO_2$

Scheme 1. Structural formula of investigated hydrazones

UV-VIS *SPECTRA*

The UV-Vis spectra of investigated hydrazones were recorded at three different ionic strengths (0.1; 0.25 and 0.5 mol/dm^3). The experimental and reconstructed spectra of hydrazone H_{13} are given in the Figs 1 and 2, respectively.

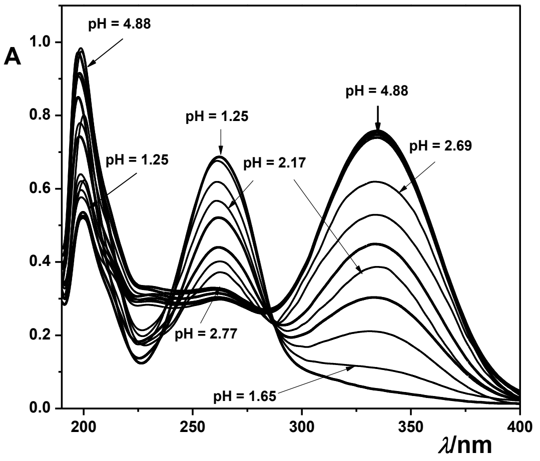


Fig. 1. Experimental UV-Vis spectra of H_{13}
(c = 3.00□10^{-5} mol/dm^3) in pH region between 1.25 and 4.88
(μ = 0.1 mol/dm^3)

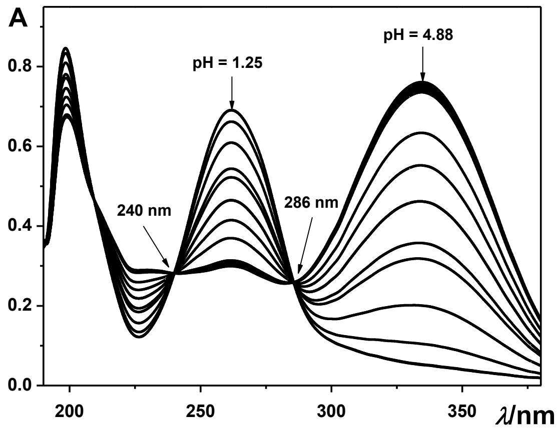


Fig. 2. Reconstructed UV-Vis spectra of H_{13}
c = 3.00·10^{-5} mol/dm^3), μ = 0.1 mol/dm^3

From the Fig. 1 it can be seen that in the weak acid media in the UV-Vis spectra of hydrazone H_{13} there are two absorption bands. The second one with a maximum at 330 nm which was due to $n \rightarrow \pi^*$ electron transitions were interesting for our further investigations [23]. In the reconstructed spectra well defined isosbestic points at 240 and 285 nm can be observed. The existence of isosbestic points confirmed that in the solution are present both neutral and protonated form of investigated hydrazones.

IONIZATION CONSTANTS

One of the main methods for determining the pK_{BH^+} values of a compound is UV-Vis spectroscopy. The stoichiometric pK_{BH^+} values were calculated by absorbance data using experimental and reconstructed spectra using the Henderson – Hasselbach equation.

$pK_{BH^+} = n \cdot pH + \log I$ .... (1)

where pK_{BH^+} is the dissociation constant of the protonated form, I is the ionization ratio, and n is number of transferred protons

The ionization ratio I ($I = c(BH^+)/c(B)$) is actually the ratio between the concentration of the protonated (BH^+) and neutral (B) form of the hydrazones. The calculations of ionization ratio were performed using the molar absorption coefficient values and the absorbance data obtained at four selected wavelengths. In accordance with Beer’s law an over determined system of four equations (absorbance data) with two unknown parameters (concentration of neutral and protonated form) was obtained. Absorbance value which corresponds to the absorption maximum was used in calculations.

Furthermore, the p$K_{BH}{}^{+}$ values were determined graphically as an intercept of the dependence of log$I$ on pH [24]. The values of stoichiometric constants were further used for determination of the thermodynamic p$K_{BH}{}^{+}$ values (Tables 1 and 2) as an intercept with extrapolation of the curve p$K_{BH}{}^{+} = f(\sqrt{\mu})$, to zero ionic strength (See Fig. 3).

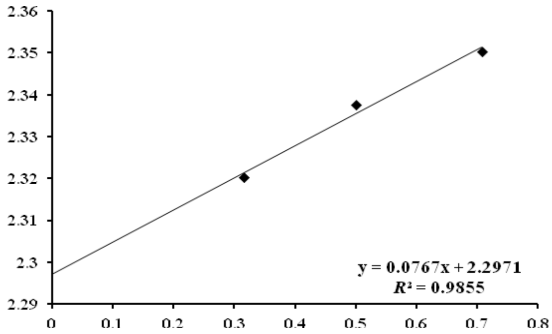


Fig. 3. The dependence of pK_{BH^+} on $\sqrt{\mu}$ for H_{11}
($c = 3.00\cdot10^{-5}$ mol/dm^3)

The p$K_{BH}{}^{+}$ values calculated from experimental spectra for hydrazones H_1 – H_6 were similar to the p$K_{BH}{}^{+}$ values calculated from reconstructed spectra. It means that the influence of the solvent on the appearance of the UV-Vis spectra is insignificant. The test solutions of hydrazones H_6 – H_{10} were prepared at the same manner as those for hydrazones H_1 – H_6, because of that it was expected that the solvent will not influence i.e. the experimental spectra of these hydrazones were not reconstructed. The volume ratio in the test solution of hydrazones H_{11} – H_{15} was 50 %, hence to investigate the influence of the solvent their experimental spectra were reconstructed.

The experimental spectra of hydrazones H_6 – H_{10} were not reconstructed because there were no significant differences between experimental and reconstructed spectra for hydrazones H_1 – H_5. Test solutions of hydrazones H_1 – H_5 and H_6 – H_{10} were prepared in the same solution.

Table 1. Thermodynamic ionization constants (pK_{BH^+}) and determination coefficient (R^2), experimental spectra

Hydrazone	numerically		graphically	
	p$K_{BH}{}^{+}$	R^2	p$K_{BH}{}^{+}$	R^2
H_1	3.44	0.993	3.42	0.991
H_2	3.12	0.996	3.10	0.994
H_3	3.25	0.997	3.23	0.989
H_4	2.56	0.989	2.58	0.999
H_5	2.74	0.990	2.77	0.988
H_6	3.26	0.996	3.26	0.963
H_7	3.37	0.991	3.41	0.998
H_8	3.46	0.99	3.48	0986
H_9	3.21	0.976	3.24	0.998
H_{10}	3.10	0.999	3.08	0.961
H_{11}	2.29	0.985	2.32	0.999
H_{12}	2.55	0.974	2.55	0.989
H_{13}	2.42	0.937	2.39	0.926
H_{14}	2.05	0.999	2.08	0.999
H_{15}	2.22	0.969	2.22	0.996

Table 2. Thermodynamic ionization constants (pK_{BH^+}) and determination coefficient (R^2), reconstructed spectra

Hydrazone	numerically		graphically	
	$pK_{BH}{}^{+}$	R^2	$pK_{BH}{}^{+}$	R^2
H_1	3.29	0.998	3.28	0.999
H_2	3.13	0.991	3.11	0.996
H_3	3.21	0.993	3.19	0.996
H_4	2.58	0.998	2.56	0.995
H_5	2.73	0.993	2.78	0.988
H_{11}	2.23	0.944	2.15	0.941
H_{12}	2.29	0.998	2.35	0.963
H_{13}	2.31	0.995	2.27	0.969
H_{14}	2.24	0.995	2.17	0.838
H_{15}	2.25	0.997	2.14	0.991

STATISTICAL DATA

In order to see how significant statistical differences are between $pK_{BH}{}^{+}$ values determined numerically and graphically, and between $pK_{BH}{}^{+}$ values calculated from absorbance data obtained from experimental and reconstructed spectra, t-test was performed with level of significance 0.05 (5 %). The obtained results showed that t critical two-tail was smaller than t statistical i.e. p critical was greater than 0.05 in following comparisons: $pK_{BH}{}^{+}$ values determined numerically from experimental spectra, $pK_{BH}{}^{+}$ values determined graphically from experimental spectra and $pK_{BH}{}^{+}$ values determined numerically from reconstructed spectra. These results suggested that here were no statistical differences between mentioned $pK_{BH}{}^{+}$ values. Insignificant statistical difference was observed between $pK_{BH}{}^{+}$ values determined graphically from reconstructed spectra. In this case p critical was 0.049, while t critical was 2.26 and t statistical 2.27. These numbers suggested that it is not about significant statistical difference.

On the other hand, for comparison of $pK_{BH}{}^{+}$ values calculated for different groups of hydrazones analyses of variance (ANOVA) test was performed. The obtained results showed that there is statistical difference between hydrazones $H_1 – H_5$, $H_6 – H_{10}$ and $H_{11} – H_{15}$. These differences are probably due to the substituents present in their structure.

CORRELATION ANALYSIS OF THE THERMODYNAMIC IONIZATION CONSTANTS WITH SELECTED DESCRIPTORS

Correlation matrix

It was important for further analysis to find correlation matrix for used descriptors. The data presented in Table 3 indicated that there isn’t the high collinearity between selected descriptors (R < 0.8), indicating that these parameters could be combined to get statistically reliably QSAR models.

Table 3. Correlation matrix between selected descriptors

	σ	π	MR	F	R	log P
σ	1.00					
π	0.10	1.00				
MR	0.14	0.17	1.00			
F	0.61	-0.26	0.64	1.00		
R	0.77	0.32	-0.36	-0.03	1.00	
log P	0.37	0.90	0.07	-0.09	0.53	1.00

ii) Mono-parametric QSAR models

The relatively good mono-parametric model was obtained only for Hammett constants indicating the importance of this descriptor in $pK_{BH}{}^{+}$ values predicting. Obtained mono-parametric QSAR models are presented in Table 4, together with statistical parameters: R^2, R^2adj., SD and F-test.

Table 4. Mono-parametric QSAR models with statistical parameters: R^2, R^2adj., SD and F-test

	$pK_{\mathrm{BH}}{}^{+}{}_{\mathbf{num}}$	$pK_{\mathrm{BH}}{}^{+}{}_{\mathbf{graph}}$
Model	$pK_{\mathrm{BH}}{}^{+}{}_{\mathrm{num}} = 2.9129 - 0.8075*\sigma$	$pK_{\mathrm{BH}}{}^{+}{}_{\mathrm{graph}} = 2.9187 - 0.8023*\sigma$
R^2	0.7206	0.7205
R^2adj.	0.6991	0.6990
SD	0.0709	0.0700
Ftest	33.5207	33.5126
	$pK_{\mathrm{BH}}{}^{+}{}_{\mathbf{num\text{-}recon}}$	$pK_{\mathrm{BH}}{}^{+}{}_{\mathbf{graph\text{-}recon}}$
Model	$pK_{\mathrm{BH}}{}^{+}{}_{\mathrm{num\text{-}recon}} = 2.8362 - 0.7674*\sigma$	$pK_{\mathrm{BH}}{}^{+}{}_{\mathrm{graph\text{-}recon}} = 2.8249 - 0.8207*\sigma$
R^2	0.6787	0.7076
R^2adj.	0.6386	0.6710
SD	0.0686	0.0685
Ftest	16.9008	19.3560

Relatively same statistical values were obtained in all cases: $R^2 = 0.67 \div 0.73$; R^2adj. $= 0.63 \div 0.7$; SD $= 0.06 \div 0.07$ and F-test = 16.0 06 ÷ 33.6. In all mono-parametric correlation, σ value is negative is negatively correlated with $pK_{\mathrm{BH}}{}^{+}{}_{\mathrm{num}}$, $pK_{\mathrm{BH}}{}^{+}{}_{\mathrm{graph}}$, $pK_{\mathrm{BH}}{}^{+}{}_{\mathrm{num\text{-}recon}}$ and $pK_{\mathrm{BH}}{}^{+}{}_{\mathrm{graph\text{-}recon}}$.

The correlations with low R^2 values (< 0.20) are not considered being statistically significant, and data shows that rest of the chosen descriptors are not linearly correlated with the activity, and they individually can’t be used for modeling the $pK_{\mathrm{BH}}{}^{+}$ values.

iii) Bi-parametric QSAR models

Since the mono-parametric regression resulted into relatively statistically significant models using the selected parameter (σ), an attempt for constructing of bi-parametric models were made.

Following statistically significant bi-parametric correlation were obtained:

$pK_{\mathrm{BH}}{}^{+}{}_{\mathrm{num}} = 2.9099 - 0.8351*\sigma + 0.2821*\pi$
$R^2 = 0.8124$
R^2adj. = 0.7811
SD = 0.0516
F-test = 25.9815

$pK_{\mathrm{BH}}{}^{+}{}_{\mathrm{graph}} = 2.9155 - 0.8311*\sigma + 0.2949*\pi$
$R^2 = 0.8222$
$R^2_{\mathrm{adj.}} = 0.7925$
SD = 0.0483
F-test = 27.7420

$pK_{\mathrm{BH}}{}^{+}{}_{\mathrm{num\text{-}recon}} = 2.8505 -0.7970*\sigma + 0.2525*\pi$
$R^2 = 0.7746$
$R^2_{\mathrm{adj.}} = 0.7102$
SD = 0.0550
F-test = 12.0261

$pK_{\mathrm{BH}}{}^{+}{}_{\mathrm{graph\text{-}recon}} = 2.8407 - 0.8537*\sigma + 0.2810*\pi$
$R^2 = 0.8157$
$R^2_{\mathrm{adj.}} = 0.7631$
SD = 0.0494
F-test = 15.4954

Initial regression analysis indicated that out of the six descriptors used, only σ and π are important descriptors in predicting of $pK_{\mathrm{BH}}{}^{+}$ values. The positive coefficients of π in all bi-parametric QSAR models, indicates that the $pK_{\mathrm{BH}}{}^{+}$ values increases as the magnitude of this descriptors increases. The correlation coefficients were found to be good (0.837 ÷ 0.77) in all cases and the standard deviations were below 0.055. According the all statistical parameters, correlation between $pK_{\mathrm{BH}}{}^{+}{}_{\mathrm{num}}$ and σ and πis the best QSAR model.

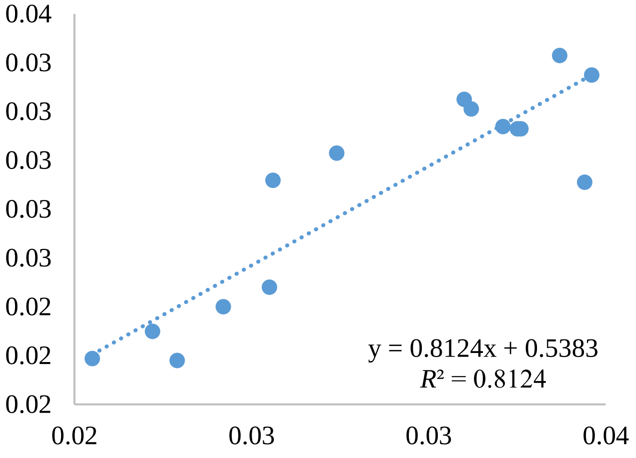


Fig. 1. Plot of experimental vs. calculated pK_{BH^+}numerical values

Finally, in order to confirm our findings, $pK_{\mathrm{BH}}{}^{+}{}_{\mathrm{num}}$ predicted were compared with the corresponding $pK_{\mathrm{BH}}{}^{+}{}_{\mathrm{num}}$ values reported in Table 1. A plot obtained between the experimental and calculated $pK_{\mathrm{BH}}{}^{+}{}_{\mathrm{num}}$

values is presented in Figure 1. Predictive correlation coefficient (*R*pre), was calculated by correlating the estimated pK_{BH^+num} values with the experimental once. The obtained predictive correlation coefficient (*R*pre = 0.8124) confirms our findings.

CONCLUSION

Ionization constants can be determined by UV-Vis spectroscopy using the absorbance data of experimental and reconstructed spectra. Reconstruction of the spectra by CVA method is useful in order to eliminate the influence of the solvent. Stoichiometric pK_{BH^+} values determined numerically and graphically at different ionic strengths were further used for evaluation of thermodynamic constants of investigated hydrazones. The obtained results showed that there are no significant statistical differences in the values of thermodynamic pK_{BH^+} values of investigated hydrazones determined from the absorbance data of experimental and reconstructed spectra, as well as for those determined numerically and graphically. The statistical differences in the pK_{BH^+} values were noticed for the hydrazones from different group $H_1 – H_5$, $H_6 – H_{10}$ and $H_{11} – H_{15}$ probably as a result of the influence of the substituents. The obtained pK_{BH^+} values were in accordance with those for similar class of compounds.

QSAR study has been made by correlation previously calculated thermodynamic ionization constants with various physicochemical parameters such: Hammett electronic parameter of substituents (σ), hydrophobic parameter (π), molar refractivity (MR), field effect (F), Verloop’s STERIMOL parameter (L) and partition coefficient (log P). Only mono-parametric regression with σ resulted into relatively statistically significant model. Bi-parametric correlation between pK_{BH^+num} and descriptors σ and π is the best statistically QSAR model, according the selected statistical parameters. Predictive correlation coefficient (*R*pre = 0.8), calculated by correlating the estimated pK_{BH^+num} values with the experimental once, confirm our conclusion for statistically best model.

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