

Spectrophotometric and electrochemical study of protolytic equilibria of some oximes-acetylcholinesterase reactivators

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Abstract

Newly synthesized oximes, mono and bis imidazole derivatives, which promise to be more effective acetylcholinesterase reactivators than standard antidotes used, were investigated by spectrophotometric and electrochemical methods. The electrochemical investigations confirmed the existence of overlapping equilibria, obtained by spectrophotometric methods. Dissociation constants of those oximes were also obtained by numerical treatment of overlapping equilibria, using the Lavendberg–Marquardt least square method, and when compared with the same for some similar compounds, were found to be very effective acetylcholinesterase reactivators. The distribution of ionic forms of the investigated oximes, as a dependence of pH values, was calculated from the obtained values of dissociation constants. The results indicated that many oxime anions will be available at physiological pH 7.4 and a relative increased ability to reactivate inhibited acetylcholinesterase could be expected. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Some mono and bis pyridinium oximes are known to be acetylcholinesterase reactivators and effective antidotes in intoxication with or-

ganophosphorous compounds. The most important standard oximes from this group are: Obidoxime chloride (Toxogonin), Pralidoxime chloride (PAM-2), Trimedoxime dibromide (TMB-4) and 1-{{4'-aminocarbonyl-1'-pyridinio}-metoxymethyl} - 2 - (hydroxyiminomethyl)pyridinium dichloride (HI-6) [1–3]. The first three are inefficient in the treatment of soman poisoning.

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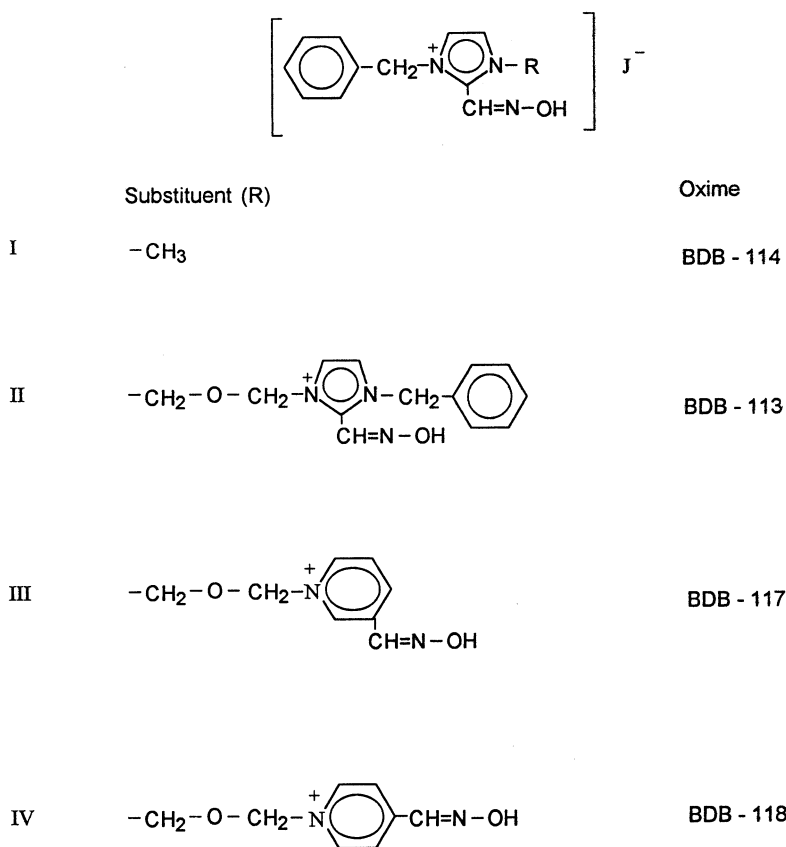
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The last one, HI-6, is efficient in soman poisoning, but it is inefficient in the therapy of tabun poisoning [4]. In attempts to find an universal antidote which would be effective against all four nerve agents (sarin, soman, tabun and VX), some mono and bis imidazole derivatives (Scheme 1) have been recently synthesized [5].

These compounds have a benzyl substituent in position three of the imidazole ring. In bis imidazole oximes one benzyl imidazole radical is substituted by 2- or 4-hydroxyiminomethylpyridine (Scheme 1). Four of the compounds investigated in this paper, 2-hydroxyiminomethyl-1-benzyl-3-methylimidazolium iodide (BDB-114), 2-hydroxyiminomethyl-3-benzyl-1-[3-(2-hydroxyiminomethyl

-3-benzyl-1-imidazolio)-2-oxapropyl]-imidazolium diiodide (BDB-113), 2-hydroxyiminomethyl-1-[3-(2-hydroxyiminomethyl-3-benzyl-1-imidazolio)-2-oxapropyl]pyridinium diiodide (BDB-117) and 4-hydroxyiminomethyl-1-[3-(2-hydroxyiminomethyl-3-benzyl-1-imidazolio)-2-oxapropyl]pyridinium diiodide (BDB-118) were effective in the reactivation of acetylcholinesterase, inhibited by soman and tabun [5] Scheme 1.

Recently, a rapid and sensitive kinetic method for the determination of these promising oximes in blood and urine, based on their action on the reaction of gold (III) with potassium iodide, was developed [6–8]. The catalytic influence of the investigated oximes on the reaction rate was most



Scheme 1. Structure of newly synthesized oximes, derivatives of benzylimidazole. (I) 2-hydroxyiminomethyl-1-benzyl-3-methylimidazolium iodide; (II) 2-hydroxyiminomethyl-3-benzyl-1-[3-(2-hydroxyiminomethyl-3-benzyl-1-imidazolio)-2-oxapropyl]-imidazolium diiodide; (III) 2-hydroxyiminomethyl-1-[3-(2-hydroxyiminomethyl-3-benzyl-1-imidazolio)-2-oxapropyl]pyridinium diiodide; (IV) 4-hydroxyiminomethyl-1-[3-(2-hydroxyiminomethyl-3-benzyl-1-imidazolio)-2-oxapropyl]pyridinium diiodide.

evident in the acidity range from pH 7–8, which is just pH range for the most potent acetylcholinesterase reactivators earlier used.

In view of the permanent potential danger of biological and chemical war and intoxication with nerve gas poisons, the study of the mentioned oximes is of interest for the pharmaceutical industry and praxis.

As previously mentioned, newly synthesized oximes are potentially more effective antidotes than some earlier used standard antidotes [1–3].

The aim of this work was to determine the dissociation constants of oxime groups in those newly synthesized oximes using spectrophotometric methods and to make a correlation with the literature data, means corresponding constants for some similar compounds determined potentiometrically. Furthermore, the scope was to find the optimal pH range for ionic forms and to compare the results of spectrophotometric measurements with the results of the electrochemical investigations in order to confirm them.

2. Experimental

2.1. Reagents

All of the solutions were prepared from analytical grade reagents and bidistilled water. Oximes BDB-114 (I), BDB-113 (II), BDB-117 (III) and BDB-118 (IV), supplied from the laboratory for Organic Chemistry and Biochemistry (Faculty of Science, Zagreb, Croatia), were used without further purification. Substrate stock solutions with concentrations of 9.4×10^{-4} M (I); 1.08×10^{-3} M (II); 1.17×10^{-3} M (III) and 4.35×10^{-4} M (IV), were prepared in bidistilled water. The other chemicals were of reagent grade quality. The acidity of the solutions in the pH range 2–12 was adjusted by the addition of Britton–Robinson buffer.

2.2. Spectrophotometric and pH measurements

Absorption spectra were recorded, against the appropriate buffer solutions, immediately after preparing the solution, on a Beckman UV VIS

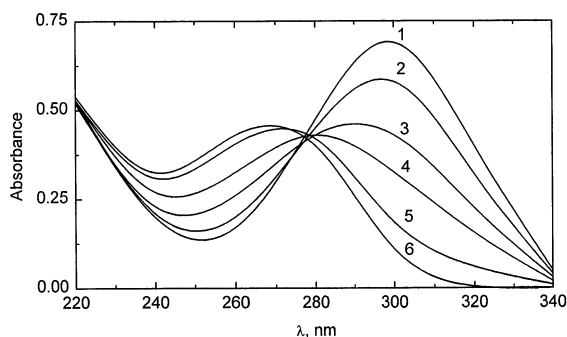


Fig. 1. Absorption spectra of BDB-113 solution (1.72×10^{-5} M) at different acidities: (1) pH 10.99; (2) pH 8.66; (3) pH 7.95; (4) pH 7.51; (5) pH 6.71; and (6) pH 5.40

2560 spectrophotometer, in a 1 cm matched, quartz cuvette with the cell holder thermostated at 25°C. The ionic strength was kept constant ($I = 0.1$ M) with the addition of KCl. To check the possible existence of the non-protolytic processes, the absorption spectra were recorded again after 1 h standing and no changes were found.

2.3. Electrochemical measurements

Electrochemical experiments were performed in a standard three-electrode cell with gold as a working electrode, saturated calomel (SCE) as a reference and platinum as a counter electrode. Cyclic voltammograms (CV) were performed at a sweep rate of 50 mV s^{-1} whereas the potential ranged from -0.8 to $+0.5$ V. The electronic equipment consisted of a PAR potentiostat/galvanostat (model 273) and a Hewlett Packard X-Y recorder (model pH 8033). The experiments were carried out at room temperature (20 ± 2)°C. Before each experiment the system was deoxygenated, for at least 5 min, by nitrogen gas bubbling.

3. Results and discussion

3.1. Spectrophotometric investigation

The absorption spectra of the oximes (I–IV, Scheme 1) were recorded in water solutions of the various acidity from pH 2 to 12. The concentra-

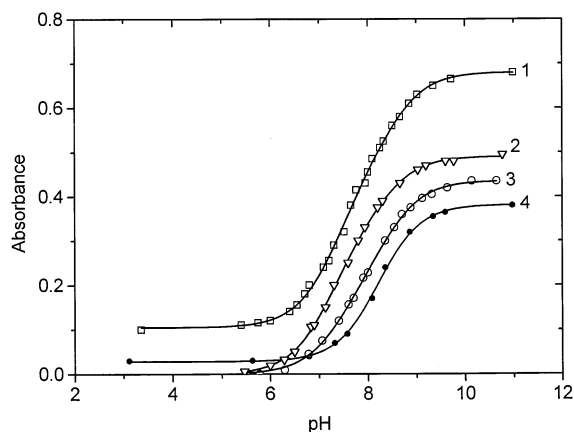


Fig. 2. Absorbance versus acidity curves for: (1) BDB-113 ($c = 1.72 \times 10^{-5}$ M), $\lambda = 300$ nm; (2) BDB-117 ($c = 2.58 \times 10^{-5}$ M), $\lambda = 360$ nm; (3) BDB-118 ($c = 1.57 \times 10^{-5}$ M), $\lambda = 330$ nm; (4) BDB-114 ($c = 1.50 \times 10^{-5}$ M), $\lambda = 300$ nm.

tions of the compounds in the measured solution were from 5.22×10^{-6} to 1.72×10^{-5} M. As an example, the absorption spectra of BDB-113, at different pH values, are shown in Fig. 1.

All the investigated compounds have, in acidic solutions ($\text{pH} < 5.5$), similar absorption spectra with the absorption maxima in the range from 260 to 280 nm. With the change of acidity, from pH 5 to 10, the absorption maxima are shifted to the range from 290 to 360 nm and molar absorptivities increase. These spectral changes are followed by the appearance of the poor isosbestic points and are similar for all dioximes. The changes in the absorption spectra with the change of pH indicate that the dissociation of two oxime

groups occurs in the investigated range of acidity. Two overlapping dissociation equilibria could produce the poor isosbestic point as shown in Fig. 1. The change of the absorption spectra of BDB-114 which is monooxime, with the acidity showed a clear isosbestic point.

The absorbance versus acidity curves were produced for the investigated acidity range (Fig. 2). Table 1 summarizes the pH intervals where no change of the absorbance takes place (optimal pH for particular molar species) together with the wavelength and molar absorptivity of the characteristic absorption band for nonprotonated and diprotonated molecular species.

3.2. Electrochemical investigations

The investigation of electrochemical behaviour of BDB-113 and BDB-114 solution was carried out in Britton–Robinson buffers, as electrolyte, for different pH values: 9.63, 8.15, 7.14 and 6.64, respectively and at a concentration of 6.4×10^{-5} M using cyclic voltammetry technique.

The results obtained by potentiodynamic measurements are presented in Table 2, and cyclic voltammograms for pH 9.63, are given in Fig. 3a and b.

It has been shown, on the basis of the cyclic voltammetry measurements, presented in Fig. 3a, that for pH 9.63 in BDB-113 solution there are two oxidation and two corresponding reduction peaks. This is in accordance with the results of the spectrophotometric investigations. The oxime BDB-113 has two overlapping dissociation equi-

Table 1
Special characteristics of the ionic forms of the compounds (I–IV)^a

Ionic form	H_2R^{2+}			HR^+			R			
	pH_{opt}	λ_{max}	$\epsilon_{\text{max}} \times 10^{-4}$	pH_{opt}	% at pH 7.4	λ_{max}	$\epsilon_{\text{max}} \times 10^{-4}$	pH_{opt}	λ_{max}	$\epsilon_{\text{max}} \times 10^{-4}$
BDB-114	–	–	–	–	–	267	1.16	>10	290	1.46
BDB-113	2–7	267	1.33	7.3–8.3	53.7	–	–	>9	298	2.00
BDB-117	2–6	278	1.31	7.2–8.2	57.6	–	–	>9	302	1.63
BDB-118	2–6	272	2.10	7.4–8.3	49.0	–	–	>9	353	2.01

^a λ_{max} (nm); $\epsilon_{\text{max}} \times 10^{-4}$ ($\text{mol}^{-1} \text{dm}^3 \text{cm}^{-1}$).

Table 2
The results of cyclo voltammetry measurements^a

pH	BDB-113			BDB-114		
	$E_0(1)/i_0(1)$	$E_0(2)/i_0(2)$	$E_r(2)/i_r(2)$	$E_r(1)/i_r(1)$	$E_0(1)/i_0(1)$	$E_r(1)/i_r(1)$
9.63	-0.20/0.017	+0.30/0.019	+0.15/-0.012	-0.40/0.025	-0.25/0.017	-0.48/-0.018
8.14	-0.20/0.017	-	-	-0.40/0.016	-0.25/0.01	-0.45/0.017
7.15	-0.20/0.017	-	-	-0.38/0.013	-0.40/0.01	-0.45/-0.01
6.64	-0.20/0.016	-	-	-0.40/0.014	-	-

^a $E_0(1)$, $E_0(2)$ (V), first and second anodic peak (oxidation) potentials, respectively, in the corresponding oxime solution. $E_r(1)$, $E_r(2)$ (V), corresponding cathodic peak (reduction) potentials. $i_0(1)$, $i_0(2)$ (mA cm⁻²), current densities for first and second anodic peak potentials, respectively. $i_r(1)$, $i_r(2)$ (mA cm⁻²), current densities for corresponding cathodic (reduction) potentials.

libria in this pH range, which could be assigned to dissociation of two -CH=N-OH groups, and therefore this oxime is in zwitterionic form. The oxygens' ions could be oxidized in the condition of cycling from negative to positive potential. The first peak at -0.24 V corresponds to the oxidation of one, but when first oxidation is finished, second oxidation is shifted to the more positive potential (+0.30 V). It could be concluded, therefore, that it is necessary, for the reaction of second oxidation, to use the higher value of the potential as a consequence of more difficult dissociation of the second oxime group. With the decreasing of pH values (from 8.14 to 6.64), voltammogram of BDB-113 exhibits the same shape as is presented in Fig. 3a and taking into account the first oxidation and corresponding reduction peak. The second oxidation and corresponding reduction peak are missing. This is in accordance with spectrophotometric investigations. For those pH values there is one dissociation constant and, therefore, only one -O⁻ is oxidized during the cycling.

The results for the other two dioximes BDB-117 and BDB-118 are not presented here because of similarity with BDB-113.

For BDB-114 oxime, as is shown in Fig. 3b for pH 9.63 one anodic peak at -0.25 V and corresponding cathodic peak at -0.48 V are observed. For pH 9.63, it is in a good agreement with spectrophotometric investigations, where one dissociation process is found. With the decreasing of pH from 8.14 to 6.64 the BDB-114 exhibits the

same electrochemical behavior during the cycling as is presented in Fig. 3b.

It could be concluded, on the basis of cyclic voltammetry investigations, that the existence of two overlapping acid-base equilibria, found by spectrophotometric measurements, have been confirmed.

3.3. Numerical treatment of overlapping equilibria

The changes in the absorption spectra, due to the variable media acidity, are the consequences of the changed ionic forms of the compound. The cyclic voltammetry investigations have also confirmed the existence of two overlapping acid-base equilibria:

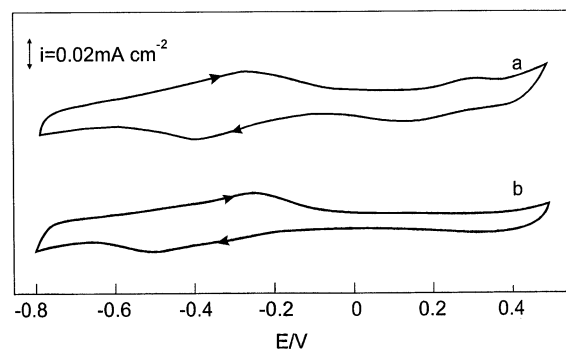


Fig. 3. Cyclic voltammograms of 6.40×10^{-5} M BDB-113 (a) and 6.40×10^{-5} M BDB-114 (b) in Britton–Robinson buffer pH 9.63 on Au electrode, sweep rate = 50 mV s⁻¹.

Table 3

The dissociation constants obtained using least square method (this work) and the literature data^a

Compound	pK_{a1}	pK_{a2}	K_{a2}/K_{a1}	Reference
BDB-114 (mono imidazol)	8.21	–	–	This work
BDB-113 (bis-BDB-114)	7.27 ± 0.05	8.32 ± 0.08	11	This work
BDB-117 (2-PAM-O-BDB-114)	7.16 ± 0.04	8.19 ± 0.08	11	This work
BDB-118 (4-PAM-O-BDB-114)	7.37 ± 0.07	8.36 ± 0.06	9.8	This work
2-PAM (mono)	7.92	–	–	[11]
4-PAM (mono)	8.44	–	–	[11]
2-PAM-O-CH ₃ (mono)	7.82	–	–	[11]
4-PAM-O-CH ₃ (mono)	8.30	–	–	[11]
4-PAM-CH ₂ -4-PAO (bis)	7.25	8.08	6.8	[11]
4-PAM-(CH ₂) ₂ -4-PAO (bis)	7.63	8.37	5.5	[11]
4-PAM-(CH ₂) ₃ -4-PAO (bis)	7.83	8.47	4.4	[11]

^a pK_{a1} and pK_{a2} from Ref. [11] were obtained by potentiometric method.

where R stands for the neutral species, HR⁺ for the intermediate monocation and H₂R²⁺ for dication. According to the structure of the investigated monooxime and dioximes (Scheme 1), it has been proposed that Eqs. (1) and (2) represent the dissociation processes of the –CH=NOH groups of the molecules.

The dissociation constants of the oxime groups were determined spectrophotometrically, from the absorbance versus pH data (Fig. 2). If the Beer–Lambert Law holds, the absorbance of a solution containing a total concentration C_R of ligand is given by [9,10]:

$$A = \frac{A_0 K_{a1} K_{a2} + A_1 K_{a1} 10^{-\text{pH}} + A_2 10^{-2 \times \text{pH}}}{K_{a1} K_{a2} + K_{a1} 10^{-\text{pH}} + 10^{-2 \times \text{pH}}} \quad (3)$$

where A is the absorbance of the solution which consists of three acidity forms: R, HR⁺ and H₂R²⁺; A_0 , A_1 and A_2 are the absorbencies of nonprotonated, monoprotonated and diprotonated ionic forms, respectively; K_{a1} and K_{a2} are dissociation constants of the Eqs. (1) and (2). Equation (3) may be derived from the equilibrium equation involving K_{a1} and K_{a2} , mass balance and characteristic of additivity of absorbance:

$$A = A_0 x_0 + A_1 x_1 + A_2 x_2 \quad (4)$$

$$C_R = [R] + [HR^+] + [H_2R^{2+}] \quad (5)$$

$$x_i = [H_i R] / C_R, \quad i = 0, 1, 2 \quad (6)$$

where x_i is the molar ratio of the particular ionic

form in the solution, and C_R is the total concentration of the corresponding compound.

Using the Lavendberg–Marquardt least square method, the absorbance versus pH data were fitted by the function (3). This method was used to determine the dissociation constants of the overlapping equilibria, as well as the absorbencies of the corresponding ionic forms of the compounds, i.e. A_0 , A_1 and A_2 . The calculations were performed at λ_{max} of the nonprotonated ionic form (R) of the compounds. The dissociation constant of monooxime (BDB-114) is also obtained using Lavendberg–Marquardt least square method. Absorbance versus pH data were fitted by function:

$$A = \frac{A_0 K_a + A_1 \cdot 10^{-\text{pH}}}{K_a + 10^{-\text{pH}}} \quad (7)$$

The calculated values of pK_{a1} and pK_{a2} are presented in the Table 3.

If K_{a1} and K_{a2} values have been calculated, it is possible to obtain x_0 , x_1 and x_2 , corresponding molar ratio of the particular ionic form R, HR⁺ and H₂R²⁺:

$$x_0 = (1 + K_{a2}^{-1} + K_{a1}^{-1} K_{a2}^{-1} 10^{-2 \times \text{pH}})^{-1} \quad (8)$$

$$x_1 = x_0 K_{a2}^{-1} 10^{-\text{pH}} \quad (9)$$

$$x_2 = 1 - (x_0 + x_1) \quad (10)$$

As an example Fig. 4 represents the distribution diagram for compound BDB-113.

From the distribution diagram as a function of pH, obtained from Eqs. (8–10), the acidity range in which 50–60% of each investigated compound is present in HR^+ ionic form in the solution, has been found (Table 1). The percent of HR^+ ionic form at physiological pH 7.4, has also been calculated for each compound and given in Table 1.

3.4. Interpretation of the results

Comparing the values of the acidity constants of BDB-114 with the acidity constants of some pyridil (2-PAM and 4-PAM) derivatives [11] (Table 3), it can be seen that the obtained pK value ($pK_1 = 8.21$) lies between the pK_{a1} values of those two oximes. The literature data show that for some mono pyridinium oximes the values of dissociation constants depend on the position of oxime groups in the heterocyclic ring. The influence of the substituent R in position one of the pyridine ring could also be strong [11].

As the results presented in Table 3. show, the dissociation constants of one of oxime groups (K_{a2}) by all investigated dioximes have values close to the dissociation constant of monooxime BDB-114. The introduction of the second oxime group in the investigated imidazolium derivatives by replacing R with II, III or IV (Scheme 1) exerted an inductive or field effect, which decreased the dissociation constant of this group enough to bring it into the required pK range (≈ 7.5). It is well known, from the literature data, that the oximes having pK_a

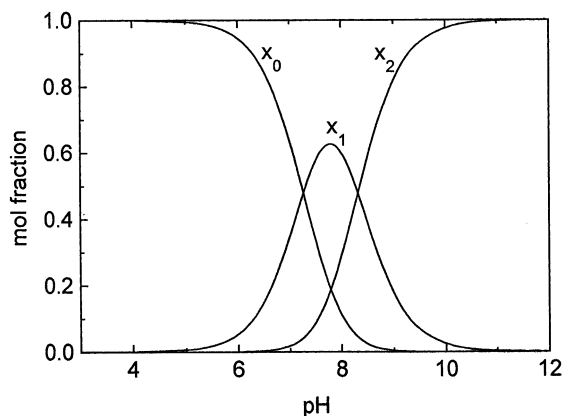


Fig. 4. The distribution diagram for BDB-113 (molar fractions of the particular ionic forms versus pH).

values in the range 7.5–8 were found to be very effective acetylcholinesterase reactivators [12]. The results obtained in this work show that the first dissociation for all investigated dioximes occurs with the pK_{a1} value of ≈ 7.1 –7.4 (Table 3). With this value of pK_a many oxime anions will be available at physiological pH and a relative increased ability to reactivate inhibited acetylcholinesterase could be expected.

For the symmetric dioxime, BDB-113, the ratio of the K_{a1}/K_{a2} was calculated and presented in Table 3. It is known, that this quotient by symmetric dioximes for statistical reasons has usually a value of about four [11]. The higher value indicates another influence on the dissociation constant of the second oxime group. It was shown earlier [11] that, by some symmetric pyridinium dioximes, the quotient of both the dissociation constants (K_1/K_2) usually has a value of four to five [11]. This value indicates the small effect of the first oxime group on the dissociation process of the second one, because of the long distance between them and the linear form of the molecules. The results presented in Table 3 indicate for BDB-113 a stronger effect on the dissociation equilibria ($K_1/K_2 = 11$). This was expected, because of the possible steric disturbance connected to the close position of two oxime groups. On the other hand, the possibility of the formation of hydrogen bonds between the dissociated and undissociated oxime groups could also increase the pK_{a2} value.

The ratio of dissociation constants (K_1/K_2) for nonsymmetric oximes was also calculated and presented in Table 3. The value obtained for BDB-117 is very close to that obtained for the symmetric BDB-113. This is in agreement with the quite similar position of two acidity groups in both of the compounds. For BDB-118 this ratio is less than for BDB-117 but greater than that for the symmetric PAM derivatives [11]. This could be ascribed to the introduction of a $-\text{CH}_2-\text{O}-\text{CH}_2$ group, as well as the asymmetrical position of oxime groups.

Acknowledgements

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