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THE PECULIARITIES OF THE BEHAVIOR OF ALENDRONIC ACID IN MICELLAR SOLUTIONS OF CETYLTRIMETHYLAMMONIUM BROMIDE

N. N. Kamneva, V. B. Kamneva*

Bisphosphonates are active substances for treatment of bone deceases. One of the representatives of this group of substances is 4-amino-1-hydroxybutylidene bisphosphonic (alendronic) acid. Micellar media of colloidal surfactants are usually considered as models of biological media for investigation of protolytic equilibria of pharmaceutical compositions. In this paper, three dissociation constants, K_{a3}, K_{a4}, and K_{a5}, of alendronic acid have been determined in micellar solution of cetyltrimethylammonium bromide (CTAB) with the concentrations of 0.01 mol/L and 0.10 mol/L via potentiometric titration.

Key words: alendronic acid, potentiometry, micellar solutions of CTAB, dissociation constants, titration curve, media effects.

Introduction

Bisphosphonates are widely used for medical treatment of metastatic bone diseases, Paget disease and osteoporosis [1-3]. They integrate into the bone structure to form a complex compound, thereby blocking the mevalonate synthesis and preventing the destruction of the bone. One of the most common bisphosphonates of the second generation is alendronic, or (4-amino-1-hydroxybutylidene) bisphosphonic acid (Fig. 1), which has zwitter-ionic structure in water solutions. The advantage of this pharmaceutic is in its resistant to enzymatic hydrolysis.

Pharmaceutical effect of the alendronic acid directly deals with its complex formation and protolytic properties.

The importance of knowledge of the dissociation of acids and bases under physiological conditions has been revealed by many authors [4,5]. Conventionally, water-organic mixtures such as water-ethanol are chosen for modeling. However, micellar solutions of different surfactants are the most appropriate analogues that meet principles of Green chemistry.

Therefore the data of the dissociation constants of alendronic acid in biological systems are of special importance for understanding the mechanism of their effects [6]. However, the latter raises the question of the choice of simple and stable systems to be simulated biological media for the study of equilibria. The most appropriate models called biomimetic systems are organized solutions including surfactants, which may also be components of the pharmaceuticals.

The present paper is devoted to the determination of the dissociation constants of the alendronic acid in micellar solutions of cetyltrimethylammonium bromide.

Protolytic equilibria of alendronic acid may be described by following equations: [5-7].

$$H_5B^+ \rightleftarrows H_4B^{+/-} + H^+, \qquad K_{a1} \qquad (1)$$

$$H_4B^{+/-} \rightleftarrows H_3B^{+/2-} + H^+, \qquad K_{a2} \qquad (2)$$

$$H_3B^{+/2-} \rightleftarrows H_2B^{+/3-} + H^+, \qquad K_{a3} \qquad (3)$$

$$H_2B^{+/3-} \rightleftarrows HB^{+/4-} + H^+, \qquad K_{a4} \qquad (4)$$

$$HB^{+/4-} \rightleftarrows B^{4-} + H^+, \qquad K_{a5} \qquad (5)$$

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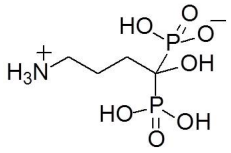

Figure 1. The molecule of alendronic acid

In water and 50 mass. % ethanol, the dissociation constants of alendronic acid have been determined earlier (Table 1).

Table 1. The indices of the dissociation constants of alendronic acid

	H_2O 0.10 mol/L KCl [5]	50 % mass. ethanol 0.10 mol/L NaCl [8]
pK_{a3}	6.38±0.03	7.40±0.02
pK_{a4}	10.68±0.06	9.58±0.04
pK_{a5}	11.4±0.2	10.45±0.07

Spectrophotometry and potentiometry are known to be used for determining the equilibrium constants in water and other media with different relative dielectric permittivity. Both methods are simple to perform, useful for routine analysis due to their accuracy, simplicity, and low cost. In the case of alendronic acid, UV-Vis or fluorescent detection cannot be used because of lack of chromophores in this molecule. Consequently, the attempted potentiometric titration proved to be a success for determining the dissociation constants [7].

The concentration-logarithmic diagram may be used for optimizing the titration conditions (Fig. 2). This diagram demonstrates the dependence of logarithm of the equilibrium concentration, [ ], of conjugated forms $H_3B^{+/2-}$ and $H_2B^{+/3-}$ on pH. If $H_3B^{+/2-}$ solution was titrated by NaOH, the stoichiometric point is located on the intersection of the graphics of $\log[H_2B^{+/3-}]$ and $\log [OH^-]$. The distance (segment) between the stoichiometric point and $\log c_t$ line corresponds to the logarithm of relative uncertainty of titration, $\log \delta$.

If the expected pK_{a3} value in micellar solution of cationic surfactant was equal to ~ 6, and titration uncertainty did not exceed 1% ($\log 0.01 = -2$), therefore, according to the concentration-logarithmic diagram, the total concentration of the alendronic acid in the equivalent point would not be less than 2.5×10^{-4} mol/L.

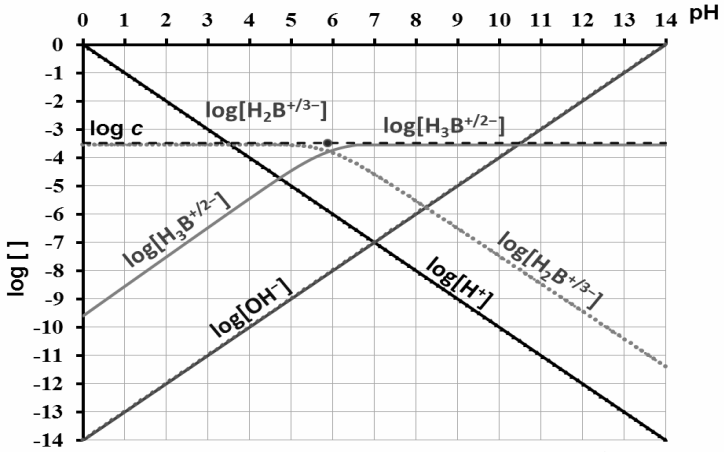

Figure 2. Concentration-logarithmic diagram for alendronic acid $H_3B^{+/2-}$ with $pK_{a3} = 5.9$ and relative uncertainty of the titration result 1%.

In the present paper, the micellar solutions of cetyltrimethylammonium bromide (CTAB) were chosen for investigations (Fig. 3). The critical micelle concentration (*cmc*) of CTAB is equal to

9×10^{-4} mol/L [9-12]. This surfactant forms spherical micelles in water solutions within the concentration region near *cmc*.

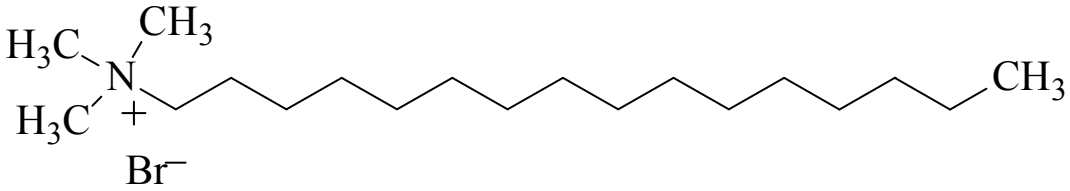

Figure 3. Cetyltrimethylammonium bromide

In the present paper, we compare the results obtained for alendronic acid in micellar solutions of CTAB and other surfactants.

The influence of the micellar media of surfactant caused by binding of the molecules and ions by micelles may be represented as a quasi-chemical reaction (eq. 6):

$$\mathrm{H}_{5-i}\mathrm{B}+\mathrm{Mic}\rightleftarrows\mathrm{H}_{5-i}\mathrm{B}\left[\mathrm{Mic}\right] \tag{6}$$

where Mic is a surfactant micelle; $H_{5-i}B$ – protolytic form in bulk water phase; $H_{5-i}B[Mic]$ – protolytic form binded by surfactant micelle.

The mass action law may be given as follows:

$$K_{b,5-i}=\frac{[\mathrm{H}_{5-i}\mathrm{B}]_m}{[\mathrm{H}_{5-i}\mathrm{B}]_w(c_s-cmc)} \tag{7}$$

Here, $K_{b,5-i}$ is the constant of $H_{5-i}B$ binded by micellar pseudophase; $[H_{5-i}B]_m$ is the equilibrium concentration in micellar phase, and $[H_{5-i}B]_w$ is the equilibrium concentration in bulk water, referred to the whole volume of the solution; c_s is the total surfactant concentration; *cmc* is the critical concentration of micelle formation.

The pK_a values in micellar media may be considered as indices of the so-called apparent constants [13,14]:

$$\mathrm{p}K_{a,i}^{app}=\mathrm{pH}_w+\lg\frac{\left[\mathrm{H}_{5-(i-1)}\mathrm{B}\right]_{tot}}{\left[\mathrm{H}_{5-i}\mathrm{B}\right]_{tot}}=\mathrm{pH}_w+\lg\frac{\left[\mathrm{H}_{5-(i-1)}\mathrm{B}\right]_w+\left[\mathrm{H}_{5-(i-1)}\mathrm{B}\right]_m}{\left[\mathrm{H}_{5-i}\mathrm{B}\right]_w+\left[\mathrm{H}_{5-i}\mathrm{B}\right]_m} \tag{8}$$

The $\mathrm{p}K_{\mathrm{a},i}^{\mathrm{app}}$ can be written in the next form using eq. (7) and (8):

$$\begin{aligned}\mathrm{p}K_{a,i}^{app}&=\mathrm{pH}_w+\lg\frac{\left[\mathrm{H}_{5-(i-1)}\mathrm{B}\right]_w\left(1+K_{b,\mathrm{H}_{5-(i-1)}\mathrm{B}}\left(c_s-cmc\right)\right)}{\left[\mathrm{H}_{5-i}\mathrm{B}\right]_w\left(1+K_{b,\mathrm{H}_{5-i}\mathrm{B}}\left(c_s-cmc\right)\right)}=\\&=\mathrm{p}K_{a,i}+\lg\frac{\left(1+K_{b,\mathrm{H}_{5-(i-1)}\mathrm{B}}\left(c_s-cmc\right)\right)}{\left(1+K_{b,\mathrm{H}_{5-i}\mathrm{B}}\left(c_s-cmc\right)\right)}\end{aligned} \tag{9}$$

The difference between $\mathrm{p}K_{\mathrm{a},i}^{\mathrm{app}}$ and the corresponding value in water $\mathrm{p}K_{\mathrm{a},i}^{\mathrm{w}}$ equal to $\Delta\mathrm{p}K_a=(\mathrm{p}K_{\mathrm{a}}^{\mathrm{app}}-\mathrm{p}K_{\mathrm{a}}^{\mathrm{w}})$ one may consider as the micellar medium effect.

Experimental

Materials. The sample of sodium alendronate trihydrate was kindly donated by the Company «Stoma», Kharkov, Ukraine. Cetyltrimethylammonium bromide (Sigma, 99 %) was used as received. Ethanol was purified by the standard procedure via rectification. Carbonate-free 0.0899 mol/L NaOH solution was prepared from the saturated NaOH solution using CO_2-free water and kept protected from the atmosphere; it was standardized by titration of weighted amounts of adipic acid. Working solutions

of NaOH were prepared by appropriate dilution of stock solutions before titrations. Analytical grade NaBr was used for preparation of the solutions of the supporting electrolyte.

Apparatus. The electromotive force was measured using a compensation scheme (R 37-01 potentiometer and pH-121 pH-meter (ZIP, Gomel, Belarus) as a null-instrument) with an ESL-43-07 glass electrode and Ag|AgCl electrode EVL-1M3 as a reference one (ZIP, Gomel, Belarus). The standard deviation was no more than 0.2 mV. The titrations were performed at 25.0 ± 0.1°C in a cell with liquid junction with glass electrode. The glass electrode was calibrated with standard buffer solutions (pH 9.18, 6.86, 4.01, 3.56 and 1.68 at 25 °C).

Procedure. The dissociation constants of alendronic acid were determined by the pH titrations in 0.01 and 0.1 mol/L micellar solution of CTAB.

Both preparation of solutions and pH measurements have been carried out at (25 ±1)°C.

For potentiometric titration, the titrated substances were prepared as follows. The concentration of alendronic acid was 1.17×10^{-3} mol/L. The volume of the titrated solutions was 15 mL. The working solution was prepared by dissolving the appropriate amount in 0.10 mol/L, 0.01 mol/L CTAB, respectively, with I = 0.05 mol/L maintained by NaBr.

The third, fourth, and fifth dissociation constants were calculated from the data of potentiometric titration with 3.60×10^{-3} mol/L NaOH in the same media with I = 0.05 mol/L (NaBr). The first two constants could not be calculated for the media under study because their values are higher than the concentration of the alendronic acid, which is limited by the solubility in the titrated solution. For pK_{a3}, pK_{a4}, and pK_{a5} calculations, the number of data points was at least 50.

Dissociation constants were calculated using the CLINP 2.1 program [15] in 20 to 80 % degree of neutralization range to eliminate the error when subtracting. Obtained constants were averaged by taking into account the variation for each constant and correlations between them for each replicate titration [16]. This approach was realized by using MATLAB 6.5 [17]. Every titration was reproduced at least four times, χ^2 was less than 50 in all cases.

Results and discussion

An example of the titration curve of alendronic acid is shown in Fig. 4.

Since the first two constants highly likely lie in strongly acidic region of pH<1, only the dissociation by the third, fourth, and fifth step has been investigated.

The K_{a3}, K_{a4}, and K_{a5} values for alendronic acid obtained in the present paper belong to the so called mixed constants, because the activity of hydrogen ions and the equilibrium concentrations of other species have been used for calculations.

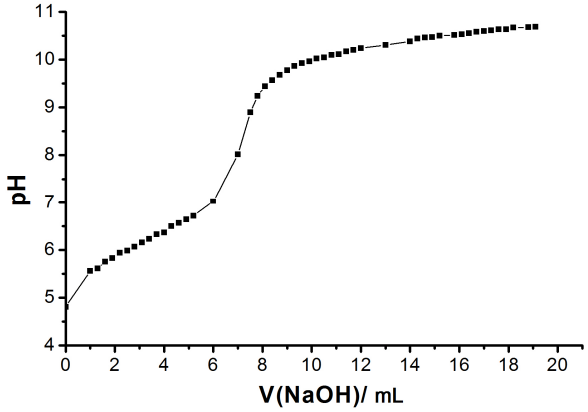

Figure 4. Experimental titration curve of 1.17×10^{-3} mol/L alendronic acid by 3.60×10^{-3} mol/L NaOH in 0.1 mol/L CTAB solution with I = 0.05 mol/L (NaBr).

The indices of the so-called apparent constants, pK_a^{app}, and the medium effects, $\Delta pK_a = (pK_a^{app} - pK_a^{w})$, of alendronic acid are gathered in Table 2.

Comparing with corresponding data with whose in aqueous solutions, pK_{a4} and pK_{a5} became smaller, that is in line with the Hartley rules [13,18,19]. There are no significant changes in dissocia-

tion of zwitter-ionic form in CTAB solutions contrary to analogous cetylpyridinium chloride (CPC) that may be explained by the differences of the binding degree.

Table 2. The indices of the dissociation constants of alendronic acid in different media

	pK_{a2}	pK_{a3}	pK_{a4}	pK_{a5}
H_2O 0.1 mol/L KCl [8]	2.24±0.01	6.38±0.03	10.68±0.06	11.4±0.2
0.1 M Brij 35 0.1 mol/L KCl [5]	3.89±0.07 (+1.65)	5.79±0.05 (–0.59)	6.75±0.05 (–3.63)	8.66±0.04 (–2.7)
0.1 mol/L CPC 0.1 mol/L KCl [8]	2.34±0.02 (+0.1)	5.97±0.02 (–0.41)	10.25±0.03 (–0.13)	10.50±0.10 (–0.9)
0.01 mol/L CTAB 0.05 mol/L NaBr	–	6.42±0.10 (+0.04)	9.76±0.08 (–0.92)	10.36±0.10 (–1.0)
0.1 mol/L CTAB 0.05 mol/L NaBr	–	6.22±0.01 (–0.16)	10.21±0.04 (–0.47)	11.04±0.10 (–0.4)

In parentheses, the media effects $\Delta pK_a = (pK_a^{app} - pK_a^{w})$ are revealed.

The higher is the concentration of CTAB, the smaller effect is observed for the pK_{a4} and pK_{a5} of alendronic acid. Probably, this originates from the increase in size of micelles and their elongation, which results in worse binding of the B^{4-} and $HB^{+/4-}$ forms.

Conclusions

The increase in the strength of alendronic acid was observed in micellar solutions of 0.01 and 0.10 mol/L CTAB. This gives evidence for the binding of B^{4-} and $HB^{+/4-}$ forms by micelles.

Micellar media of CTAB displays the significant effect on the strength of the alendronic acid. The pK_{a4} and pK_{a5} values decrease by one unity as compared with those in aqueous solutions (Table 2), indicating the significant binding of protolytic B^{4-} and $HB^{+/4-}$ forms by micellar aggregates of cationic surfactants. There is an analogy in the change of pK_{a4} and pK_{a5} values of alendronic acid in solutions of CPC and CTAB, due to similar structure and properties of these surfactants.

Meanwhile, the pK_{a3}^{app} value only slightly depends on the concentration of CTAB, but subsequent pK_a^{app} ones markedly increase on going from 0.01 mol/L to 0.10 mol/L of CTAB solution.

It is likely that there is an effect that was mentioned previously [13]. The values of pK_a^{app} corresponding to the equilibria between multiply charged anions, may even increase on going from aqueous solution to the micelles of cationic surfactant, if the value of pK_a^i [13,14] outweighs the contribution of the summand containing ψ. In this case, these effects may be amplified with increasing concentrations of CTAB, and the resulting restructuring of the micelles and further hydrophobization of the micellar pseudophase.

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Н. Н. Камнева, В. Б. Камнева. Особенности поведения алендроновой кислоты в мицеллярных растворах бромида цетилтриметиламмония.

Бисфосфонаты являются активными веществами для лечения заболеваний костей. Одним из представителей этого класса соединений является 4-амино-1-гидроксибутилидендифосфоновая (алендроновая) кислота. В качестве моделей биологических систем для исследования протолитических равновесий фармацевтических препаратов обычно рассматривают мицеллярные среды коллоидных поверхностно-активных веществ. В настоящей работе методом потенциометрического титрования были определены три константы диссоциации, K_{a3}, K_{a4} и K_{a5}, алендроновой кислоты в мицеллярном растворе бромида цетилтриметиламмония (ЦТАБ) с концентрациями 0.01 моль/л и 0.10 моль/л.

Ключевые слова: алендроновая кислота, потенциометрия, мицеллярные растворы ЦТАБ, константы диссоциации, кривая титрования, эффекты среды.

Н. М. Камнєва, В. Б. Камнєва. Особливості поведінки алендронової кислоти в міцелярних розчинах броміду цетилтриметиламонію.

Бісфосфонати є активними речовинами для лікування хвороб кісток. Одним з представників цього класу сполук є 4-аміно-1-гідроксибутилідендифосфонова (алендронова) кислота. У якості моделей біологічних систем для досліджування протолітичних рівноваг фармацевтичних препаратів зазвичай розглядають міцелярні середовища колоїдних поверхнево-активних речовин. У даній роботі методом потенціометричного титрування було визначено три константи дисоціації, K_{a3}, K_{a4} та K_{a5}, алендронової кислоти в міцелярному середовищі броміду цетилтриметиламонію (ЦТАБ) з концентраціями 0,01 моль/л та 0,10 моль/л.

Ключові слова: алендронова кислота, потенціометрія, міцелярні розчини ЦТАБ, константи дисоціації, крива титрування, ефекти середовища.