Determination of Protonation Constants and Stability Constants of Lanthanides with Derivative of H_4DOTA Complexes by the UV-VIS Spectrophotometry and Potentiometry

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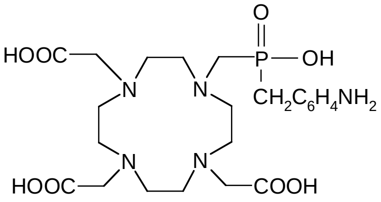
Abstract: Methods of static potentiometry and UV-VIS spectrophotometry were applied for the determination of protonation constants of (10-[4-aminobenzyl(hydroxyl)phosphonylmethyl]-1,4,7,10-tetraazacyclo-1,4,7-triacetic acid) (DOTA(NH_2)) and stability constants of holmium and yttrium complexes with DOTA(NH_2). The DOTA(NH_2) protonation constants of [HA] (log β_1 = 12.7); [H_2A] (log β_2 = 21.4) and [H_3A] (log β_3 = 27.9) were experimentally determined by the static potentiometry. The stability constants of Ho-DOTA(NH_2) (log $K_{\text{Ho DOTA(NH2)}}$ = 18.0) and Y-DOTA(NH_2) (log $K_{\text{Y-DOTA(NH2)}}$ = 17.8) complexes were determined by UV-VIS spectrophotometry using the competitive reaction of the DOTA(NH_2) – metal complexes with Arsenazo III.

Keywords: Lanthanide complexes, protonation, stability constants, UV-VIS spectrophotometry, potentiometry, Arsenazo III, 10-[4-aminobenzyl (hydroxyl) phosphonylmethyl]-1,4,7,10-tetraazacyclo-1,4,7-triacetic acid.

1. INTRODUCTION

Chelation agents, for instance DTPA-diethylenetriaminepentaacetic acid [1-3], MDP – methylenediphosphonic acid [4], HEDP – 1-hydroxyethylenediphosphonic acid [4], EDTMP – ethylenediamine-tetra-methylenephosphonic acid [5-7], DOTA – 1,4,7,10-tetraazadodecane-N,N',N'',N'''-tetraacetic acid [1], [8] and their derivatives DO3A-B - 10-[2,3-dihydroxy-(1-hydroxymethyl)-propyl]-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid [9-11], DO3A-ME – 1,7-bis(carboxymethyl)-4,10-bis(1-hydroxymethyl-2,3-dihydroxypropyl)-1,4,7,10-tetraazacyclododecane [12] and DOTA (NH_2) - 10-[4-aminobenzyl(hydroxyl)phosphonylmethyl]-1,4,7,10-tetraazacyclo-1,4,7-triacetic acid (Scheme **1**) are widely used in the preparation of radiolabeled monoclonal antibodies for radiodiagnostical and therapeutical purposes. Before covalent binding with biomolecules chemical modification of the chelating agent is often used. The resulting bifunctional chelating agents have the specific group forming very strong chelating bond with metals and the reactive group for interaction with antibody.



Scheme 1. Structure of DOTA(NH_2).

These bifunctional chelation agents enable the labeling of antibodies by various radionuclides with suitable nuclear properties for therapeutical applications. e.g. ^{166}Ho and ^{90}Y [1, 13]. The system radionuclide - bifunctional chelator - monoclonal antibody is very complex, and, consequently, understanding the mutual interactions of all components involved would lead to better characterization of the conjugate. Stability constants of chelating agents with the respectively metal ions are very useful for these characterizations.

Various methods have been employed for the determination of stability constants of metal - chelator complexes, namely potentiometry [8, 14], voltammetry [15], spectroscopic techniques [12], capillary electrophoresis [16, 17] and capillary isotachophoresis [1].

Although a few DOTA derivatives have been synthesized the stability constants of these ligands with lanthanide cations have not been published [18-21].

The aim of the present work was the determination of protonation constants of DOTA(NH_2) using the potentiometric method and the determination of the stability constants of Ho-DOTA(NH_2) and Y-DOTA(NH_2) complexes applying competition spectrophotometric method. To the best of our knowledge the latter two stability constants have not been reported yet.

2. EXPERIMENTAL

2.1. Chemicals

The chelating agent, 10-[4-aminobenzyl (hydroxyl) phosphonylmethyl]-1,4,7,10-tetraazacyclo-1,4,7-triacetic acid (DOTA(NH_2), was prepared at the Department of Inorganic Chemistry of the Charles University in Prague. Purity of the product checked by ^{1}H and ^{13}C NMR was better than 98%.

Following chemicals were purchased from Lachema (Czech Republic): NaCl, $KHCO_3$, H_2SO_4, NaOH, $KHC_4H_4O_6$, $KHC_8H_4O_4$, KH_2PO_4, Na_2HPO_4, $Na_2B_4O_7.10H_2O$ and acetic acid. Holmium and yttrium salts, $Ho(NO_3)_3.5H_2O$ and

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$YCl_3.6H_2O$, and indicator 3,6-bis-[(2-arzenofenyl)-azo-]-4,5-dihydroxy-2,7-naftalendisulfonic acid (Arsenazo III; AA, 97.1%, batch: 447130/1) were obtained from Sigma-Aldrich (Czech Republic). Deionized water was used for the preparation of all solutions. All chemicals were of analytical grade.

2.2. Procedure

pH meter PHM 84 (Radiometer. Copenhagen. Denmark) with G 2040 B glass electrode and K 4040 calomel electrode was used for the static potentiometry. Method of the static titration [4], [14] based on the long-term (several days) pH measurement of the studied reagents was applied for the determination of protonation constants of the chelator. The static titration of DOTA(NH_2) was performed with a set of various solutions. Each of these solutions contained DOTA(NH_2) (1 ml, c = 10^{-3} M), titration reagent (NaOH. from 0 to 2 ml, c = 2.4×10^{-3} M) and sodium chloride (1 ml, c = 1 M). Finally, the total volume of each individual reaction mixture was adjusted to 10 ml in a volumetric flask. These experiments were carried out in a box with N_2 atmosphere to eliminate the effect of carbon dioxide. The pH values of the reaction mixtures were measured for 160 hours (40 hours is need for the reach of equilibrium). The calibration was made using standard buffers ($KHC_4H_4O_6$, pH = 3.56; $KHC_8H_4O_4$ pH = 4.01; KH_2PO_4 + Na_2HPO_4, pH = 6.88; KH_2PO_4 + Na_2HPO_4 pH = 7.41; $Na_2B_4O_7.10H_2O$, pH = 9.18).

Spectrophotometric titrations were carried out with UV-VIS spectrophotometer Cary 50-Bio (Varian. USA). UV-VIS spectra were recorded from 200 nm to 800 nm in 1 cm quartz cell.

The individual reaction mixtures of the total volume 2 ml were prepared using Arsenazo III (AA) stock solution (0.050 ml, c = 10^{-3} M), solution of the respective metal ion (0.050 ml, c = 10^{-3} M) (AA / metal ratio 1:1) and sodium acetate buffer (1.90 ml, c = 0.1 M) at pH values 5 and 7, respectively. The DOTA(NH_2) (0.100 ml, c = 5×10^{-4} M) was added to these reaction mixtures.

Spectra were measured in pre-selected time span and intervals, specifically, immediately after mixing and then after 1, 2, 3, 4, 5, 28, 55, 72 and 96 hours. Conditional stability constants of metal-AA complexes were determined in pH = 5.6 sodium acetate buffer. The constants were determined by titration of AA solution (0.050 ml, c = 10^{-3} M) with metal ion solution (0.010 ml to 0.300 ml, in 10 μl increments, c = 5×10^{-4} M) in sodium acetate buffer (1.95 ml, c = 0.1 M; pH = 5.6) and the changes of the absorbance during the titration were measured.

This complexation was studied by UV-VIS titration of metal-Arsenazo III complex (0.050 ml, c(AA) = 10^{-3} M; 0.050 ml, c(metal) = 10^{-3} M) in sodium acetate buffer (1.90 ml, c = 0.1 M; pH = 5.6) with a solution of the chelator (0.010 ml to 0.300 ml, in 0.010 ml increments, c = 5×10^{-4} M). The reaction mixture of ion metal solution with AA solution was titrated by DOTA(NH_2) solution with 0.010 ml additions.

The IUPAC recommendations have been fulfilled in all experiments [22, 23].

3. RESULTS AND DISCUSSION

3.1. Static Potentiometry

The “static titration curve” for the mixtures of a 10^{-4} M solution of DOTA(NH_2) (**1**) with various amounts of carbonate-free NaOH at constant ionic strength (0.1 M NaCl) is depicted in Fig. (**1**).

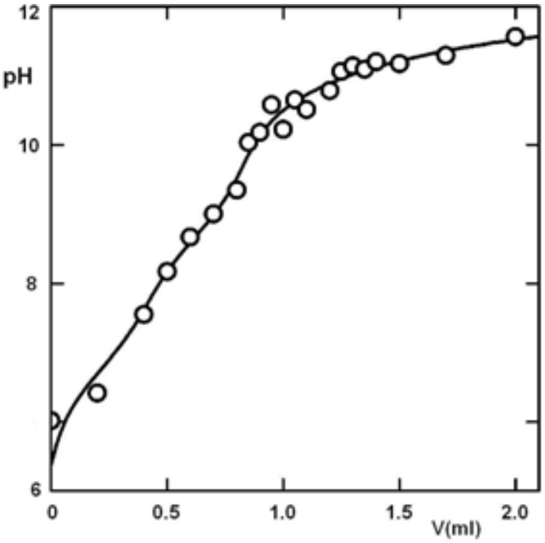

Fig. (1). Titration curve of DOTA(NH_2) (in box under atmosphere of nitrogen). Reaction mixture: total volume 10 ml composed of DOTA(NH_2) (*C* = 10^{-4} *M*), NaOH (concentration from 0 to 4.8 $\times10^{-4}$ *M*), NaCl (*C* = 0.1 *M*), reaction time: 160 hours. The curves were calculated using the constants given in Table **1**.

The pH range of the titrations was 2-10. Each point was prepared and measured three times and the average value was used for further calculations. It was found that DOTA(NH_2) reached its protonation equilibrium after 64 hours under experimental conditions. Maximum difference between the data of each point was 0.05 pH unit.

Table 1. The Protonation Constants of DOTA(NH_2) Ligand Determined by the Static Potentiometry. The Standard Deviations s were Calculated from Eq. 14

Specie	log β
[HL]	12.7 (*s* = 0.3)
[H_2L]	21.4 (*s* = 0.3)
[H_3L]	27.9 (*s* = 0.3)
Coefficient log α (at pH = 5.6)	11.2

The system H^+ - $\mathbf{1}^{3-}$ ($\mathbf{1}^{3-}$ is the DOTA(NH_2) anion) can be characterized by equations

$$\mathrm{i}\,H^+ + \mathbf{1}^{3-} \longleftrightarrow H_i\mathbf{1}^{(3-i)-} \qquad (1)$$

the equilibrium constants of which are defined as

$$\beta_i = \frac{[H_n\mathbf{1}^{(3-i)-}]}{[\mathbf{1}^{3-}]\cdot[H^+]^i}. \qquad (2)$$

The protonation constants were calculated from the potentiometric data with the computer program LTGW ETITR, derived from LETAGROP ETITR [24]. The results are summarized in Table **1**. Only three functional groups on the ligand were protonated under experimental conditions and the kinetics of protonation was slow.

The stability constants of Ho^{3+} and Y^{3+} complexes of $DOTA(NH_2)$ were determined by spectrophotometric measurement from a competition reaction with Arsenazo III. All measurements were taken at a constant pH of 5.6, so conditional stability constants could be used instead of thermodynamic ones.

The reaction of M^{3+} ($M^{3+} = Ho^{3+}, Y^{3+}$) with Arsenazo III (abbr. **2**) can be described by Eq. (3) [25]

$$M^{3+} + n\,\mathbf{2}^{8-} + i\,H^+ \Leftrightarrow M{\cdot}\mathbf{2}_nH_i^{(8n-3-i)-} \qquad (3)$$

the stability constant of which can be written as (charges are omitted for simplicity)

$$\beta_{n,i} = \frac{\left[M\cdot\mathbf{2}_nH_i\right]}{[M][\mathbf{2}]^n[H]^i} \qquad (4)$$

If the experiments proceed at constant pH, the conditional stability constant can be used instead of the thermodynamic one, hence

$$\beta_n^{'} = \frac{\left[M\cdot\mathbf{2}_n\right]}{[M][\mathbf{2}]^n} \qquad (5)$$

where n = 1 or n = 2. The protonization of Arsenazo and metal - Arsenazo complexes are involved in the value of the conditional stability constant, so the pH value during experiments was held constant at pH 5.6.

Using mass balances, equation (5) can be rewritten as

$$\beta_n' = \frac{\left[M\cdot\mathbf{2}_n\right]}{\left[M\right]*\left\{C(\mathbf{2})-\left[M\cdot\mathbf{2}\right]-2\left[M\cdot\mathbf{2}_2\right]\right\}^n} \qquad (6)$$

The conditional stability constant of the metal - ligand is defined as follows:

$$K' = \frac{\left[M\cdot\mathbf{1}\right]}{\left[M\right]\left\{C(\mathbf{1})-\left[M\cdot\mathbf{1}\right]\right\}}\,. \qquad (7)$$

where [M·**1**] is the equilibrium concentration of the metal-ligand complex, [M] is the equilibrium concentration of free metal ions and C(**1**) is the molar concentration of the ligand.

The stability constant of the metal-ligand complex is described as follows:

$$K = \frac{\left[M\cdot\mathbf{1}\right]}{\left[M\right]\left[\mathbf{1}\right]} \qquad (8)$$

where [M·**1**] is the equilibrium concentration of the metal-ligand complex, [M] is the equilibrium concentration of free metal ions and [**1**] is the equilibrium concentration of the ligand.

The stability constants (*K*) were determined as a product of the conditional stability constant of the metal-ligand complex (*K*') and the coefficient α (at pH 5.6), so

$$K = K' * \alpha_{(pH=5.6)} \qquad (9)$$

The coefficient α is defined as follows:

$$\alpha = \left(1+\sum_{i=1}^{n}\left[H^+\right]^i * \beta_i\right) \qquad (10)$$

where β_i is the overall protonation constant of an $H_i\mathbf{1}$ specie defined by Eq. (2) and $\mathbf{1}^{3-}$ represents the $DOTA(NH_2)$ anion. The obtained protonation constants of $DOTA(NH_2)$ (see Table **1**) were used to calculate the coefficient α (log α = 11.2 at pH 5.6).

3.2. UV-VIS Spectrophotometry

Time dependencies of the absorbance changes were constructed from the data obtained at pH 5.2 and pH 7.1. The kinetics was followed over the time range 0 – 192 hours (Figs. **2** and **3**).

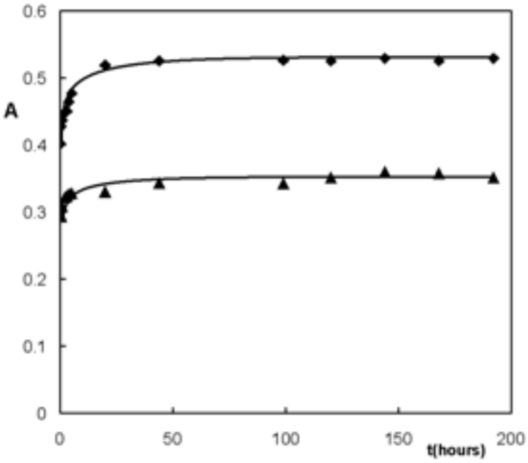

Fig. (2). The time-dependence of absorbance values of the Ho-$DOTA(NH_2)$-Arsenazo III reaction mixture measured by UV-VIS spectrophotometry. *C*(**2**) = $2.5\times10^{-5}M$, $C(Ho(NO_3)_3) = 2.5\times10^{-5}M$, $C(DOTA(NH_2)) = 2.38\times10^{-5}$ *M*, 0.1 *M* sodium acetate buffer, λ = 548 nm. ◆ pH = 5.2, ▲ pH = 7.1.

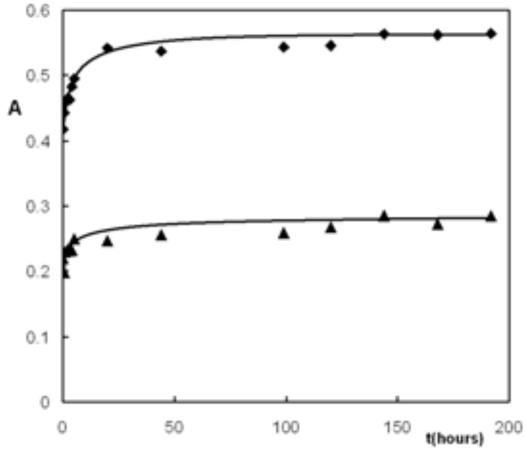

Fig. (3). The time-dependence of absorbance values of the Y-$DOTA(NH_2)$-Arsenazo III reaction mixture measured by UV-VIS spectrophotometry. *C*(**2**) = 2.5×10^{-5} *M*, *C* $(YCl_3) = 2.5\times10^{-5}$ *M*, *C* $(DOTA(NH_2)) = 2.38\times10^{-5}$ *M*, 0.1 *M* sodium acetate buffer, λ = 558 nm. ◆ pH = 5.2, ▲ pH = 7.1.

Further experiments were carried out at pH 5.6 because maximal changes in absorbance were achieved. Reaction time of 3 days was used for all further spectrophotometric measurements.

The spectra of Arsenazo III and Arsenazo III – metal complexes are depicted in Figs. (**4** and **5**).

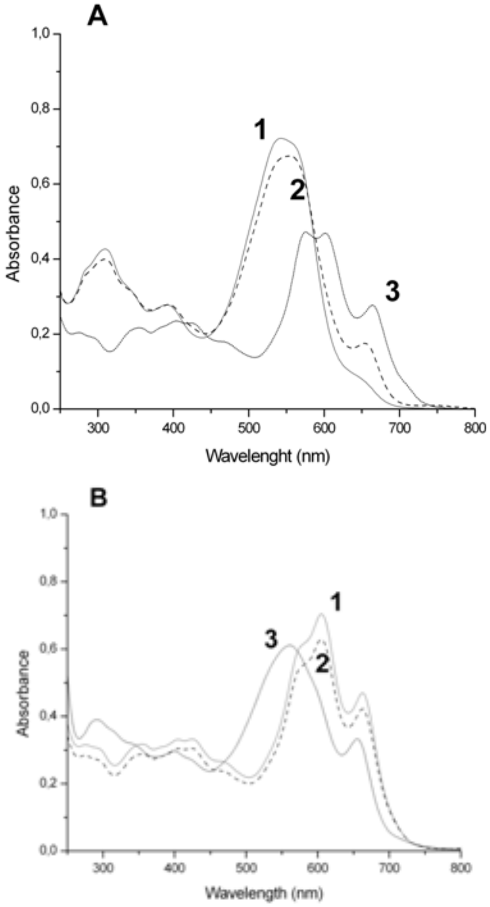

Fig. (4). A- UV-VIS spectrum of titration of Arsenazo III by $Ho(NO_3)_3$ solution. Reaction mixture: Arsenazo III ($C = 2.5\times10^{-5}$ *M*), sodium 0.1*M* sodium acetate buffer, pH = 5.6; 1 – **2** only, 2 - **2** + 2.4×10^{-6} *M* $Ho(NO_3)_3$, 3 - **2** + 6.52×10^{-5} *M* $Ho(NO_3)_3$.

B- UV-VIS spectrum of titration of Ho-Arsenazo III complex by DOTA(NH_2) solution. Arsenazo III (2.5×10^{-5} *M*) + $Ho(NO_3)_3$ (2.5×10^{-5} *M*), 0.1 *M* sodium acetate buffer, pH = 5.6); reaction time: 3 days; 1 - **2** + $Ho(NO_3)_3$ only, 2 - **2** + $Ho(NO_3)_3$ + 2.4 X 10^{-6} *M* DOTA(NH_2), 3 - **2** + $Ho(NO_3)_3$ + 6.52×10^{-5} *M* DOTA(NH_2).

The conditional stability constants of metal-Arsenazo III were determined by titrating AA III solution with a solution of the metal ion. Absorbance at wavelengths 558 nm (Fig. **4A**) and 548 nm (Fig. **5A**) corresponding to the free concentration of [**2**] decreased during the experiment.

On the other hand, absorbance at 663 and 603 nm, corresponding to the Y·**2** complex (Fig. **4A**) and at 665 and 603 nm corresponding to Ho·**2** (Fig. **5A**) increased.

The formation of a metal-**1** complex was observed at optimum complexation conditions, i.e. pH 5.6. One wide peak at 554 nm, which belongs to **2** can be clearly seen in the spectrum of a mixture of complexes Y.1, Y.2 and Y.2_2 (Fig. **4B**). **2** was released due to metal transchelation with **1,** so the absorbance of **2** increased during titration experiments. The two narrow peaks at 658 and 605 nm belong to Y·**2** complexes.

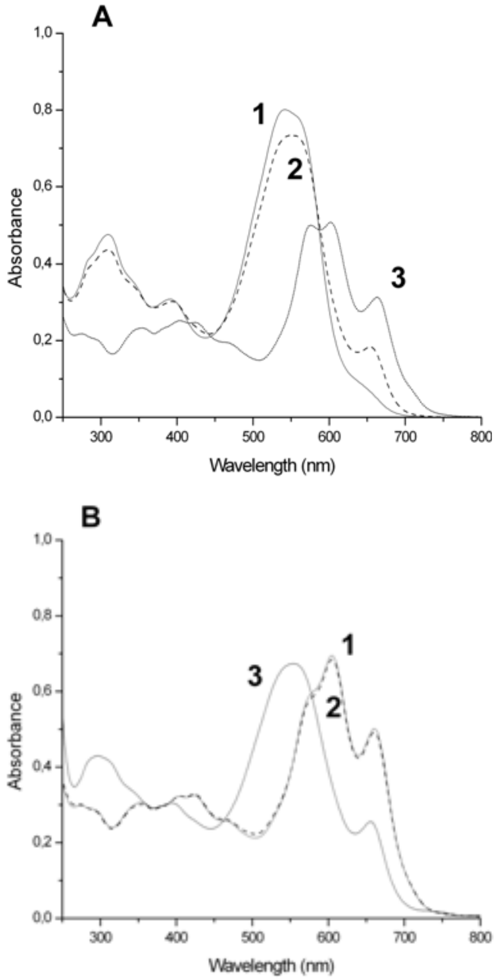

Fig. (5). A- UV-VIS spectrum of titration of Arsenazo III by YCl_3 solution. Reaction mixture: total volume 2 cm^3 prepared of Arsenazo III (c = 2.5×10^{-5} *M*), sodium 0.1 *M* sodium acetate buffer, pH = 5.6; 1 – **2** only, 2 - **2** + 2.4×10^{-6} *M* YCl_3, 3 - **2** + 6.52×10^{-5} *M* YCl_3. **B**- UV-VIS spectrum of titration of Y - Arsenazo III complex by DOTA(NH_2) solution. Reaction mixture: Arsenazo III (*C* =2.5×10^{-5}*M*) + YCl_3 (2.5×10^{-5}*M*), 0.1 *M* sodium acetate buffer, pH = 5.6); reaction time: 3 days; 1 - **2** + YCl_3 only, 2 - **2** + YCl_3 + 2.4 $\times10^{-6}$ *M* **1**, 3 - **2** + YCl_3 + 6.52×10^{-5} *M* **1**.

The absorbance of Y - **2** decreased with increasing concentration of **1** in the reaction mixture**.** Similarly, one wide peak at 552 nm that belongs to **2** and two narrow peaks at 663 and 605 nm, which correspond to the Ho·**2** complex can be seen in the spectra for a Ho – **1** - **2** mixture (Fig. **5B**). The time required to form the metal-ligand complex (Ho·**1** or Y·**1)** was equal to 71 hours (Figs. **2** and **3**). If the difference in the stability constants of metal-ligand complex is high enough (i.e. more than K = 10^4), two peaks are formed in the spectra in Figs. (**2** and **3**).

The conditional stability constants of Ho·**2** were calculated from data at wavelengths 665, 603, 548, 429 and 310 nm. All solutions were prepared separately and the absorbencies were measured after 14 days. Some of the results are depicted in Fig. (**6**). The conditional stability constants of Ho**2** and Ho**2**$_2$ complexes were calculated by the least squares minimizing program LTGW-SPEFO, derived from Letagrop Spefo [26].

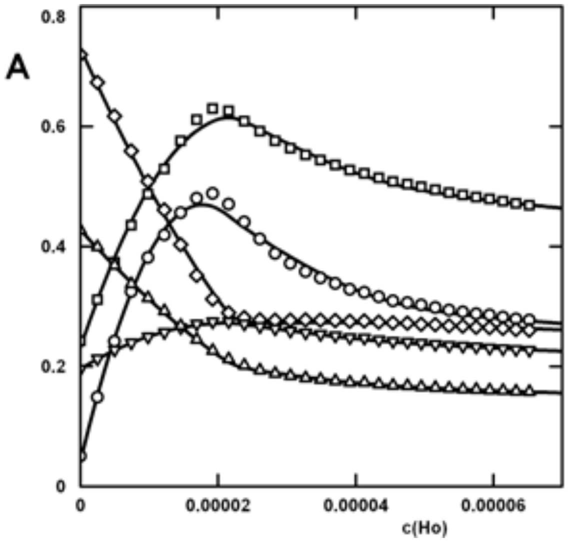

Fig. (6). Spectrophotometric titration of Arsenazo solution by the solution of Ho(NO$_3$)$_3$. $C(\mathbf{2})_0 = 2.35\times10^{-5}$ *M*, 0.1*M* sodium acetate buffer, pH = 5.6; λ (nm): ○ - 665, □ - 603, ◇ - 548, ▽ - 429, △ - 310.

This program can calculate stability constants and molar absorption coefficients for any system that can be described by the reactions

$$p\,P + q\,Q + r\,R \Leftrightarrow P_pQ_qR_r \tag{11}$$

If a set of experimental data A = f (c_P, c_Q, c_R) is available where A are absorbance values and c_P, c_Q, c_R the total analytical concentrations of the components P, Q and R, the stability constants of the complexes $P_pQ_qR_r$, defined as

$$K(P_pQ_gR_r) = \frac{[P_pQ_gR_r]}{[P][Q][R]} \tag{12}$$

and molar absorption coefficients of all the colored species can be calculated simultaneously. A set of M·**2**, M·**2**$_2$ and M·**1** complexes are of course also $P_pQ_qR_r$ – type.

The sum of the deviation of measured (A_{exp}) and calculated (A_{calc}) values of absorbances was minimized, i.e.

$$U = \Sigma(A_{exp} - A_{calc})^2 \tag{13}$$

The calculation covers all complexes formed in this system and includes the amount of uncomplexed ligand and free metal ion. The best values of stability constant and molar absorption coefficients for all wavelengths are calculated from all experimental data and all wavelengths using a single computer run. Each data set, i.e. static titration curve, was prepared and measured three times and the average values of the calculated constants are given in Table **2** with a standard deviation, defined as

$$s_i = \sqrt{\frac{1}{n-1} * \sum\left(\beta_i - \overline{\beta_i}\right)^2} \tag{14}$$

where $\overline{\beta_i}$ is the average value of the constant β_i and n = 3.

The conditional stability constants of Y-**2** complexes were measured and calculated from absorbance data at wavelengths 663, 603, 558, 416 and 316 nm (Fig. **7**) in the same manner (Table **2**).

Table 2. The Conditional Stability Constants log β' of Metal-Arsenazo III Complex Determined by UV-VIS Spectrophotometry. The Standard Deviations *s* were Calculated from Eq. 14

Specie	log β' Ho·2	log β' Y·2
Metal-2	5.7 (*s* = 0.2)	6.1 (*s* = 0.2)
Metal-2$_2$	10.0 (*s* = 0.3)	10.4 (*s* = 0.2)

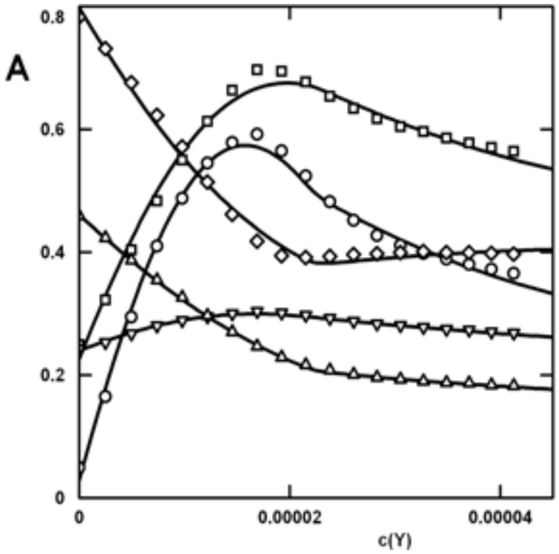

Fig. (7). Spectrophotometric titration of Arsenazo solution by the solution of YCl$_3$. $C(\mathbf{2})_0 = 2.35\times10^{-5}$ *M*. 0.1*M* sodium acetate buffer, pH = 5.6; λ (nm): ○ -663, □ - 603, ◇ - 558, ▽ - 416, △ - 317.

Determination of the conditional stability constants of metal-**2** complexes demonstrated that both complexes exhibit similar stability.

The major objective of this work was to determine the stability constants of metal – **1** complexes. The conditional stability constants of metal-ligand complexes were calculated from experimental data depicted in Figs. (**8** and **9**) in the same way as the conditional stability constants of M**2** and M**2**$_2$ (Table **3**). The conditional stability constants of metal **2** complexes could be used for calculating the conditional stability constants of a metal – ligand complex because the pH values were identical in both cases (pH = 5.6). The values of the conditional stability constants of metal – **1** complex *K*' were obtained by these calculations. Three sets of data were used for these calculation and the average values of constant K' with standard deviation calculated from Eq. 14 are summarized in Table **3**.

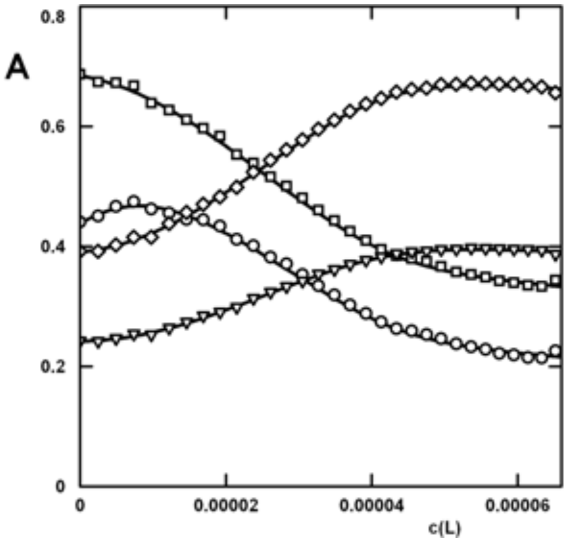

Fig. (8). Spectrophotometric titration of Ho – Arsenazo III solution by the solution of DOTA(NH_2). $C(2)_0 = C(\text{Ho})_0 = 2.35\times10^{-5}$ *M*. λ (nm): ○ - 663, □ - 605, ◇ - 552, ▽ - 316.Solid curves were calculated for constants given in Table **3**.

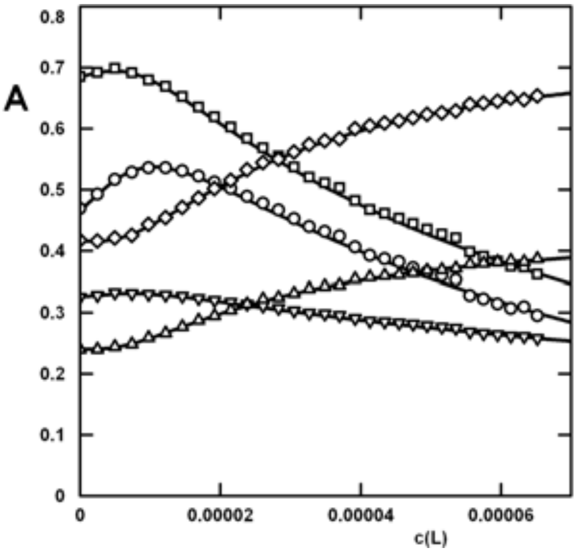

Fig. (9). Spectrophotometric titration of Y – Arsenazo III solution by the solution of DOTA(NH_2). $C(\mathbf{2})_0 = C(\text{Y})_0 = 2.35\times10^{-5}$ mol/l. λ (nm): ○ - 658, □ - 605, ◇ - 554, ▽ - 425, △ - 316. Solid curves were calculated for constants given in Table **3**.

The stability constants (*K*) were determined as the product of the conditional stability constant of the metal-ligand complex (*K*') and coefficient α at pH = 5.6.

Table 3. The Conditional Stability Constants log *K*' and Stability Constants Log *K* of Metal-Ligand Complexes Determined by UV-VIS Spectrophotometry. The Standard Deviations *s* were Calculated from Eq. 14

Specie	*log K'*	*log K*
Ho-DOTA(NH_2)	6.8 (*s* = 0.3)	18.0 (*s* = 0.3)
Y-DOTA(NH_2)	6.6 (*s* = 0.2)	17.8 (*s* = 0.2)

The Ho-DOTA(NH_2) complex is characterized by a higher stability constant (log *K* = 18.0) than the Y-DOTA(NH_2) complex (log *K* = 17.8).

Due to the absence of DOTA(NH_2) stability constants in available literature, DO3A-B (10-[2,3-dihydroxy-(1-hydroxymethyl)-propyl]-1,4,7,10-tetraazacyclododekan-1,4,7-triacetic acid) was chosen as the ligand most closely resembling DOTA(NH_2) for comparison [9], [12]. The determined stability constant of the Ho-DOTA(NH_2) complex (log *K* = 18.0), was compared with the stability constant of the Ho-DO3A-B complex (log *K* = 18.9 (0.02) [9]). It follows that the Ho-DOTA(NH_2) complex is less stable than Ho-DO3A-B.

4. CONCLUSIONS

The static potentiometry was used for determination of the protonation constants of DOTA(NH_2) and calculation of coefficient log α. The study of DOTA(NH_2) with Ho^{3+} and Y^{3+} complexation was performed by using the UV-VIS spectrophotometry.

It was found that in pH range 2 – 10 dissociation of DOTA(NH_2) reached the equilibrium after 64 hours. The values of protonation constants determined by static potentiometry are log β_1 = 12.7; log β_2 = 21.4 and log β_3 = 27.9.

Coefficient log α = 11.2 at pH = 5.6 was calculated using the above protonation constants.

It was found that the metal-DOTA(NH_2) complex was formed in three days under given conditions. The stability constants of Ho^{3+} and Y^{3+} complexes using UV-VIS spectrophotometric titration were log $K_{\text{Ho-DOTA(NH2)}}$ = 18.0 and log $K_{\text{Y-DOTA(NH2)}}$ = 17.8.

The knowledge of the protonation and stability constants of DOTA(NH_2) will be utilized in our future experiments focused on the preparation of radioimmunoconjugates labeled with derivatives of ^{166}Ho-DOTA(NH_2) and ^{90}Y-DOTA(NH_2) complexes.

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