

Isotachophoretic determination of stability constants of Ho and Y complexes with diethylenetriaminepentaacetic acid and 1,4,7,10-tetraazadodecane-N,N',N'',N'''-tetraacetic acid

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Abstract

The method of capillary isotachopheresis with conductivity detection was applied for the determination of the physico-chemical characteristics (conditional stability constants $\log \beta'$) of holmium and yttrium complexes with DTPA (diethylenetriaminepentaacetic acid) and DOTA (1,4,7,10-tetraazadodecane-N,N',N'',N'''-tetraacetic acid). The $\log \beta'$ determination is based on the linear relation between the stability constants of lanthanide–DTPA (lanthanide–DOTA) complexes and the reduction of the zone of the complex owing to the bleeding phenomena (liberating free metal ion). The stability constants calculated using this relationship are comparable with the literary data obtained by other methods for both holmium ($\log \beta'_{\text{Ho-DTPA}}=21.9$, $\log \beta'_{\text{Ho-DOTA}}=24.5$) and yttrium complexes ($\log \beta'_{\text{Y-DTPA}}=21.2$, $\log \beta'_{\text{Y-DOTA}}=24.4$). Capillary isotachopheresis was applied for the determination of the optimum composition of the reaction mixture (metal:ligand ratio) as well.

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1. Introduction

DTPA (diethylenetriaminepentaacetic acid), DOTA (1,4,7,10-tetraazadodecane-N,N',N'',N'''-tetraacetic acid) and their derivatives, are widely applied in the field of radioactively labeled monoclonal antibodies for radiopharmaceutical purposes [1–5]. These bifunctional chelation agents enable the indirect labeling of antibodies by various radionuclides with their nuclear properties suitable for therapeutical use, e.g. ^{166}Ho , ^{90}Y , $^{186,188}\text{Re}$. The system radionuclide–bifunctional chelator–monoclonal antibody is very complex, and, consequently,

understanding the mutual interaction of all components involved would allow formulating better characteristic of the conjugate as a whole.

A variety of methods have been applied for the determination of stability constants of metal–chelator complexes as e.g. potentiometry [6,7], voltammetry [8], spectroscopic techniques [9] and capillary electrophoresis [10,11]. The application of isotachopheresis for the determination of the conditional stability constants in the case of stable complexes having large $\log \beta'$ values (as metal–EDTA complexes) was first discussed by Gebauer et al. [12]. Under proper operating conditions (sufficiently low pH of leading electrolyte), the isotachophoretic zone of the metal–EDTA complex anion gradually liberates free metal cation due to zone-bleeding phenomena and

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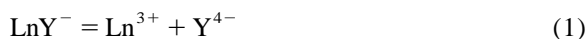
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the zone length of the complex is reduced. It was shown that the zone reduction depends on the conditional stability constant and the decomposing zones are predicted to be isotachophoretically stable and can be utilized for quantitative analysis. Linear relation between $\log \beta'$ and \log [zone reduction] was successfully proven experimentally [13] for EDTA complexes of lanthanides.

In this communication, according to the known fact that lanthanides form stable complexes with derivatives of acetic acid (DTPA, DOTA) we attempted first to verify a linear relation $\log \beta' - \log$ [zone reduction] for lanthanide complexes with DOTA and DTPA and, consequently, to determine conditional stability constants of holmium and yttrium complexes by capillary isotachopheresis. The second part of our paper deals with the determination of the optimum metal:ligand ratio to reach maximum complexation yield of studied complexes. Results obtained in this study were compared with those found in the literature.

2. Theory

The experiment assumes that the dissociation equilibrium of the DOTA (or DTPA) complexation with lanthanide is sufficiently fast in comparison with electrophoretic migration and the system can be described by the following equation:



where Ln^{3+} is lanthanide cation and Y^{4-} is DOTA (or DTPA) anion.

Eq. (1) is sufficient for the present purpose in spite that the formed Y^{4-} in the equilibrium in fact immediately undergoes hydrolysis to form H_xY^{x-4} , depending on pH. It can be assumed that, in the isotachophoretic zone of complex LnY^- , the equilibrium concentration of free metal cation $[\text{Ln}^{3+}]$ is equal to the equilibrium concentration of ligand anion $[\text{Y}^{4-}]$. Therefore, the definition of conditional stability constant can be written as follows:

$$\beta' = \frac{[\text{LnY}^-]}{[\text{Ln}^{3+}][\text{Y}^{4-}]} = \frac{[\text{LnY}^-]}{[\text{Ln}^{3+}]^2} \quad (2)$$

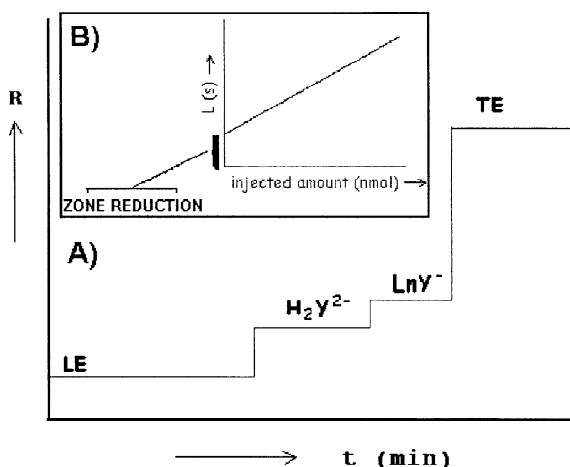


Fig. 1. (A) Illustrative isotachopherogram of lanthanide complexation with DOTA and DTPA; H_2Y^{2-} , free complex agent ion (DOTA, DTPA); LnY^- , lanthanide complex; LE, leading electrolyte (20 mmol/l HCl–glycine, pH 3.1); TE, terminating electrolyte (20 mmol/l NH_3 –acetic acid, pH 4.8); R, specific resistance. (B) Example of the determination of zone reduction value; L, length of the zone of complex LnY^- .

and in the logarithmic form:

$$\log \beta' = \log [\text{LnY}^-] - 2 \log [\text{Ln}^{3+}] \quad (3)$$

The equilibrium concentration of $[\text{LnY}^-]$ is almost constant for different lanthanide ions, thus the $\log \beta'$ is proportional to the concentration of free metal cation, $[\text{Ln}^{3+}]$, and this is directly related to the instability of the complex zone [13]. So the linear relation between conditional stability constant and zone reduction must exist as generally described in the following equation:

$$\log \beta' = b - 2 \log [\text{zone reduction}] \quad (4)$$

In order to plot this dependence, it is necessary to determine the values of zone reduction for different Ln–Y complexes. This can be done by measuring the length of the zone of the complex for various sample amounts injected (molar ratio of lanthanide:ligand = 1:1). The dependence obtained this way can be described by the linear equation where the intercept is the zone reduction, as illustrated in Fig. 1.

3. Experimental

The experiments of capillary isotachopheresis (ITP) were carried out in a CS capillary electrophoretic analyzer EA 100 (Labeco, Spišská Nová Ves, Slovakia) with two separation columns. Both pre-separation (FEP, 90 mm length \times 0.8 mm I.D.) and analytical (FEP, 160 mm length \times 0.3 mm I.D.) columns were equipped with conductivity detection. Current in the analytical column was set at 50 μ A.

All experiments were performed in the following set of electrolytes: 20 mmol/l HCl buffered by glycine at pH 3.1 was used as the leading electrolyte, the terminating electrolyte contained 20 mmol/l ammonia solution buffered by acetic acid at pH 4.8. Chemicals for preparation of buffers except glycine (Merck, Darmstadt, Germany) were purchased from Lachema (Brno, Czech Republic) and were of analytical grade. All chemicals used for preparing samples were obtained from the following: DOTA (Sigma–Aldrich, Prague, Czech Republic), DTPA (Koch & Light, Hatfield, UK), holmium chloride, yttrium chloride, europium chloride, lanthanum chloride, cerium nitrate, ytterbium nitrate, praseodymium nitrate, gadolinium nitrate, erbium nitrate and neodymium nitrate (all Sigma–Aldrich). Their purity was of analytical grade or the highest available.

The calibration samples for the determination of the zone reduction and for the plot of $\log \beta' - \log$ [zone reduction] were prepared from the 10 mmol/l stock solution of Ln^{3+} ($\text{Ln} = \text{La, Ce, Pr, Nd, Eu, Yb}$ in the case of the complexation with DTPA; $\text{Ln} = \text{La, Ce, Eu, Er, Gd, Yb}$ for the complexation with DOTA) and added at an equimolar amount of DTPA and DOTA, respectively. The samples containing holmium and yttrium (chlorides, Sigma–Aldrich) were prepared in the same way. The pH of the samples was adjusted to 4.8 by 1 mol/l NaOH (Lachema). The values of $\log \beta'$ for Ln –DOTA and Ln –DTPA complexes were obtained from the SC Query Database [14].

The samples for the determination of the optimum metal:ligand ratio were prepared by mixing of calculated volumes of 1 mmol/l stock solutions of HoCl_3 (YCl_3) and DOTA, resp. DTPA. After pH adjustment the sample volume was made up to 5 ml by Milli-Q water. The concentration of Ho^{3+} resp. Y^{3+} in final solution was $1 \cdot 10^{-4}$ mol/l, concentration of

ligand ranged from $2 \cdot 10^{-5}$ to $7 \cdot 10^{-4}$ mol/l (metal: ligand ratio 5:1–1:7).

4. Results and discussion

4.1. Determination of stability constants

Data for the estimation of conditional stability

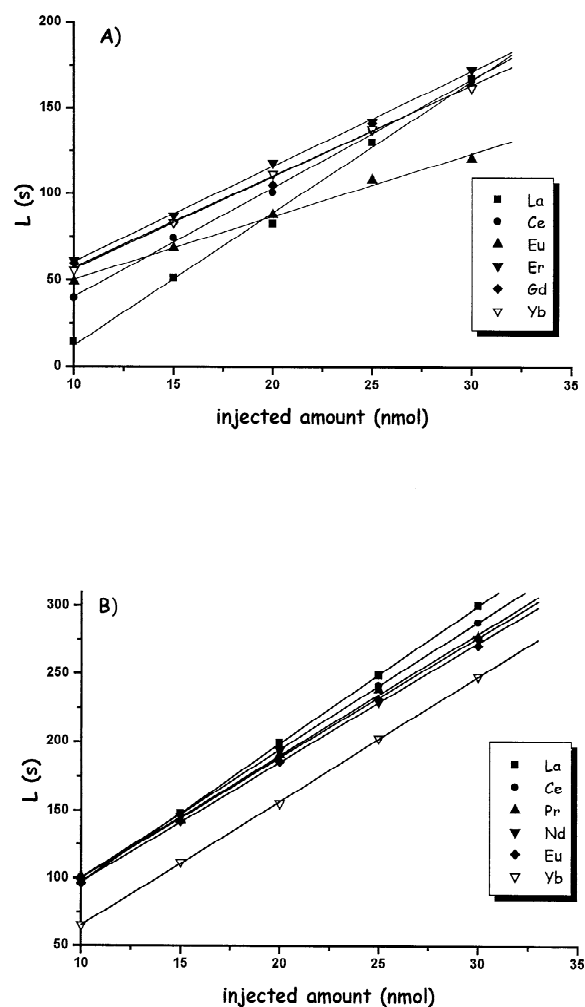


Fig. 2. Calibration curves for the determination of the zone reduction values for lanthanide complexes with (A) DOTA; (B) DTPA [15]. LE, 20 mmol/l HCl–glycine, pH 3.1; TE, 20 mmol/l NH_3 –acetic acid, pH 4.8, $I = 50 \mu\text{A}$. Samples: $c(\text{Ln}^{3+}) = c(\text{DTPA}) = c(\text{DOTA}) = 0.33\text{--}1.0$ mmol/l, pH 4.8, injection 30 μl .

constants of Y–DOTA, Ho–DOTA, Y–DTPA and Ho–DTPA are summarized in Figs. 2–4. Fig. 2 shows the relation between the length of the LnY⁻ zone and the sample amount injected for the obtaining of the LnY⁻ zone reduction values of calibration sets of both lanthanide–DOTA and lanthanide–DTPA complexes. According to the linear character of all dependencies the zone reduction values were determined from the intercept of calibration lines. Using results obtained the dependencies of $\log \beta'$ on $\log [\text{zone reduction}]$ for DOTA and DTPA complexes were plotted (see Fig. 3). Both of them confirmed the prediction of linearity and thus they were used for the determination of conditional stability con-

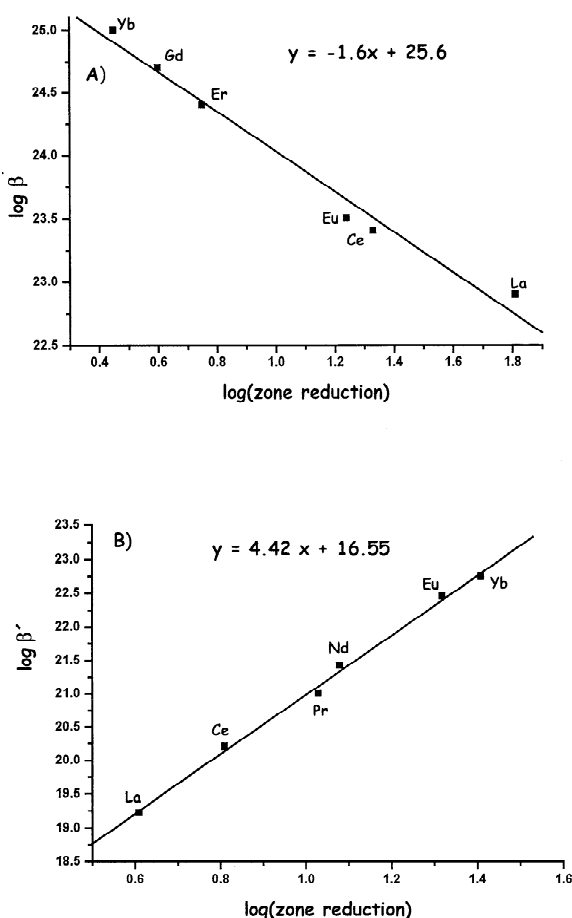


Fig. 3. The relationship between $\log \beta'$ and $\log [\text{zone reduction}]$ for (A) Ln–DOTA; (B) Ln–DTPA [15] complexes. Source for $\log \beta'$: SC Query Database [14]; for experimental conditions see Fig. 2.

stants of DOTA and DTPA complexes with studied metals. The reason for the reverse orientation of Ln–DOTA and Ln–DTPA curves can be found in the character of studied ligands. Both complexation agents belong to the derivatives of acetic acid, however, their behavior differs owing to their structural dissimilarity that can influence the instability of the Ln–DOTA zone during the isotachopheric separation under selected separation conditions.

Fig. 4 summarizes calibration data for the determination of the zone reduction of studied yttrium and holmium complexes. Using data obtained from these dependencies, the equations describing the $\log \beta' - \log [\text{zone reduction}]$ relations were applied to calculate the conditional stability constants of DOTA and DTPA complexes with holmium and yttrium. Considering the similar behavior of holmium and yttrium complexes as demonstrated in our previous study [15] we decided to apply the same $\log \beta' - \log [\text{zone reduction}]$ relation of lanthanide complexes for the determination of $\log \beta'$ of both holmium and yttrium complexes, in spite of the fact that yttrium does not belong to the group of lanthanides. The resulting conditional stability constants of yttrium and holmium complexes were compared with available literary data and summarized in Table 1. From the present data it can be concluded that the conditional stability constants of all complexes studied are in good agreement with the literary data and the

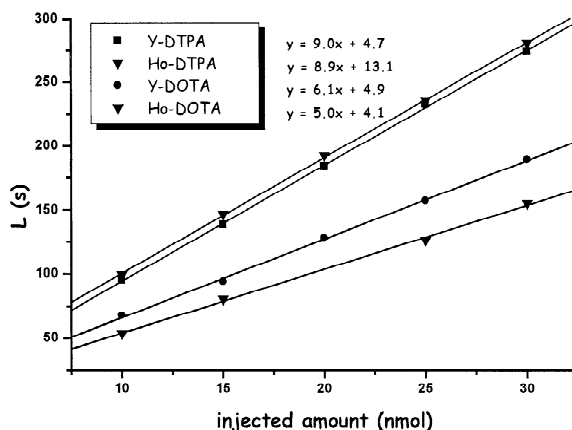


Fig. 4. Calibration curves for the determination of the zone reduction of Ho and Y complexes with DOTA and DTPA ($c(\text{Ho}^{3+}) = c(\text{Y}^{3+}) = 0.33\text{--}1.0 \text{ mmol/l}$); for experimental conditions see Fig. 2.

Table 1
Conditional stability constants of Ho and Y complexes with DOTA and DTPA

Complex	Log β'^a	Log $\beta'^{b,c}$
Y–DTPA	21.2±0.2	21.9 ^b
Ho–DTPA	21.9±1.2	22.7 ^b
Y–DOTA	24.4±0.1	24.9 ^c
Ho–DOTA	24.5±0.5	24.5 ^c

^a Results obtained in this work.

^b Results published in Ref. [14].

^c Results published in Ref. [16].

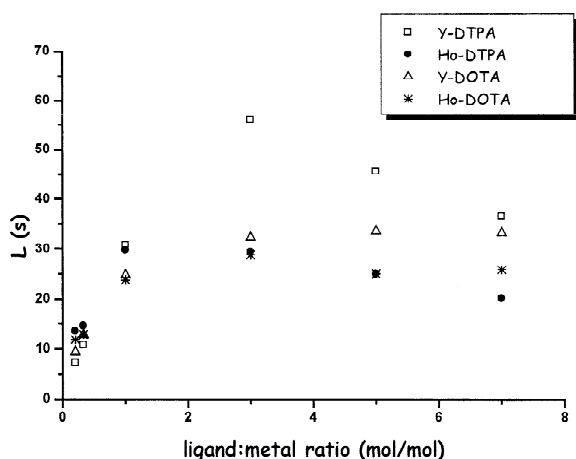


Fig. 5. The influence of ligand:metal ratio on the length of the zone of complex $c(\text{Ho}^{3+})=c(\text{Y}^{3+})=0.1$ mmol/l; $c(\text{DTPA})=c(\text{DOTA})=0.02\text{--}0.7$ mmol/l; for experimental conditions see Fig. 2.

method of capillary isotachopheresis can also be applied for the estimation of the stability constants of rare earth metal complexes.

4.2. Determination of optimum composition of reaction mixture

For the development of the radiopharmaceuticals it is very important to find the proper preparation conditions, which would lead to the maximum complexation yield. The optimum composition of the reaction mixture is one of them. The influence of ligand:metal ratio on the length of the LnY^- zone is demonstrated in Fig. 5 for all studied complexes. It can be seen that to get a maximum length of the complex zone it is necessary to reach ligand:metal ratio at least 1:3 in all cases. The detailed results are shown in Table 2. By comparing the present results with data revealed by the thin-layer chromatography with radiometric detection [15], a good agreement was obtained.

5. Conclusions

Capillary isotachopheresis proved a suitable method, which by utilizing the $\log \beta' - \log [\text{zone reduction}]$ relation, allows the determination of the conditional stability constants of DOTA and DTPA complexes of lanthanides. This approach can be exploited also for the estimation of the stability

Table 2
Optimum metal:ligand ratios for the Ho and Y complexation with DOTA and DTPA; a comparison of ITP and TLC with radiometric detection [15]

Complex	ITP		TLC [15]	
	Metal:ligand ratio	c (mol/l)	Metal:ligand ratio	c (mol/l)
Y–DTPA	1:3	Y ³⁺ : $1 \cdot 10^{-4}$ DTPA: $3 \cdot 10^{-4}$	1:2–1:9	Y ³⁺ : $9.7 \cdot 10^{-4}$ DTPA: $0.9\text{--}3.9 \cdot 10^{-4}$
Ho–DTPA	1:1–1:3	Ho ³⁺ : $1 \cdot 10^{-4}$ DTPA: $1 \cdot 10^{-4}\text{--}3 \cdot 10^{-4}$	1:3–1:10	Ho ³⁺ : $1.8 \cdot 10^{-4}$ DTPA: $2.6 \cdot 10^{-4}\text{--}8.5 \cdot 10^{-4}$
Y–DOTA	1:3–1:7	Y ³⁺ : $1 \cdot 10^{-4}$ DOTA: $3 \cdot 10^{-4}\text{--}7 \cdot 10^{-4}$	1:3–1:7	Y ³⁺ : $2.7 \cdot 10^{-4}$ DOTA: $2.6 \cdot 10^{-4}\text{--}6.2 \cdot 10^{-4}$
Ho–DOTA	1:3	Ho ³⁺ : $1 \cdot 10^{-4}$ DOTA: $3 \cdot 10^{-4}$	1:2–1:9	Ho ³⁺ : $2.1 \cdot 10^{-4}$ DOTA: $1\text{--}9 \cdot 10^{-4}$

constants of rare earth metal complexes as it was demonstrated with yttrium complexation with the studied ligands. The conditional stability constants of Ho–DOTA, Ho–DTPA, Y–DOTA and Y–DTPA are all in very good agreement with the literary data.

Capillary isotachopheresis was also successfully applied for the determination of the optimum metal:ligand ratio and the results obtained in this part of our work confirmed the values obtained by thin-layer chromatography with radiometric detection.

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