

INVESTIGATION ON THE INTERACTION BETWEEN $^{111}\text{In}^{3+}$ AND DTPA IN WATER BY ELECTROMIGRATION ANALYSIS

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ABSTRACT

Gamma radionuclide ^{111}In is often used in radiodiagnostics due to his suitable characteristics. Its typical clinical form is as a chelate of some polyaminopolycarboxylic acids, in most cases - DTPA. Therefore, it is of a practical interest to understand the chemical stability of the complex In-DTPA, as well as the Indium-DTPA equilibrium system kinetic. In this paper some results of investigations of behaviour of $^{111}\text{In(III)}$ in aqueous solutions in the presence of DTPA carried out by the method of horizontal zone electrophoresis in a free electrolyte are presented. Some thermodynamic parameters have been estimated as follows: effective charge of the complex In-DTPA $z_{\text{eff.}} = -1,7$; stability constant $\beta = (1,5 \pm 0,3) \cdot 10^{+29}$; kinetic constants for reactions of formation (k_1) and degradation (k_2) of chelate: $\lg k_1 = -1,0$ and $\lg k_2 = -2,6$.

Key words: InDTPA, ^{111}In , radiodiagnostics, kinetics, electromigration

INTRODUCTION

^{111}In is a well-known radioisotope due to his suitable characteristics such as convenient half-life, energy of gamma-emission, high photo efficiency, as well as availability of methods for his production and radiochemical purification. In most cases it is used for generating labeled monoclonal antibodies (14,18,16), somatostatin analogues (11) and lipoproteins (12) as well as in radionuclide tumour diagnostic (13,15) and cisternography (5,17). It is a proven technique to introduce the radioactive Indium as complexes with polyaminopolycarboxylic acids (*complexons*), because of their extreme stability and solubility in water systems. Some of the most popular complexons used are: EDTA (ethylenediaminetetraacetic acid), DTPA (diethylenetriaminepentaacetic acid), DCTA (1,2-diaminocyclohexanetetraacetic acid), NTA (nitrilotriacetic acid), etc.

It is possible to use many standard techniques to analyze thermodynamic as well as kinetic characteristics of Indium radiopharmaceuticals used in practice. The matter of these radioanalytical problems draws attention to the usefulness of the electromigration methods. From the experimental point of view, advantage is a nanogram amount of substance needed (in case of ^{111}In - usually no more than 10 μCi) as well as the wide range of the experimental conditions available (pH = 0÷14, temperature 0÷100°C, ionic

strength of solution 0÷10 mol.l⁻¹, Indium concentration 10⁻⁵ ÷ 10⁻⁹ mol.l⁻¹). Moreover, the electromigration method in a free electrolyte traditionally ensures analyzing the aqueous systems in their genuine state, because there are no side processes detected into the system (e.g., phase surface or capillary effects). There should be mentioned that the ^{111}In isotope is ideal for the electromigration technique due to its soft gamma energy radiation. From the methodical point of view, advantage is the fact that investigations of a different kind could be done using a single device, which substantially reduces the time consumption as well as the risk of experimental error accumulation.

In the present paper an experimental cycle on investigation of the behaviour of Indium ions in aqueous solutions in the presence of DTPA as well as on characterization of the radiopharmaceutical In-DTPA complex stability and kinetics is discussed.

MATERIAL AND METHODS

A carrier-free ^{111}In [$T_{1/2} = 2,83$ d, $E_{\gamma} = 171\text{keV}$ (90,3%), 245keV (94,0%)] was used. It was produced by the irradiation of the natural silver target with a 30MeV α -particles on the U-200 accelerator at JINR-Dubna, Russia. After the extraction of ^{111}In from the silver target and the appropriate radiochemical purification (10) $^{111}\text{In}(\text{NO}_3)_3$ of a high specific activity was obtained.

The experimental results concerning the state and behaviour of ^{111}In ions in the analyzed solutions are collected using a fully automatized device for horizontal zone electrophoresis (6). In each experimental cycle 5-10 Ci $^{111}\text{In(III)}$ were injected into the electromigration cell filled-in with the suitable electrolyte. The temperature of the liquid media

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was maintained constant with an accuracy of 0,05°C, pH of a solution was set and measured with an accuracy of 0,05. The gradient of the electric field was set to 10V.cm⁻¹ and maintained constant with an accuracy of 0,03V.cm⁻¹. Bidistilled water as well as *Suprapur*® *Merck*® reagents was used in order to prepare the solutions.

The experimental results were processed by specialized numerical methods software - *Equilibrium* (1), *Matrix* (2), *Microcal*™ *Origin*®.

RESULTS AND DISCUSSION

The formation of the radiopharmaceutical complex In-DTPA²⁻ could be described by the net equilibrium reaction as follows:

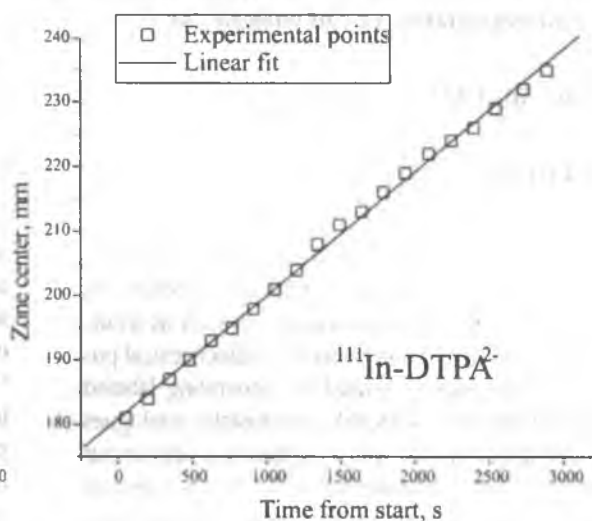
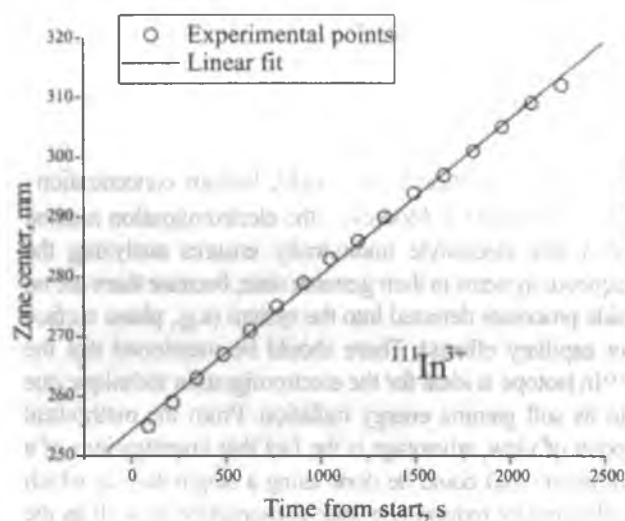


Fig. 1. Tracking the electromigration of zones

Studying equilibrium systems of that kind one could apply the method of electrodiffusion developed recently (9) in order to collect kinetic data needed. According to the theory, the kinetic constants of an equilibrium process (k_1 - forward, k_2 - reverse direction) are connected to the electrodiffusion coefficient of the system (D_e , m².s⁻¹), the individual ion mobilities of the concurrent ionic forms (u_1 - metal ions, u_2 - complex ions, m².V⁻¹.s⁻¹), the gradient of the electric field (E , V.m⁻¹), and the equilibrium concentration of the ligand ($[L]$, in our case - [H₃DTPA], mol.l⁻¹):

$$(2) \quad D_e = \frac{E^2 k_1 k_2 [L] (u_1 - u_2)}{(k_1 [L] + k_2)^3}$$

In order to find out the kinetic constants k_1 and k_2 we need to experimentally determine the values of the individual ion mobilities as well as system electrodiffusion coefficient under precisely identical conditions (pH, solution's ionic strength and temperature, gradient of the electric field and

ligand concentration). In addition, we should have at least one more relation between parameters k_1 and k_2 . For such an additional relation the definition of the equilibrium constant (K) of the chemical system (1) could be used:

$$(3) \quad K = k_1/k_2.$$

The equilibrium constant K of the process (1) is connected to the full protolytic constant of the H₃DTPA [$K_a = 2,6 \cdot 10^{-28}$, (3)] and the stability constant of the complex In-DTPA²⁻ (β):

$$(4) \quad K = K_a \beta.$$

The stability constant β is a parameter that could be experimentally found using a routine electromigration method. As mentioned above, one of the advantages of the electromigration approach to the problem is that all the val-

ues needed are found using a single device that reduces the level of the experimental error.

In³⁺ and In-DTPA²⁻ individual ion mobility determination

The ionic form being examined was injected into the migration tube, forming in such a way a narrow radioactive zone in the solution. After the application of the constant electric field, an electrophoretic movement of the whole zone was detected. The constant velocity of the movement was measured by periodically scanning the tube using a moveable gamma detector (Fig. 1).

Indium(III) ions mobility (u_1) was measured in a H₂O/HNO₃ solution with pH = 1,00; ionic strength 0,100; temperature 25,0°C; gradient of the electric field 8,0V.cm⁻¹. The value was found to be:

$$u_1 = (3,25 \pm 0,04) \cdot 10^{-4} \text{ cm}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$$

In-DTPA^{2-} ions mobility (u_2) was measured in a $\text{H}_2\text{O}/\text{HNO}_3/\text{DTPA}$ solution with $\text{pH} = 1,00$; ionic strength 0,100; temperature $25,0^\circ\text{C}$; gradient of the electric field $10,0 \text{ V}\cdot\text{cm}^{-1}$; $[\text{DTPA}] = 1 \cdot 10^{-4} \text{ mol}\cdot\text{l}^{-1}$. The value was found to be:

$$u_2 = (-1,93 \pm 0,03) \cdot 10^{-4} \text{ cm}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$$

In-DTPA^{2-} stability constant determination

The overall mobility (u) of the system $^{111}\text{In}^{3+}/\text{DTPA}$ in correlation of the chelate concentration was studied (Fig. 2).

The values of the individual ion mobilities u_1 and u_2 as well as the equilibrium ligand concentration in each experiment are known; therefore the value of the system stability constant β could be retrieved. Though a numerical methods should be used.

The determination of the $^{111}\text{In-DTPA}^{2-}$ stability constant β was done in a $\text{H}_2\text{O}/\text{HNO}_3/\text{KNO}_3/\text{DTPA}$ solution with ionic strength 0,500; temperature $25,0^\circ\text{C}$; gradient of the electric field $10,0 \text{ V}\cdot\text{cm}^{-1}$; $[\text{DTPA}] = 1 \cdot 10^{-4} \text{ mol}\cdot\text{l}^{-1}$. The estimated value was:

$$\beta = (1,5 \pm 0,3) \cdot 10^{29}$$

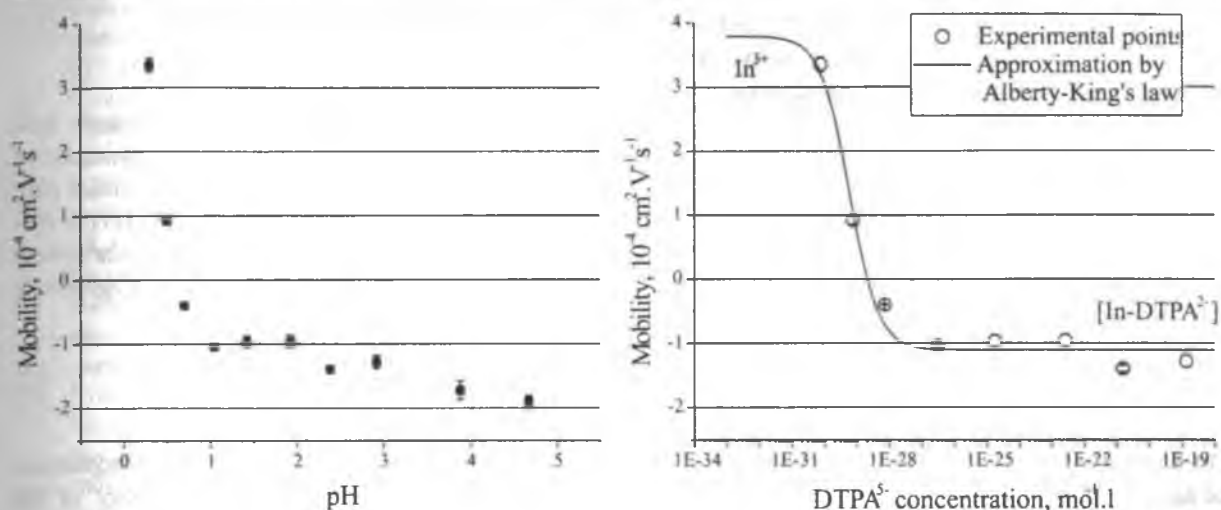


Fig. 2. $^{111}\text{In}^{3+}/\text{DTPA}$ system overall mobility vs. pH of a solution (left) and vs. DTPA^{5-} equilibrium concentration (right)

The concentration of the chelate was controlled by fine adjustment of the solution's pH . According to Alberty-King's equation (8),

There was no literature data for comparison, except a theoretical estimation $\beta \approx 10^{27}$ (4) which showed an excellence agreement.

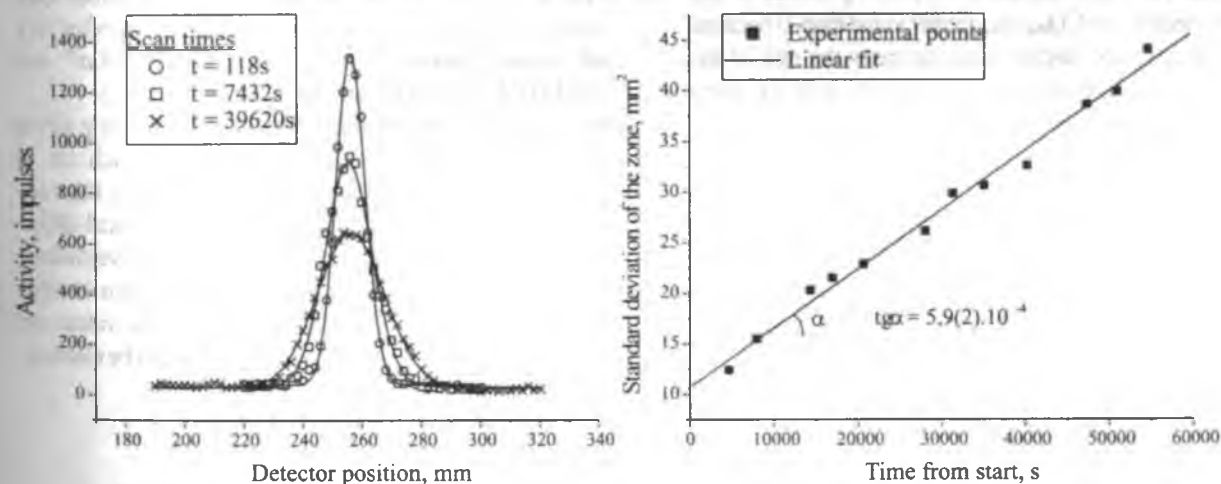


Fig. 3. Diffusion expansion of a zone, containing In-DTPA^{2-} . Some concentration profiles (left) and the linear model (right)

$$(5) \quad u = \frac{u_1 + u_2 \beta [L]}{1 + \beta [L]}$$

$\text{In}^{3+}/\text{DTPA}$ system diffusion constant determination

The diffusion coefficient D of In-DTPA^{2-} ions in the specific solution could be determined measuring the speed of

the spontaneous radioactive zone expansion with the electric field switched off (9). It has been shown (ibid.) that there is a linear relation between the time and the half-width square of the zone as demonstrated on Fig. 3.

The determination of the $^{111}\text{In-DTPA}^{2-}$ diffusion coefficient D was done in the $\text{H}_2\text{O}/\text{HNO}_3/\text{DTPA}$ solution with $\text{pH}=4,50$; ionic strength 0,020; temperature $25,0^\circ\text{C}$; gradient of the electric field $12,1\text{ V}\cdot\text{cm}^{-1}$; $(\text{DTPA})=5\cdot 10^{-3}\text{ mol}\cdot\text{l}^{-1}$. The value was:

$$D = (2,93 \pm 0,09) \cdot 10^{-6}\text{ cm}^2\cdot\text{s}^{-1}$$

It should be mentioned that the data concerning ion mobility and coefficient of diffusion could be used to derive information about ionic effective charge (z_{eff}) according to the *Nernst-Einstein's* equation:

$$(6) \quad D = \frac{RT}{z_{\text{eff}}F} u,$$

where:

- R - universal gas constant, $8,314\text{ J}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$;
T - temperature, K;
F - the Faraday constant, $96\,485\text{ C}\cdot\text{mol}^{-1}$.

Hence, for the effective charge of $^{111}\text{In-DTPA}$ ion we found the value $z_{\text{eff}} = -1,7$. One could notice that finding the effective charge of the complex has no relation with the topic, but could be treated as an easy-obtained side result. Knowing that complexons such as DTPA interact with metal ions usually at ratio 1:1 the value obtained is an expected one.

After measuring the ordinary diffusion coefficient D we proceed to the determination of the diffusion coefficient D_{exp} of the same system, this time in the presence of the constant electric field. D_{exp} has greater value than D because the presence of the electric field increases the rate of the zone expansion. The experiment follows exactly the same procedure described above; the difference is that the electric field is switched on.

Determination of the diffusion coefficient D_{exp} of the $^{111}\text{In-DTPA}^{2-}$ ion in the electric field was carried out into $\text{H}_2\text{O}/\text{HNO}_3/\text{DTPA}$ solution with $\text{pH} = 1,00$; ionic strength 0,100; temperature $25,0^\circ\text{C}$; gradient of the electric field $10,0\text{ V}\cdot\text{cm}^{-1}$; $(\text{DTPA}) = 1\cdot 10^{-4}\text{ mol}\cdot\text{l}^{-1}$. The value was found to be:

$$D_{\text{exp}} = (4,73 \pm 0,38) \cdot 10^{-5}\text{ cm}^2\cdot\text{s}^{-1}$$

Consequently, the value of the electrodiffusion coefficient D_e is equal to the difference between measured diffusion coefficients in the presence (D_{exp}) and absence (D) of electric field:

$$(7) \quad D_e = D_{\text{exp}} - D.$$

Therefore, the electrodiffusion coefficient of the $^{111}\text{In}^{3+}/\text{DTPA}$ system under the condition mentioned was estimated to be:

$$D_e = (4,44 \pm 0,39) \cdot 10^{-5}\text{ cm}^2\cdot\text{s}^{-1}$$

In-DTPA²⁻ kinetic constants determination

Finally, after the collection of all the data needed, the kinetic parameters of the system (1), i.e., the kinetic constants of formation (k_1) and degradation (k_2) of the complex $^{111}\text{In-DTPA}^{2-}$ could be calculated, according to equations (2)-(4). Here are the values found:

$$k_1 = 9,3 \cdot 10^{-2} \quad (\lg k_1 = -1,0)$$

$$k_2 = 2,4 \cdot 10^{-3} \quad (\lg k_2 = -2,6)$$

The relative error in the determination of the kinetic constants was estimated as 32%. Error in the determination of the complex stability constants represents the major part. There was no kinetic literature data about In/DTPA systems, so we can cite the value of k_2 for the similar system $\text{Eu}^{3+}/\text{DTPA}$: $\lg k_2 = -2,9$ (7), which shows a good agreement with our results.

CONCLUSION

Applying the method of the horizontal zone electrophoresis in a free electrolyte for studying the behavior of the $^{111}\text{In}/\text{DTPA}$ system in aqueous solutions leads to gathering of valuable thermodynamic and kinetic data as well. It has been proven that $^{111}\text{In-DTPA}^{2-}$ complex ion is a very stabile one and, hence, it is the basic formation existing in the In(III) solutions in DTPA presence in a wide range of concentrations, temperature and pH of the solution. Chemical equilibrium consisting of the formation and destruction of the complex $^{111}\text{In-DTPA}^{2-}$ can be described as a labile type because both forward and backward reactions are relatively fast (mean lifetimes of the hydrated particles $^{111}\text{In}^{3+}$ and $^{111}\text{In-DTPA}^{2-}$ are 10,8 s and 6,9 min, respectively).

We should draw attention to the necessity of keeping all the experimental conditions strictly identical in each set of measurements. Unfortunately, in the present work this condition was partially observed due to some chemical differences between the electrolyte systems used. Nevertheless, valuable results on studying the In/DTPA system kinetics have been presented. Therefore, if more precise values are required, the slight condition differences should be cleared.

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