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STUDY OF THE ^{111}In-**DTPA** COMPLEX BY THE ELECTROMIGRATION METHOD

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1. Introduction

The aqueous chemistry of Indium, the behaviour of its simple and complex ion forms in microconcentrations, is of considerable interest because of the application of cyclotron produced carrier-free ^{111}In as a radiopharmaceutical in the field of nuclear medicine, especially for labeling monoclonal antibodies (Loetscher, 1986; Zoghbi *et al.*, 1986; Reilly *et al.*, 1992), somatostatin analogues (Hildebrandt *et al.*, 1994), low density lipoproteins (Jasanada *et al.*, 1996) and imaging cancers (Kulasegaram *et al.*, 2001). Indium-111 diethylenetriaminepentaacetic acid (^{111}In-DTPA) was described as an ideal and the most commonly used agent for radionuclide cisternography (Vora, 1991; Luyken *et al.*, 1994; Locher, 2001).
The method of horizontal zone electrophoresis in a free electrolyte is suitable for investigating on the state of no-carrier-added radionuclides in aqueous solutions in concentrations of as low as 10^{-8}-10^{-12} M and to obtain a large number of physico-chemical, thermodynamical and kinetic data. In a previous work (Bonchev *et al.*, 2000) the electrophoretic behavior of ^{111}In(III) ions was studied, the presence of a single $[InDTPA]^{2-}$ complex form in the interval of pH 2-11 was established and the stability constant of the complex was estimated to be greater than $2 \cdot 10^{21}$. In the present work investigation of the migration and diffusion behavior of the In-DTPA complex has been carried out. The stability constant and the diffusion coefficient of the complex were determined.

2. Experiment

No-carrier-added ^{111}In ($T_{1/2}$ = 2.83 d, E_γ: 171 keV (90.3%), 245 keV (94.0 %)) was used in the electrophoretic runs. The ^{111}In used was produced by irradiation of a natural silver target with 30 MeV alpha particles on the U–200 accelerator (FLNR, JINR). After radiochemical separation and purification, $^{111}In(NO_3)_3$ with high specific activity was obtained.

The temperature of the electrophoretic cell was kept constant with an accuracy of 0.05°C. The ionic strength of the electrolyte was adjusted by adding potassium nitrate. The necessary value of pH was obtained by adding the appropriate volumes of HNO_3 (*Suprapur, Merck*) and in each measurement was controlled by means of a digital pH-meter with a precision of 0.05 pH. The analytical concentration of DTPA was determined by the gravimetric and titration methods. The equilibrium concentrations of the existing ionic form in the solution were calculated taking into account the pH, the analytical concentration and the protonation constants of DTPA.

The electrophoretic runs were performed by the following procedure. In each experiment a volume of 5 μl (200 kBq) of ^{111}In was injected to the electromigration cell. The gradient of the electric field has been set to 10 $V \cdot cm^{-1}$. The migration of the formed allocation zone was observed by continuously scanning the electrophoretic tube with the CsI(Tl) detector of the completely automated electrophoresis device, described elsewhere (Priemyshev *et al.*, 2000).

In order to avoid hydrodynamic fluxes, the electrode cells and the electromigration tube were connected through a nucleopore filter (ϕ=100 nm).

The electrolyte in the electrode cells was continuously refreshed by a peristaltic pump to counteract any influence of the electrolytic processes.

3. Results and discussion

Determination of the stability constant. In a cycle of electrophoretic experiments the stability constant of the $[^{111}InDTPA]^{2-}$ complex was determined. The dependence between the pH and electrophoretic mobility was studied at the constant temperature, ionic strength and analytical concentration of the DTPA. The experimental results obtained are shown in Fig. 1.

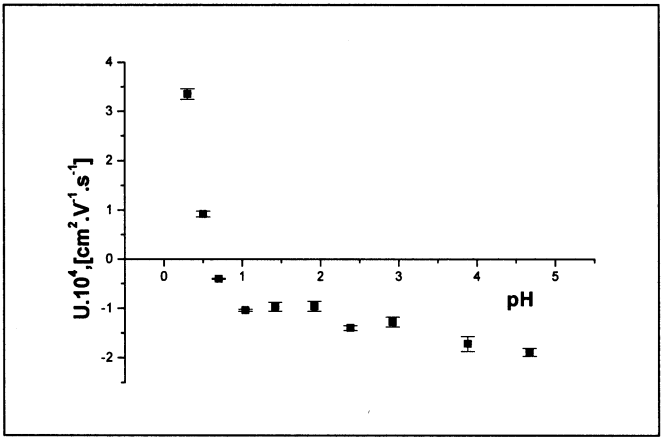

Fig. 1. *Electrophoretic mobility of $[InDTPA]^{2-}$ complex vs. pH. Ionic strength 0.5; analytical concentration of DTPA is $1 \cdot 10^{-4}$ M; temperature– 25.00 ± 0.05 ^0C; gradient of electric field– 10 $V \cdot cm^{-1}$. Error bars represent the standard deviation of the linear fit.*

In the presence of complexing agents in the solution, a dynamic equilibrium between a cation and complex forms takes place. The formed single electromigration zone moves under an electric-field gradient with the average mobility given by the basic electrophoretic equation :

$$\overline{U} = \frac{U_M + U_{ML}[L]\beta}{1 + [L]\beta}, \qquad (1)$$

where:

$\overline{U}$ is the electrophoretic mobility of the zone;

U_M is the electrophoretic mobility of the cation form;
U_{ML} is the electrophoretic mobility of the complex form;
[L] is the equilibrium concentration of the ligand;
β is stability constant of the complex.

The experimental data were mathematically processed in accordance with (1), using the Newton– Gauss method, and the stability constant of the complex was determined to be $\beta=(1.5 \pm 0.3)\cdot 10^{29}$. The obtained value is in reasonable agreement with theoretical calculations (Kodina and Levin, 1974), which evaluate the In-DTPA stability constant as close to 10^{27}.

Temperature coefficient of the electrophoretic mobility of $[InDTPA]^{2-}$ in isotonic solution. The temperature dependence of the electrophoretic mobility is given by the equation:

$$U(t) = U(t_0)\,[1 + \alpha\Delta t + \beta(\Delta t)^2 + \ldots], \qquad (2)$$

where:
U(t) is the electrophoretic mobility at temperature t;
$U(t_0)$ is the electrophoretic mobility at temperature t_0;
α is the first order temperature coefficient;
β is the second order temperature coefficient;
t is the temperature.

In a relatively narrow temperature range it is sufficient to take into account only the first order temperature coefficient because $\beta << \alpha$.

The temperature dependence of the electrophoretic mobility of complexes of In(III) with DTPA was studied in isotonic solutions and the results are shown in Fig. 2.

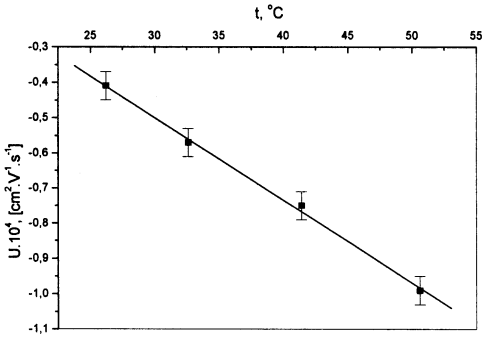

Fig. 2. *Temperature– mobility dependence obtained for $[InDTPA]^{2-}$ complex. Analytical concentration of DTPA is $3\cdot10^{-5}$ M, concentration of NaCl is 0.154 M, pH=7.10 ± 0.05; electric field gradient is 10 $V\cdot cm^{-1}$.*

The determined value of the first order temperature coefficients α for $[InDTPA]^{2-}$ is $0.0234 \pm 0.0008 \cdot 10^{-4}$ $cm^2 \cdot V^{-1} \cdot K^{-1} \cdot s^{-1}$. From the linearity of the temperature-mobility dependence, the conclusion can be made that the complex is stable in this temperature range.

Diffusion coefficient of $[InDTPA]^{2-}$. The electromigration method has been recently developed for determining the diffusion coefficients of ions in aqueous solutions (Bozhikov *et al.*, 2001). In diffusion experiments a volume of 5 µl (200 kBq) of ^{111}In containing solution was injected into the electrophoresis tube and high voltage was applied. The formed active zone moved from the injection point to the homogeneous part of the electromigration tube under a constant electric field gradient of 10 $V \cdot cm^{-1}$. Then the high voltage was switched off and the scanning procedure started. Due to the diffusion process, the zone width increase, as is seen in Fig. 3.

The formed allocation zone is described by the Gaussian distribution function with the standard deviation:

$$(\sigma(t))^2 = 2Dt, \qquad (3)$$

Scanning the zone distribution profiles at different moments t_1, t_2,...,t_n and determination of respective standard deviations σ_1, σ_2,...,σ_n made it possible to linearly fit the function $\sigma^2 = f(t)$ and to calculate the diffusion coefficient of the complex.

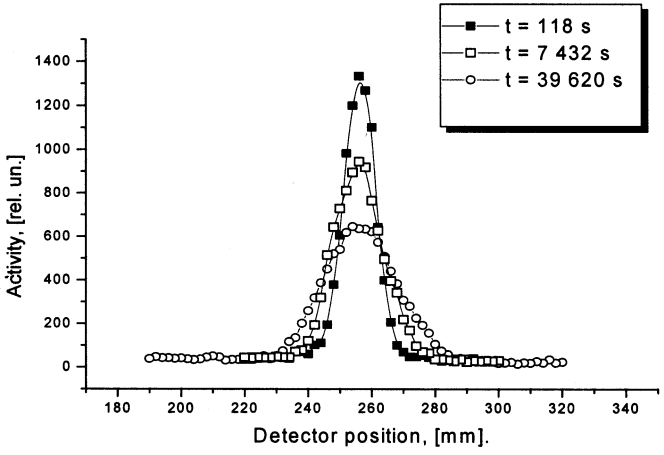

Fig. 3. *Distribution profiles of $[InDTPA]^{2-}$ allocation zone at different moments. The time from start of the diffusion experiment is given.*

The linear fit of the standard deviation– time dependence obtained for $[^{111}InDTPA]^{2-}$ is given in Fig. 4.

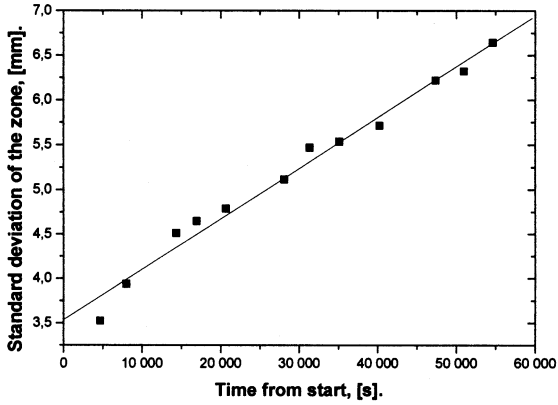

Fig. 4. Standard deviation– time dependence. Analytical concentration of DTPA is $5 \cdot 10^{-3}$ M, pH = 4.50 ± 0.05, temperature 25.00 ± 0.05 ℃, ionic strength is $7.02 \cdot 10^{-3}$.

After processing the experimental data, the diffusion coefficient of the In-DTPA complex in aqueous solution was calculated to be
$D = (2.03 \pm 0.09) \cdot 10^{-6}$ $cm^2 \cdot s^{-1}$.

Effective charge of $[InDTPA]^{2-}$. The effective charge of a complex form can be calculated in accordance with the Nernst-Einstein law:

$$D = u \frac{RT}{zF}, \qquad (4)$$

where:

D is the diffusion coefficient;
u is the electrophoretic mobility;
R is the gas constant;
F is the Faraday constant;
z is the effective charge.

The electrophoretic mobility u was determined simultaneously with the diffusion coefficient under the conditions described above and the value
$u = (-1.95 \pm 0.05) \cdot 10^{-4}$ $cm^2 \cdot V^{-1} \cdot s^{-1}$ was found. Taking into account (4), the effective charge of the In-DTPA complex form at pH = 4.50 was calculated
$z = -1.78$.

The obtained value is smaller than the formal charge of $[InDTPA]^{2-}$ and a possible explanation can be found in the influence of the positive charged ion atmosphere around the complex.

4. Conclusion

The migration and diffusion behavior of the In-DTPA complex has been investigated by the method of horizontal zone electrophoresis in a free electrolyte. Cyclotron-produced ^{111}In in ultramicroconcentrations was used in the experiments. In a cycle of electromigration runs the stability constant of $[InDTPA]^{2-}$ was determined. The diffusion coefficient and effective charge of the complex as well as the temperature dependence of the electrophoretic mobility were measured.

5. Acknowledgements

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Иванов П. И. и др. E12-2002-38
Изучение комплекса ^{111}In-ДТПА электромиграционным методом

Исследовано электромиграционное поведение радиофармацевтического препарата ^{111}In-ДТПА. Определены коэффициент диффузии, эффективный заряд, константа нестойкости комплекса и температурная зависимость электрофоретической подвижности.

Работа выполнена в Лаборатории ядерных реакций им. Г. Н. Флерова ОИЯИ.

Препринт Объединенного института ядерных исследований. Дубна, 2002

Ivanov P. I. et al. E12-2002-38
Study of the ^{111}In-DTPA Complex by the Electromigration Method

The electrophoretic behavior of the ^{111}In-DTPA radiopharmaceutical has been investigated. The stability constant, diffusion coefficient and effective charge of the complex as well as the temperature dependence of the electrophoretic mobility were determined.

The investigation has been performed at the Flerov Laboratory of Nuclear Reactions, JINR.

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