TM-167: AN ELECTROPHORETIC INVESTIGATION OF ITS PROPERTIES IN ULTRADILUTED AQUEOUS SOLUTIONS

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ABSTRACT

The gamma-radionuclide ^{167}Tm is often used in the clinical radiodiagnostics and nuclear medicine. The usual form of its application is as a citrate complex. Nevertheless, there are considerable amounts of the simple hydrated Thulium (III) species that can be expected in the blood and body tissue in some cases. Main reasons for that fact are metabolic and hydrolytic processes as well as failures in radiopharmaceutical preparation (kit engagement). Therefore, it is of a practical interest to know chemical properties as well as the behavior of the $Tm_{aq.}^{3+}$ in water solutions at microconcentrations as low as $10^{-7} - 10^{-8}$ mol.l^{-1} This will help understanding how to prevent an undesired side effect, especially for *in-vivo* treatment with radioactive Thulium drugs. In this paper the highly suitable electromigration technique is discussed and applied. A set of collected radioanalytical data, including values of parameters such as ion mobilities, molar volumes, *Stocks* radii, hydration numbers and diffusion coefficients in water solutions is presented.

INTRODUCTION

Clinical radiodiagnostics traditionally uses gamma-radioactive nuclide ^{167}Tm as a tracer in bond scintigraphy (16). As Thulium is a typical lanthanide, simple Tm^{3+} species are not suitable for *in-vivo* treatment. Mainly, this can be explained by its hydrolytic properties: Thulium ions can easily form insoluble hydroxides, which do not have appropriate biochemical characteristics for application. Therefore, clinical form of radioactive Thulium is as more or less stable organic complexes, usually - citrates or lactates. Unfortunately, lanthanide complexes of citric and lactic acids, despite their relative stability (stability constants of Ln-citrates are approximately $2 \div 8.10^{10}$; Ln-lactates - $2 \div 6.10^{6}$ (12) suffer hydrolysis in a noticeable extent, especially in an acidic media. Thus, a free hydrated ion $Tm_{aq.}^{3+}$ can be expected in the tissue, especially in those cases in which the radionuclide is not firmly engaged by the chelate kit. Understanding the behavior of uncomplexed Thulium (III) ions in aqueous solutions at microconcentration levels is essential in order to avoid incidents in radioclinical practice, such as internal gamma rays overdosing due to radionuclide precipitation and inappropriate *in-vivo* radionuclide targeting. Investigations on properties of

$Tm_{aq.}^{3+}$ species at microconcentration levels should be made by appropriate techniques regarding the well-known fact, that there are serious differences in behavior of radioisotope in trace, µM and mM amounts. The specific features of microconcentrated radioactive solutions are being usually connected to formation of pseudocolloids as well as polymer associates (6,10,11). Thus, electrochemical methods as electrophoresis, electromigration, transference numbers and potentiometric analysis are extremely suitable, offering tracer working concentrations down to 10^{-10}-10^{-11} M. Moreover, these methods guarantee a unique, direct measurement of basic thermodynamic parameters, such as ion velocity, diffusion and electrodiffusion rates (4). Most reliable results can be retrieved by the method of horizontal zone electrophoresis in free electrolyte systems, by which a major part of side effects (electroosmosis, rheophoresis, phase equilibrium etc.) are avoided (2).

MATERIAL AND METHODS

Experimental part of this work was done at the Joint Institute for Nuclear Research (JINR) of Dubna, Russia, in the Flerov Laboratory of Nuclear Reactions (FLNR) and Djelepov Laboratory of Nuclear Problems (DLNP).

A carrier-free ^{167}Tm ($T_{1/2}$=9,24d, E_γ=49,13keV (X, 48,3%), 48,22keV (X, 27,2%), 57,1keV (3,53%), 207,8keV (41%), 531,5keV (1,58%)) with high specific activity was used. It has been produced via (α, xn) reaction with energy of the α-particles near 30MeV on the cyclotron U-200 at JINR. After chemical extraction from the target, Thulium was pu-

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rified and concentrated by the methods of radiochemical separation and ion-exchange chromatography.

The electromigration analysis was done by fully automated device for horizontal zone electrophoresis in a free electrolyte (7). In each separate experiment 5-10μCi ^{167}Tm(III) was injected into the migration tube filled with an appropriate electrolyte system. In this way into the migration cell a radioactive cylindrical zone was being formed. Concentration of the radioisotope within the zone was about 10^{-8} M. The temperature of the electrolyte was set to 25°C and maintained constant with an accuracy of 0,05°C; pH of the solution was set and measured with an accuracy of 0,05. Bidistilled water as well as chemical reagents of grade "purissimum" and Suprapur® Merck® were used. The gradient of the electric field was about 10V.cm^{-1} and maintained uniform with an accuracy of 0,03V.cm^{-1}.

Collected experimental data were mathematically processed using numerical methods by means of specialized software - Equilibrium (1) and Microcal™ Origin® (15).

RESULTS AND DISCUSSION

A cycle of well-planned electrophoretic experiments has been done. In each separate experiment four specific radioactive zone parameters are traced: direction of movement, shape, width and velocity. Direction of movement reveals the sign of a ions charge; geometrical parameters and their stability show the presence or absence of a side interaction (adsorption, hydrolysis and pseudocolloid formation); velocity itself appears to be the base quantitative electrochemical characteristic of the ion being investigated. By the help of the existing electromigration methodic (2) ion velocity can be used to derive information about important physicochemical ion characteristics, such as ion mobility, relative ion conductance, *Stoks* radius, molar volume, hydration number as well as diffusion coefficient under particular experimental conditions.

Determining the Tm^{3+} ion mobility

In each experiment, after injection of the radionuclide the electric field has been switched on, which results in electrophoretic traveling of the zone. Periodically, the position of the zone (here: the maximum zone activity) has been determined, tracing in such a way its movement along the tube. Since the ions in uniform electric field migrate with a constant velocity, there should be a linear correlation between the time and position of the zone. Results can be used to find out the ion velocity, and, consequentially, ion mobility, as it is shown on Fig. 1 and Fig. 2.

The values obtained for the hydrated Thulium (III) ion velocity and ion mobility within a perchlorate medium (ionic strength 0,0017mol.l^{-1}, pH=3,16, ΔE=9,90V.cm^{-1}) are: $(6,79 \pm 0,05).10^{-3}$cm.s^{-1} and $(6,86 \pm 0,05).10^{-4}$cm^2.V^{-1}.s^{-1}, respectively.

The values obtained for the hydrated Thulium (III) ion velocity and ion mobility within a nitrate medium (ionic strength 0,2000mol.l^{-1}, pH=1.00, ΔE=10.04V.cm^{-1}) are:

$(4,63 \pm 0,03).10^{-3}$cm.s^{-1} and $(4,61 \pm 0,03).10^{-4}$cm^2.V^{-1}.s^{-1}, respectively.

It has been detected that Thulium zone migrates to the anode showing a strictly constant speed and keeping its initial narrow, regular Gaussian shape. Hence, one can conclude that the zone consists of cations Tm_{aq}^{3+} which were not en-
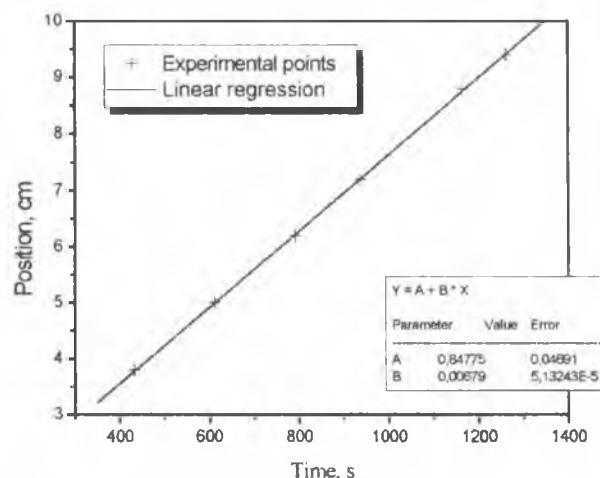

Fig. 1. Determining the Tm^{3+} ion velocity in NaClO$_4$/HClO$_4$ solution. Ionic strength 0,0017mol.l^{-1}; pH=3,16; electric gradient 9,90V.cm^{-1}
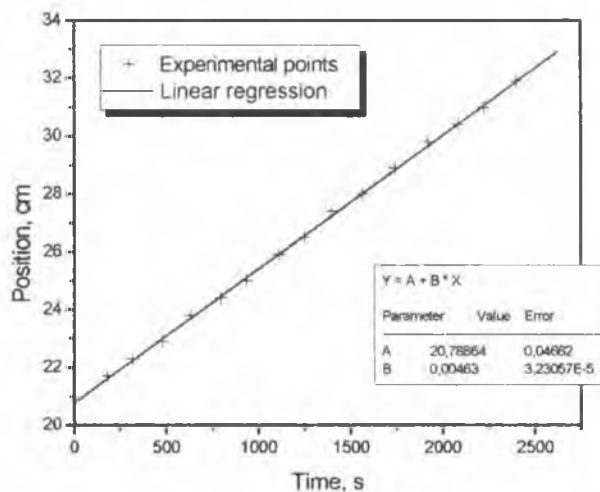

Fig. 2. Determining the Tm^{3+} ion velocity in KNO$_3$/HNO$_3$ solution. Ionic strength 0,2000mol.l^{-1}; pH=1,00; electric gradient 10,04V.cm^{-1}

gaged in side processes as hydrolysis and adsorption to the inner cell's surface, therefore the values obtained should be adequate ones. For comparison, ion mobilities for trivalent hydrated ^{169}Yb and ^{173}Lu under the same conditions in perchlorate medium are $(6,20 \pm 0,10).10^{-4}$ cm^2.V^{-1}.s^{-1} and $(6,21 \pm 0,10).10^{-4}$ cm^2.V^{-1}.s^{-1}, respectively; ion mobility of hydrated ^{111}In^{3+} in identical nitrate solution is $(4,77 \pm 0,01).10^{-4}$cm^2.V^{-1}.s^{-1} (2). It is clearly shown that there is a significant level of agreement.

Ion mobility measured in the electrolyte solutions with a different ionic strength, shows different values, which is in agreement to the Onzager's limiting law (4):

(1) $\qquad u = u_0 - A\mu^{\frac{1}{2}}$

where u - ion mobility $cm^2.V^{-1}.s^{-1}$, u_0 - ion mobility at infinite dilution $cm^2.V^{-1}.s^{-1}$, μ - ionic strength, $mol.l^{-1}$, A - an empiric factor. Applying this equation toward the found results we obtain for the $Tm_{aq.}^{3+}$ $u_0=(7.09 \pm 0.06).10-4$ $cm^2.V^{-1}.s^{-1}$ and $A=+5.54$, which is in accordance to the reference data: $u_0(Ln^{3+})=(6,6 \div 7,0).10^{-4}cm^2.V^{-1}.s^{-1}$ (3). These data can be used to predict Thulium mobility in vast variety of electrolyte systems.

Determining the Tm^{3+} equivalent conductance
One of the most conventional electrochemical parameter is ion equivalent conductance λ, $m^2.\Omega^{-1}.mol^{-1}$. Its value is often used as a result of routine electrophoretic analysis and plays a major role in physico-chemical investigations. There is a simple relation between λ and ion mobility u (9):

(2) $\qquad \lambda = Fu,$

where $F=96\ 485 C.mol^{-1}$ is a Faraday constant. According to this equation we have calculated values of equivalent $Tm_{aq.}^{3+}$ conductance for solution used to be as follows: $(6.62 \pm 0.05).10^{-3}$ $m^2.\Omega^{-1}.mol^{-1}$ (in the perchlorate solution) and $(4.45 \pm 0.03).10^{-3}$ $m^2.\Omega^{-1}.mol^{-1}$ (in the nitrate solution). Extrapolation to ionic strength zero leads to value $\lambda_0=(6.62 \pm 0.05).10^{-3}$ $m^2.\Omega^{-1}.mol^{-1}$. Again, this is in excellent agreement with the literature data: for Yb_{aq}^{3+} corresponding value was reported to be $\lambda_0=6.52.10^{-3}$ $m^2.\Omega^{-1}.mol^{-1}$.

Determining the Tm^{3+} Stocks radius
The *Stocks* radius rs of ions gave us extremely useful information about the state of the ion in dissolute form and can be used for characterization of its hydration shell (8). Although attention should be drawn to the fact that the electrotransport methods are practically unique in describing kinetically stable ion formations (4, 13). For calculation of the *Stocks* radii a *Stocks* relation can be used (9):

(3) $\qquad r_s + \dfrac{Ze}{6\pi\eta u},$

where Z is the absolute charge of the ion, $e=1.602.10^{-19}C$ is the electron charge, η is the dynamic viscosity of solution (for a dilute water systems at 25°C $\eta \approx 0.000895$ Pa.s). Having values of Thulium mobility determined, we have calculated Stocks radii for the hydrated Tm (III) ions as follows: 4.2 ± 0.1 Å (in the perchlorate solution) and 6.2 ± 0.1 Å (in the nitrate solution). Extrapolation to ionic strength zero leads to value $r_s^0=4.0 \pm 0.1$ Å. Reference value found for comparison: $r_s^0(La_{aq.}^{3+})=3.95$ Å (12). Obtained values do not need any corrections, because Stocks relation (3) looses its linearity beneath 4 Å (9).

Determining the Tm^{3+} absolute and molar volumes

The absolute ion volume V_s as well as the molar ion volume V_s^0 are important parameters, significant for the solutions' structure investigation. They could be connected to the Stocks radius rs by the following equations (4):

(4) $\qquad V_s = \dfrac{4}{3}\pi k r_s^3$

(5) $\qquad V_s^0 = \dfrac{4}{3}N_A \pi k r_s^3$

where $N_A=6,022.10^{23}mol^{-1}$ is the Avogadro constant and k - packaging factor (for a simple hydrated ions $k=1$). Using the values obtained for the $Tm_{aq.}^{3+}$ Stocks radii we have calculated absolute volume and molar volume of the hydrated Thulium (III) ions respectively as follows: 310 ± 7 Å^3 and 187 ± 4 Å$^3.mol^{-1}$ (in the perchlorate solution); 999 ± 20 Å^3 and 602 ± 12 Å$^3.mol^{-1}$ (in the nitrate solution). Extrapolation to ionic strength zero leads to corresponding values: 268 ± 7 Å^3 and 161 ± 4 Å$^3.mol^{-1}$. Reference data for comparison shows the absolute volume of the $La_{aq.}^{3+}$ equal to 258 Å^3 (12).

Determining the Tm^{3+} hydration number
Comparing the *Stocks* r_s and the crystallographic r_k radii of the given ion one can estimate the ion hydration number n_s - the mean amount of water molecules coordinated to the solvated ion. Described estimation could be done by the following equation (4):

(6) $\qquad n_s = \dfrac{4}{3}\pi\dfrac{\left(r_s^3 - r_k^3\right)}{V_L},$

where V_L is the solvent's molecule volume (for the water corresponding value it is approximately 30 Å^3). Crystallographic radius for the Tm^{3+} is known to be 0,87 Å. Hence, the values of the hydration numbers of $Tm_{aq.}^{3+}$ in the investigated solutions were found to be 10 (for the perchlorate solution) and 33 (for the nitrate solution). The difference could be expected due to correlation between the concentration of solution and thickness of the ion's shell (13). For comparison, hydration number for the La^{3+} in diluted aqueous systems has been reported to be approximately 13-14 (12), which is in a good agreement, although there is a slight difference between the Lanthanum and lanthanide radii.

Determining the Tm^{3+} diffusion coefficient
The most important kinematical ion parameter is its coefficient of diffusion. Though there exist techniques for its measurement (14), most of them are complicated, need a long experimental cycle and are applicable for specialized aims only. However, it is possible to find out an estimation of the diffusion coefficient, using the Nernst-Einstein law which gives a connection between micro-kinematical properties of the ion (such as ion mobility) and its macro-features (diffusion coefficient) (9):

$$(7) \qquad D = \frac{uRT}{ZF},$$

where $\mathbf{D}$ is the coefficient of diffusion, $cm^2.s^{-1}$, $\mathbf{T}$ - temperature, K, and $\mathbf{R}=8,314 J.mol^{-1}.K^{-1}$ is the universal gas constant. Strictly, the Nernst-Einstein law is obeyed in infinitely diluted solutions only (5); nevertheless an accuracy of 10-15% is often achieved for real solutions [9].

Using the Nernst-Einstein law we have found the diffusion coefficient of the Tm_{aq}^{3+} in investigated solutions to be as follows: $(5,87 \pm 0,04).10^{-6}$ $cm^2.s^{-1}$ (in the perchlorate solution) and $(3,94 \pm 0,03).10^{-6}$ $cm^2.s^{-1}$ (in the nitrate solution). Extrapolation to ionic strength zero leads to value of the diffusion coefficient at infinitely diluted solution $(6,07 \pm 0,05).10^{-6}$ $cm^2.s^{-1}$.

CONCLUSION

Using a device for a horizontal zone electrophoresis in a free electrolyte system as well as an existing method for the electrochemical analysis of the behavior of ions in diluted aqueous solutions most important physico-chemical properties of the hydrated Tm (III) ions have been determined. It is shown that in ultradiluted (μM range) water solutions Tm_{aq}^{3+} reports to be a typical lanthanide, which significant charge and relatively small dimensions leads to deep hydration, thick solvation shell and considerable affinity for hydrolysis. Products of Tm (III) hydrolysis are mainly insoluble Thulium hydroxides $Tm(OH)_3(aq)_x$, which can be easily precipitated onto the vessel surfaces (especially when in microamounts), what obviously can not be allowed during *in-vivo* treatment. Stability of Thulium-kit complexes (as citrates) may not be sufficient to fully avoid the risk of emitting the free Tm^{3+} ions in blood and body tissue. Hence, using substantially more stable chelates such as EDTA, DTPA or their conjugates with the appropriate amino acids/peptides/polypeptides/peptide-chains should be proposed. As a typical lanthanide, it can be expected that Tm should form an extremely stable 1:1 polyaminopolycarboxylic complex which will assure the chemical permanence of [167]Th radiopharmaceuticals under conditions of *in-vivo* applications.

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