Stability of ^{111}In-ligand Complexes Studied by TDPAC *

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The TDPAC technique has been applied to study the stability of ^{111}In complexes with NTA and DTPA in solutions with different concentrations of stable In at pH = 7. A sample of In-DTPA complexes attached to microspheres of albumin (MSA) has been measured at temperatures of 293 and 130 K. The results show that the products formed after ^{111}In → ^{111}Cd decay and following Auger-effect are determined by the stability of In(Cd)-complexes with organic ligands. The daughter Cd behaviour depends on the In : ligand mole ratio, from 1 : ∞ to 1 : 1. The possibility of Cd-ligand complex destruction and following Cd rechelating is discussed. The results indicate that the rechelating probability correlates with the stability of the parent and daughter complexes.

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1. Introduction

The time differential perturbed angular correlation (TDPAC) method is believed to be a versatile technique in solid state physics. Relatively recently the method has been applied in chemical and biological studies and proved to be a useful tool for coordination and molecular dynamics studies in conjunction with other traditional techniques. TDPAC is attractive for chemists and biologists because of i) the high sensitivity to the charge density distribution around the probe atom, that is of great importance for coordination studies and identification of probe sites (see, for example the review by Bauer [1]); ii) the applicability to systems with extremely low concentrations of probe atoms (for example, carrier free radiopharmaceuticals); iii) the unambiguous difference between static and dynamic type of interaction (that, for instance, allowed Hwang [2] to differentiate between the intact and disintegrated liposomes in living mice); iv) the independence of the method's sensitivity on temperature, aggregation state, external fields, vibrations and sample container. A short summary of TDPAC applications for studies lying on the Physics-Chemistry-Biology border is given in the article by Lerf and Butz [3] (see also references therein).

We concentrate on the application of TDPAC to studies of low molecular weight complexes which are used as radiopharmaceuticals in diagnostic medicine. In particular, in [4] we proposed to determine the structure of ^{111}In-complexes using an approach based on a combination of TDPAC and molecular mechanics calculations. A comparative stability estimation for In-complexes with newly synthesised chelates – derivatives of hydroxyamino acids in aqueous solutions with pH = 1 to 7.5 has been done by the TDPAC technique in [5]. In addition, the integral parameter of stability including the hydrolytic stability of ^{111}In-complexes and resistance to Auger after-effects has been introduced in [6, 7].

The radionuclide ^{111}In, which is frequently used for preparing radiopharmaceuticals, is one of the most suitable TDPAC isotopes. ^{111}In decays into ^{111}Cd by electron capture, and the well known 172–247 keV γ-ray cascade in ^{111}Cd is used for hyperfine interaction measurements of the Cd nucleus with the surrounding electron charge density. The electron capture after-effects are mainly determined by Auger-electron emission and filling holes in the electron shells of the daughter Cd ion. The vacancy cascade moving towards outer shells gives rise to a shock ionisation of Cd resulting in a highly positive ion charge up to 10^+, or higher.

After-effects of electron capture are usually handicaps in the experiments because an abrupt change of the ion charge can cause a damage or even a destruc-

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tion of the molecules under study. As a result, Cd ions can appear in different surroundings, depending on the probability and degree of initial complex destruction. In fact, in [7] two components with essentially different relaxation constants λ_c were observed in TDPAC spectra measured for aqueous solutions of ^{111}In-complexes with the following ligands: diethylentriaminpentaacetic acid (DTPA), nitrilotriacetic acid (NTA), glycine-N-dimethylenphosphonic acid, oxabiphoric acid and a derivative of hydroxyamino acid. The component with the relaxation constant $\lambda_c > 10^7\ s^{-1}$ was assigned to the intact complexes, and another component with much smaller λ_c to unidentified light molecules formed after the complex destruction.

It is known [8] that the relaxation of highly charged ions depends on the chemical surrounding, and it can be proposed that the complex destruction probability is a function of the strength and type of metal ion bonds with the ligand. Thus, the resistance to Auger after-effects, expressed via the population ratio of two observed fractions $S = a_1/(a_1 + a_2)$, can be regarded as a measure of complex stability.

The Cd behaviour, following the complex destruction, will be determined by the rate of its interaction with the excess ligands and hydroxy groups and the stability of the formed compounds. To study the possibility of binding Cd ions by molecules of excess ligands, it is useful to measure the dependence of the parameter S on the relative In and ligand concentration.

The present work is aimed at the investigation of ^{111}In-complexes with DTPA and NTA in solutions with different concentrations of carrier (stable In). The choice of the ligands has been determined by the difference of their stability constants which could be manifested in different relative fractions of intact complexes surviving after the Auger-process. The constants (log K) for the In and Cd complexes with DTPA and NTA are listed in Table 1 [9]. Also the sample of ^{111}In-DTPA attached to albumin microspheres has been studied and the results have been compared with those for In-DTPA.

Table 1. Stability constants for In and Cd complexes with DTPA and NTA.

Complex	Log K	Complex	Log K
In-DTPA	29.0	In-NTA	16.9
Cd-DTPA	19.0	Cd-NTA	9.78

2. Experimental

The ^{111}In isotope was produced via the ^{109}Ag(α, 2n) ^{111}In reaction by irradiation of a metallic silver foil by 25 MeV α-particles in the cyclotron of the Institute of Nuclear Physics of Moscow State University. After irradiation, ^{111}In was extracted from the target foil as a carrier-free solution of $InCl_3$ in 0.3 N HCl.

Samples of ^{111}In-ligand complexes were prepared by dilution of 1 mg of DTPA or NTA in 0.3 N HCl, adding the initial $^{111}InCl_3$ solution with subsequent adding of 0.1 N NaOH for solution neutralisation. The radioactivity of the samples was 5–7 μCi. The samples were analysed by electrophoresis using the Cellagram acetate-cellulose film (250 V, 15–20 min) for 0.1 N HCl and 0.1 N NaCl solution, pH = 7.3. The initial solution of $^{111}InCl_3$ was used for a check. Stable indium chloride solution was added to the prepared radioactive samples to get the required In : ligand concentration ratio. Samples of NTA and DTPA with In : Ligand concentration ratios 1 : infinity (1 : ∞), 1 : 5 and 1 : 2 were prepared. An additional sample with a ratio 1 : 1 was prepared for NTA.

Microspheres of albumin (MSA) are spherical particles produced by albumin thermal denaturation [10]. The aqueous albumin solution was emulsified in olive oil and heated up to 130 °C. After cooling MSA were separated, treated by acetone for removing the surface oil film and fractionated in accordance with particle dimensions. The fraction with 10–30 μm particles is used for the sample preparation. To attach DTPA to the MSA surface, MSA are treated by DTPA dianhydride in dimethylsulfoxide with adding pyridin. The modified MSA contain 5 wt.% of DTPA. The initial $^{111}InCl_3$ solution was added to the prepared sample with subsequent neutralisation by NaOH.

The 172–247 keV γ-ray cascade in ^{111}Cd goes through the intermediate state with an energy of 247 keV and the following nuclear parameters: $I = 5/2$, $T_{1/2} = 84$ ns, $Q = 0.8$ barn. TDPAC spectra were measured using a 3-detector coincidence spectrometer with time resolution ~2 ns. BaF_2 scintillator with a PMT XP2020Q was used for the registration of the 172 keV radiation, and two scintillators NaI(Tl) with a PMT RCA 8575 were used for the registration of the second successive γ-ray with an energy of 247 keV.

From the coincidence spectra $N(\Theta, t)$ collected at the angles $\Theta = 90$ and 180° between the detectors,

anisotropy spectra of the angular correlation were obtained:

$$R(t) = -2\frac{N(180, t) - N(90, t)}{N(180, t) + 2N(90, t)} = -A_{22}\, Q_2\, G_2(t), \quad (1)$$

where the correlation coefficient $A_{22} = -0.18$ for the given γ-ray cascade in ^{111}Cd; Q_2 is a solid angle and sample size correction factor, and $G_2(t)$ is the perturbation function containing information about hyperfine interactions.

The mathematical expression for $G_2(t)$ depends on the type of hyperfine interaction (static or dynamic, electric quadrupole (EQI) or magnetic dipole). In solids (crystals, powders or frozen solutions) the nuclei experience a static interaction, and for EQI $G_2(t)$ can be written as

$$G_2(t) = \sum_{l=0}^{3} \sigma_{2l}(\eta) \cos\{\omega_l(\eta)\, t\} \exp\{-\Lambda\, \omega_l(\eta)\, t\}, \quad (2)$$

where η is the electric field gradient (EFG) asymmetry parameter, σ_{2l} are coefficients calculated in [11] for the spin of the intermediate state $I = 5/2$, $\omega_l(\eta)$ are frequencies that are functions of η and the quadrupole frequency $\omega_0 = \frac{3}{20} eQV_{zz}/\hbar$ (for $I = 5/2$), Q is the nuclear electric quadrupole moment for the intermediate state and V_{zz} is the largest component of the EFG. Λ is the full width at half-maximum of the V_{zz} distribution (assuming it is a Lorentzian) around a mean value due to variations of charge distributions around the probe nuclei.

For a dynamic perturbation the perturbation function is exponential:

$$G_2(t) = \sum_{i=1}^{m} a_i \exp(-\lambda_{ci}\, t), \quad (3)$$

where i is the number of inequivalent nuclear sites (e.g. there can be different complexes dissolved in solution) and a_i are amplitudes proportional to their populations, $\lambda_c = 2.8\, \omega_0^2\, \tau_c$ is the damping constant (for nuclear spin = 5/2), ω_0 the quadrupole frequency. The reorientational correlation time is determined by the expression

$$\tau_c = \frac{4}{3}\pi\, f\, r_{\text{eff}}^3/kT, \quad (4)$$

where f is the solution viscosity at temperature T, r_{eff} the effective radius of the molecule assuming a spherical shape. To determine the effective radius one has to measure the EQI frequency. This can be done with immobilised molecules, e.g. by freezing the solution. Calculations of the effective radii for probable complex structures using the molecular dynamics methods and comparison with the experimentally determined value allow one to choose the most probable structure of the complex under study.

Anisotropy spectra were analysed using the least squares method using (2) or (3).

3. Results and Discussion

The TDPAC spectra of the 172–273 keV γ-ray cascade in ^{111}Cd for all samples were measured at room temperature (Figs. 1 and 2), pH = 7. For all concentrations a dynamic perturbation of the angular correlation was observed. The experimental spectra were satisfactorily fitted by two exponents, the results of the fitting procedure are shown in Table 2. The amplitudes of these two components fully account for the observed anisotropy, since $|A_{22}\, Q_2| = 0.14(1)$, as was shown by the measurements of the unperturbed correlation with $InCl_3$ in concentrated hydrochloric acid.

As was done previously [6, 7], the fraction with the large λ_c value was assigned to the intact molecules. We proved that this was correct by calculations of the effective radius of In(Cd)-NTA complexes and comparison with the one determined by the TDPAC data.

To estimate the effective radius using (4), one should measure the quadrupole frequency ω_0. We solved this problem, first, by freezing a solution, attaching a sample container to the liquid nitrogen dewar, and, second, by sorption of In-NTA complexes by a molecular sieve with 3 Å cells. Both experiments gave identical result. The correlation was strongly perturbed by a static EQI with an average quadrupole frequency $\omega_0 = 105(5)\, 10^6 \times s^{-1}$ and a wide frequency distribution $\Lambda \approx 41\%$. Assuming a spherical shape of the molecules and a solution viscosity of $f = 10^{-3}$ Pa × s, we obtained $r_{\text{eff}} = 8.3 \pm 1.6$ Å.

The results of the calculations on the basis of molecular dynamics [12] for several possible In complexes with NTA are given in Table 3. The value nearest to the experimental is $r_{\text{eff}} = 7.83$ Å. The model of the complex In-H_3NTA is given in Figure 3. This estimation shows that our assumption that the fraction with the large λ_c represents the intact molecules of the Cd-NTA complex, is realistic. The second fraction represents some small molecules. Their appearance seems to be a result of electron capture after-effects, since the

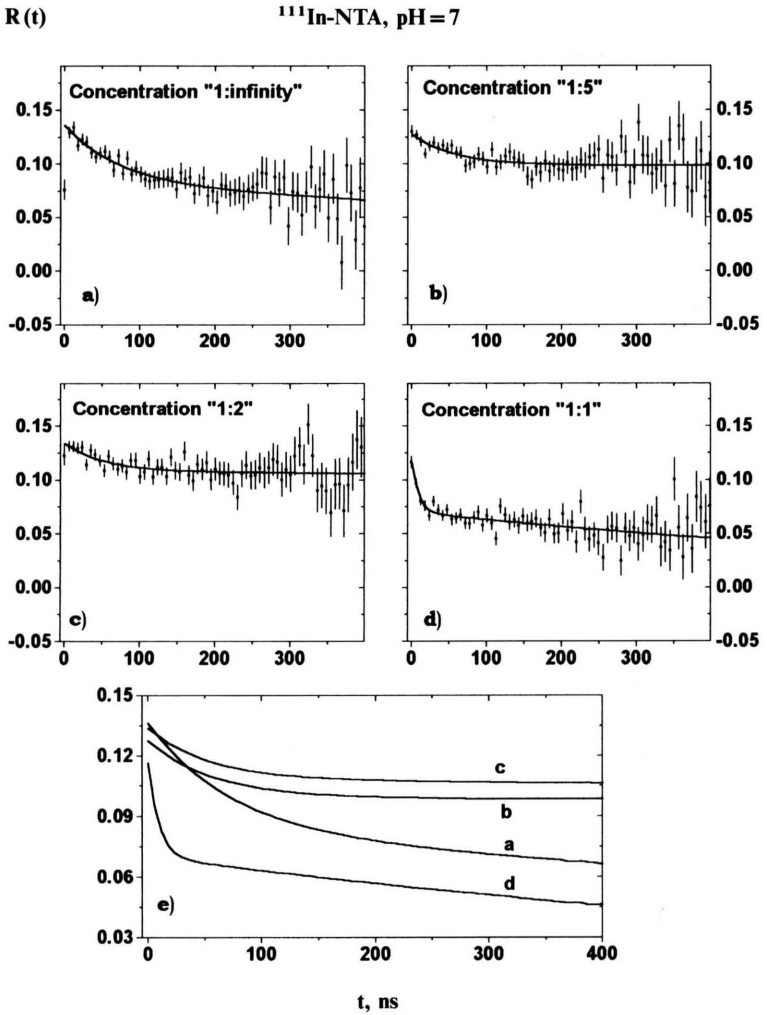

Fig. 1. TDPAC spectra of the 172–247 keV γ-ray cascade in ^{111}Cd, measured with NTA-complexes at room temperature for In-NTA concentration ratio: a) 1 : ∞, b) 1 : 5, c) 1 : 2, d) 1 : 1. All fitting curves are given together in e).

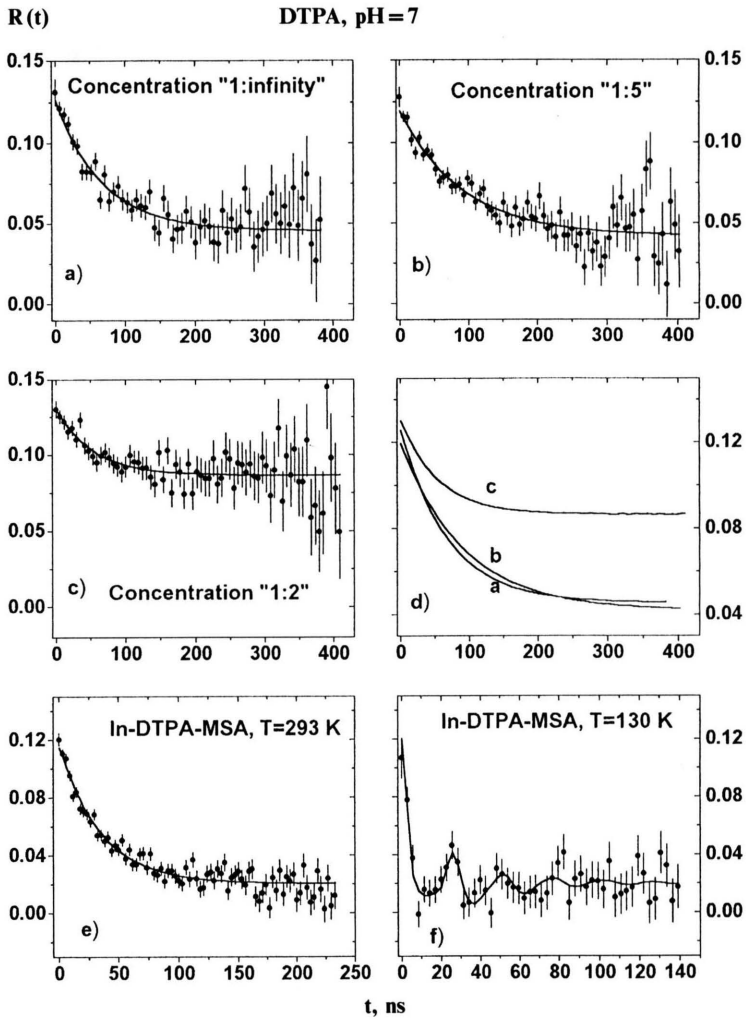

Fig. 2. TDPAC spectra of the 172–247 keV γ-ray cascade in ^{111}Cd, measured with DTPA (a–d) and DTPA-MSA (e, f) complexes. In-DTPA concentration ratio: a) 1 : ∞, b) 1 : 5, c) 1 : 2, d) all fitting curves. Spectra of In-DTPA-MSA were measured at temperature e) 293 K, f) 130 K.

Table 2. Fitting parameters of dynamic and static EQI perturbations for In(Cd) complexes with NTA, DTPA and DTPA-MSA.

Ligand	Concentration	a_1	λ_1, 10^6 s^{-1}	a_2	λ_2, 10^6 s^{-1}	$S=a_1/(a_1+a_2)$	ω_0, 10^6 s^{-1}	Λ, %
NTA	1:∞	0.05 (2)	14 (7)	0.08 (2)	1(1)	0.39	105 (5)	41 (7)
	1:5	0.032 (3)	15 (4)	0.097(3)	0(1)	0.32	–	–
	1:2	0.026 (8)	19 (9)	0.109(9)	0(1)	0.24	–	–
	1:1	0.049 (6)	115(25)	0.071(2)	1(1)	–	–	–
DTPA	1:∞	0.081 (4)	15 (2)	0.046(3)	0(1)	0.64	94 (5)	40(10)
	1:5	0.079 (3)	11 (1)	0.042(3)	0(1)	0.65	–	–
	1:2	0.044 (3)	19 (4)	0.086(2)	0(1)	0.34	–	–
DTPA-MSA	1:∞	0.097 (2)	29 (1)	0.021(1)	0(1)	–	235(10)	12 (4)

Table 3. Effective radii, calculated for the In complexes with NTA.

Complex	r_{eff}, Å
$[In(NtaH)_2(H_2O)]^{-1}$	6.89
$[In(NtaH_2)_3]^0$	7.53
$[In(NtaH_2)_2(NtaH)]^{-1}$	7.83
$[In(NtaH_2)(NtaH)(H_2O)]^0$	5.20
$[In(OH)(Nta)(H_2O)]^{-1}$	3.95

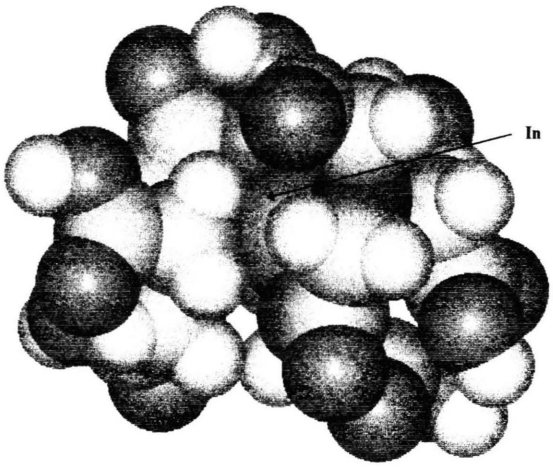

Fig. 3. Model of the In-H_3NTA complex structure. The central atom is In.

radioactive decay of ^{111}In into ^{111}Cd accompanied by the cascade Auger-effect can cause a complete complex disintegration. The population of the latter fraction increases with In concentration. This means that in the presence of excess ligands (concentration 1:∞) the Cd ion can be rechelated in a short enough time, say $\sim 10^{-9}$ s. With increasing In concentration (1:2) the probability to find "free" ligands by released Cd reduces and, evidently, Cd binds to OH-groups forming molecules of much smaller size than the initial complex.

The spectrum for the concentration ratio 1:1 also shows the presence of two fractions, but this time the "heavy" fraction can not represent the intact complexes since the value of the damping constant is too high and, consequently, the radius of those molecules is much larger. We think that at pH > 7 for such concentrations the polymerisation process of In-ligand complexes starts, resulting in partial In binding in polymer chains $In_a(OH)_b(NTA)_c$. Possible channels of In-ligand transformations after the In decay are shown in Fig. 4 both for the infinite concentration and equal indium and ligand concentrations.

The spectra measured with the samples ^{111}In-DTPA for the concentration ratios 1:∞ and 1:5 are

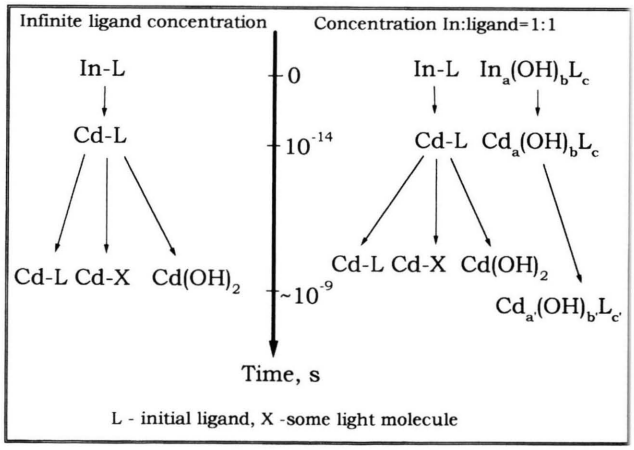

Fig. 4. Possible transformation channels of initial In-ligand complex after In decay considering after-effect complex destruction

almost identical, while for the NTA sample with a concentration ratio 1 : 5 the spectrum showed a significant increase of the component with $\lambda_c \sim 0$. This difference reflects the different stability constants for In(Cd)-NTA and In(Cd)-DTPA complexes (Table 1).

For the MSA sample we also observed a 2-component spectrum at room temperature, but the "heavy" fraction was dominant (Fig. 2e) indicating a higher stability of In-DTPA-MSA complexes in comparison with In-DTPA. In-DTPA is bound to microspheres via amide bonds which can rotate. The lower number of degrees of freedom in comparison with the free In-DTPA complex results in slower movement and larger values of λ_c and, consequently, larger reorientational correlation times (see Table 2). The results obtained with the frozen sample (Fig. 2f) showed that, contrary to the DTPA complex where $\Lambda = 40\%$, the static perturbation with a relatively narrow frequency distribution, $\Lambda = 12\%$, characterises the major component in the MSA spectrum. This means that the charge distribution around Cd in MSA-DTPA is much more uniform than that for the frozen DTPA solution. It is interesting to note that the structural transformations of the initial ligand in the series DTPA – DTPA-MSA leads to a significant difference in molecular dynamics and stability of the formed complexes.

In conclusion, the results of this work confirmed the validity of the parameter S for the estimation of the complex stability. Considering the possibility of a rapid check for the possible applicability of a new complex as a radiopharmaceutical (depending on the type of angular correlation perturbation, static or dynamic, in a neutral solution), evaluation of an effective radius of the complex under study, and estimation of its stability, the TDPAC technique provides a unique method of carrier-free ^{111}In-labelled pharmaceutical investigations and opens the way of studying processes of complex formation of In with different organic ligands.

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