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Computer Simulation of Speciation of Trivalent Aluminum, Gadolinium and Yttrium Ions in Human Blood Plasma

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Abstract

The speciation of Al^{3+}, Gd^{3+} and Y^{3+} ions in human plasma has been studied by computer simulation using the program HySS2009. A literature computer model of blood plasma was updated and comprised 9 metals, 43 ligands and over 6100 complexes. To this model critically evaluated data of Al^{3+}, Gd^{3+} and Y^{3+} constants with blood plasma ligands have been added. Low molecular mass (LMM) speciation of Al^{3+} ion strongly depends upon the chosen equilibrium model of the metal – phosphate and metal – citrate systems. The obtained computer simulation of LMM speciation data of Al^{3+} ion were: $AlPO_4Cit$ (40.7%), $AlPO_4CitOH$ (22.9%), AlCitOH (19.2%) and $AlPO_4(OH)$ (12.7%) (% of total LMM Al species pool); for Gd^{3+} ion: GdAspCit (30%) and $GdCit(OH)_2$ (20%) (% of total [Gd]) and for Y^{3+} ion: YCit (48%), $Y(CO_3)_2$ (32%) and $Y(CO_3)$ (11%) (% of total [Y]). Citrate appears as the important binding and mobilizing ligand for all examined ions, while the dominating species are the ternary ones.

Keywords: Aluminum, gadolinium, yttrium, speciation, blood plasma

1. Introduction

Aluminum is generally regarded as toxic or detrimental element.[1] Nevertheless, its compounds are widely used in areas from medicine to car industry. Normally, despite oral intake ranged from 5 to 10 mg daily (food, food additives, drinks, atmospheric dust) aluminum is very little absorbed into serum and tissues (less than 1% of intake dose).[1,2] Normal serum levels are lower than 0.05 μmol L^{-1}.[1,2] However, high levels of aluminum (>3 μmol L^{-1}) may accumulate in tissues of patients who have renal insufficiency or kidney failure and are treated by dialysis fluid that contains aluminum or are given aluminum based gels to control high plasma phosphate level. These patients may develop blood, bone, brain diseases which at least in part may be linked to the excess of the aluminum.[1,2]

In blood, aluminum is transported by transferrin to lungs, liver, bones and other tissues including brain.[3] In blood aluminum may exist as bound to proteins (transferrin, albumin), low-molecular mass ligand complexes (LMM) and as free ion.[4] Its chemical form is important for its transport to tissues and cells, accumulation and excretion, thus the knowledge of identity, stability and concentration of various aluminum species is necessary for understanding its metabolic pathways.[5]

Gadolinium and yttrium ions may be present in blood as a result of medical treatment from imaging diagnostic procedures in MRI where gadolinium is used as a contrast agent and during the therapy of cancer where ^{90}Y is widely used.[6,7] In nature, ^{90}Y cannot be found, except in the case of contamination or uncontrolled and rapid clearance of the patient. Toxic effects of parenterally introduced gadolinium and yttrium chelates are numerous.[8,9] Non-complexed gadolinium is unsuitable for clinical use as it may form precipitates which could exist for long periods in the body. The biochemical effects induced by simple gadolinium salts involve the interference with calcium-depending processes and calcium entry into cells. Free gadolinium ion can form mineral emboli in the circulation which may be deposited in tissues like muscle, skin, liver, bone and other organs. The emboli

consist of a complex gadolinium phosphate, carbonate or hydroxide.[10,11] Phagocytosis of emboli may lead to cell death due to blocking of macrophage function. Insolubility and toxicity of gadolinium is eliminated by forming either macrocyclic or linear chelates. The chelates are used as MRI contrast agents and believed to be safe. The side effects may occur owing to dissociation of Gd-ligand complex into metal ion and ligand. This process is facilitated by endogenous metals like iron, zinc, copper and calcium and by endogenous acids.[12] Gadolinium contrast agents may cause nephrotoxicity and acute renal failure. Moreover after exposure to gadolinium based contrast agents, patients with renal insufficiency may develop nephrogenic systemic fibrosis with scleroderma-like changes in the skin and connective tissues which has sometimes been fatal.[8]

^{90}Y obtained from the ^{90}Sr–^{90}Y generator system finds widespread use in the cancer treatment in the form of radiopharmaceutical chelate.[13] As other radiopharmaceuticals yttrium is administered by intravenous injection. Yttrium chelates (DTPA, DOTA, etc.) are very stable and usually safe for use. However in blood plasma dissociation may occur by the similar mechanism as in the case gadolinium chelates. Yttrium may form particular emboli consisting mainly of phosphate, carbonate or hydroxide species. The chemical forms and their concentration levels determine the fate of both gadolinium and yttrium ions in the organism.[14,15] It is the reason of detailed study of their speciation by computer simulation.

Direct measurement of concentrations of various forms of aluminum, gadolinium and yttrium in blood and other human tissues is difficult owing to low concentration of these ions. Some well-established methods and their properties used for the analysis of Al were reviewed in several excellent reviews and are summarized in Table 1.[16–19]

Data presented in Table 1 show that main HMM Al species is its transferrin complex, while Al – citrate species were identified as main LMM complexes. Significant number of studies determined only percentage of total Al bound in LMM complexes without any speciation. Any attempt to obtain the chemical speciation information by using direct analytical way with methods given in Table 1 results in uncontrolled changes of labile Al species so that the obtained results reflect more the analytical procedure than the distribution of the native species in the samples. In such cases the sole possible speciation techniques is of indirect nature, through calculations based on the simulation models.

In the literature, numerous methods for the analysis of Gd-based MRI contrast agents in several biological matrices are described. To investigate Gd-based contrast agents with the aim to study toxicity, only the total concentration of gadolinium were commonly determined in biological fluids, such as plasma, serum, urine and faces, by elemental techniques, such as ICP-OES, ICP-MS or AAS.[30–35] To gather more detailed information about the Gd species, chromatographic or electrophoretic techniques are required to separate the particular gadolinium compounds and to detect them individually. Several approaches are described in the literature, employing a variety of separation techniques, coupled to optical, element and mass detectors.[36–45] Experimental data for the determination of gadolinium in plasma are based on the addition of gadolinium contrast agents in the blood plasma of healthy persons with subsequent determination of total Gd^{3+}. Achieved limit of detection was at best 0.01–0.1pg mL^{-1} while limit of quantitation was in the range 1.3–5.8 ng mL^{-1} depending on applied method.

Experimental data for determination of yttrium in human samples and blood are not developed as those for

Table 1. Overview of experimental methods for the determination of Al species in human serum

Analytical Method	Type of sample	Al-HMM Species (%)	Al-LMM Species (%)	Ref.
ICP-ETAAS	Uremic serum: Stored/Fresh	–	12.9/13.3	20
HPLC-ETAAS	Healthy controls (Spiked serum)	90 (Al-Tfn)	12±5 (Al-Cit)	21
^{26}Al and AMS	Healthy controls (Spiked serum)	–	<5%	22
GFC-FAAS	Healthy controls Normal renal function Renal-dialysis patients	–	14.5±3 16.2±4 19.7±4	23
FPLC-ETAAS	Healthy controls (Spiked serum)	–	15–19 (Al-Cit)	24
HPLC and Zeeman AAS	Healthy controls Exposed	79.1±7.0 91.3±3.3	19.6±3.6 8.7±3.2	25
HPLC	Healthy controls	–	20	26
–	Healthy controls (Spiked serum)	–	< 5 (Al-Cit)	27
–	Healthy controls	80 (Al-Tfn)	5	28
FPLC-ICP-MS	Healthy controls	–	10	29

aluminum and gadolinium. So far limited data exist for the biodistribution of yttrium in mice using radioactive methods.[46,47]

Existing experimental methods cannot identify and quantify most of the Al-, Gd- and Y-LMM species in serum, thus the speciation must be based on the computer simulation. These simulations were subject of intensive interest by various research groups. The study of Al(III) speciation in human blood plasma by computer simulation was performed by several research groups mainly using the program ECCLES.[48–55] These calculations were based on the assumption that aluminum belongs to the class of non-exchangeable elements (ie with slow uptake or dissociation from transferrin) according to May et al.[56] This means that percentage of metal appearing in a given LMM species is constant regardless of the exact free metal concentration that exists in equilibrium with transferrin. Practically, the concentration of LMM complex is directly proportional to the free metal concentration since the free ligand concentration is not significantly affected by complexation owing to very low free metal concentration. Thus, the distribution of Al^{3+} is independent of metal concentration in the concentration range 10^{-15}–10^{-5} mol L^{-1}. So far obtained results for speciation of aluminum in human plasma are summarized in Table 2. From Table 2 it can be seen that speciation is dependent on the assumed aluminum ion binding to transferrin and on the stability constants of citrate and soluble phosphate complexes. Controversy still exists whether phosphates or citrates are dominating species.

Majority of the studies identified Al – citrate species as the dominating ones, however, in some studies Al – phosphate species were found to be dominant ones. Careful analysis of blood plasma database data is needed to resolve the problem. It seems that inclusion in the database only the species relevant to physiological conditions, and large number of ternary species may rectify the problem. Indeed, Harris et all have reconsidered previous Harris's data and concluded that if Al –phosphate equilibrium data are limited only to species relevant to physiological conditions and low Al free concentration in plasma, then Al – citrate species are the dominant ones while Al – phosphates become insignificant.[53,55]

The study on Gd(III)-ion speciation in human blood plasma was performed by Jackson et al but they used a single phase model in which the insoluble species of Gd were not considered and some important macromolecular and ternary complexes were not included.[57] Webb et al studied Gd(III) speciation in GI tract, but precipitates were again not considered.[59] Yue Wang et al studied Gd(III) speciation in human interstitial fluid with precipitate species and some important new complexes being considered.[60] They used a total concentration of gadolinium in range from 1.2×10^{-9} mol L^{-1} to 2.2×10^{-2} mol L^{-1} and the results are given in Table 3.

Table 2. Literature data on bio-distribution of Al(III) in blood plasma by computer simulations

Total Al concentration (mol L^{-1})		**5×0^{-1} -5×10^{-3}**	**$(1.8–2.5) \times 10^{-13}$**	**3×10^{-6}**	**9×10^{-8}**	**3×10^{-6}**	**2.2×10^{-13}**	**1×10^{-6}**	**3×10^{-6}**
% of total Al bound to transferrin		57	63	77	80	80	81	83	93
% Al-LMM Species	$Al(OH)_3$	51	–	–	–	–	4	–	–
	$AlPO_4$	41.5	62	–	1.5	–	2	–	–
	$Al_2PO_4(OH)_2$	7.2	–	–	–	–	–	–	–
	AlCitOH	23	–	–	3	–	10	–	–
	$AlPO_4CitH$	10	–	–	–	–	–	–	–
	$AlCit(OH)_2$	–	–	–	94	51	–	–	–
	$AlOxa(OH)_2$	1.4	–	–	–	–	–	–	–
	$AlPO_4OH$	–	–	–	–	21	80	–	–
	$AlPO_4Cit$, $AlPO_4CitOH$	–	–	–	–	28	–	–	–
	$Al(OH)_4$	–	–	–	–	–	3	–	–
	Al-Citrate (all forms)	–	–	80	–	–	–	98	88
	Al-Hydroxide (all forms)	–	–		–	–	–	2	8
	Al-Phosphate (all forms)	–	–	20	–	–	–	–	2
	Reference	48	49	50	51	52	53	54	55

Table 3. Literature data on bio-distribution of Gd(III) in blood plasma by computer simulations

Total Gd concentration (mol L^{-1})		**1.2×10^{-9}**	**1.0×10^{-7}**	**5.99×10^{-4}**	**2.07×10^{-2}**	**2.2×10^{-2}**
% Gd-LMM Species	Gd(HSA)	29.6	29.6	29.8	33.6	8.5
	Gd(Oxa)	18.2	18.2	18.3	14.9	1.2
	Gd(Cit)(Lac)	10.0	10.0	9.9	9.5	1.7
	Gd(Cit)(Leu)	7.9	7.9	7.8	7.4	1.2
	Gd(Cit)(Asp)	7.7	7.7	7.6	5.3	<1
	$Gd_3(OH)_4$	<1	<1	<1	1.0	78.9
	Gd_{free}	5.4	5.4	5.5	6.5	2.6

From the Table 3 one can see that the main ligands which complexed gadolinium are albumin, oxalate and ternary complexes citrate with lactate, leucinate and aspartate. With increasing the total concentration of gadolinium to 2.2×10^{-2} mol L^{-1} the main ligand becomes hydroxide.

There are not many papers in which a computer speciation of yttrium in blood plasma was described. De Witt et al performed computer modeling of complex yttrium-EDTMP in blood plasma.[61] Concentrations used in simulation far exceed concentrations that are employed in the treatment of bone cancer treatment. Results of computer modeling by program ECLLES are given in Table 4.

If **F** is column vector of f_i then improved values of [Ri](=X) are calculated by Newton-Raphson method:

$$F(X + \delta X) = F(X) + J * \delta X \quad (3)$$

$$J * \delta X = -F(X) \quad (4)$$

The method requires the computation of the Jacobian matrix **J,** that is the matrix of partial derivatives of each functions **f**, with respect to each unknown variables *X*. The shift vector δX is obtained by solving the equation 3 applying the method of LU decomposition. The solution vector has the form:

Table 4. Literature data on bio-distribution of Y(III) in blood plasma by computer simulations

Total Y concentration (mol L^{-1})		1×10^{-3}	1×10^{-2}	1×10^{-1}	1×10^{0}	1×10^{1}	1×10^{2}
% Y-LMW Species	Y- EDTMP	0.0	0.3	2.4	19.9	71.3	96.1
	Y- Citrate	98.2	98.2	98.2	98.2	98.2	98.2
	Y- Oxalate	0.8	0.8	0.8	0.8	0.8	0.8
	Y- Lactate	0.4	0.4	0.4	0.4	0.4	0.4
	Y-Amino acids	0.2	0.2	0.2	0.2	0.2	0.2

1. 2. The human blood plasma model and speciation calculation

There are two general approaches to simulate complex equilibria systems (a) Gibbs energy minimization and (b) equilibrium constant method. The equilibrium constant method is widely used and is based on the solution of a set of equilibrium conditions satisfying stoichiometric mass balance equations. To this end we used Windows based computer program HySS2009 with graphical interface.[62] The program is readily available, data input is straightforward and simple, and output is produced as both graph and table of concentrations.

The mathematical algorithm of the HySS program is based on solving the stoichiometric equation

$$T_{Ri} = \sum_{j=1}^{n} \nu_{ji} \beta_j \prod_{i=1}^{m} [R_i]^{\nu_{ji}} + \sum_{k=1}^{s} \nu_{i,k} A_k \quad (1)$$

$t = 1 + m$ number of components
$j = 1 + n$ number of reactions (products)
$\beta_j = 1 + n$ – formation constant of particular product, j
ν_{ji} – stoichiometric coeficients, $\nu_{ji} = 1$ for $j = i$ and $j \leq m$ and $\nu_{ji} = 0$ for $j \neq i$ and $i \leq m$ (first m products are identical to components)
A_k – relative amount of the insoluble species, k, formed
$[R_i]$ – free concentration of components

To calculate m free concentrations $[R_i]$, $i = 1 + m$ the equation (2) is solved

$$f_i = T_{R_i}^{calc} - T_{R_i} = 0 \quad (2)$$

where T_{Ri}^{calc} is calculated from right-hand side of Eq(1). T_{Ri} in Eq (2) are experimental total concentration of reactants.

$$X^{(h+1)} = X^{(h)} + \delta X \quad (5)$$

where h is indicator of the iteration. Equations (4) and (5) are applied repeatedly until convergence of functions **F** and variable **X** is reached. The Jacobian **J** is calculated algebraically and numerically by finite approximation equation. The method is subject to problems if **J** is nearly singular or if highly nonlinear system with more than five equation is present. These troubles are partially overcome by using the numerical procedures of dumping. scaling and convergence forcer.

In developing the computer modeling of blood plasma we improved May et al model of blood plasma and constructed multi-phase model including 9 metals, 43 ligands and over 6100 complexes. Total concentrations of all components were taken from published papers and Geigy tables.[56,48–58,63] Almost all stability constants of binary and ternary complexes were abstracted from published databases (JESS, IUPAC, NIST) and where necessary converted to physiological conditions (T=310 K, I= 0.15 mol L^{-1} NaCl) using the program SIT(Specific Interaction Theory).[64–66] Part of the stability constants was updated on the basis of recent literature data.

2. Results and Discussion

2. 1. Aluminum in Human Blood Plasma Model

The physiological model of human blood plasma is based on May et al computer model of blood plasma.[56] To

this model aluminum species were added either from reference databases or published data. If no constants were available in the literature, values were estimated using LFER approach between Al^{3+} and Fe^{3+} ions. Essential step in simulations is selection of total Al-concentration in plasma and extent of its binding to transferrin. Inclusion of transferrin binding in calculations leads to decrease of LMM species concentration but their relative distribution remains constant. The effect of transferrin is to decrease the amount of Al^{3+} available for the LMM species pool. Jackson found that at pH 7.4 within the total Al concentration range 10^{-13} – 10^{-5} mol L^{-1} free Al concentration in plasma is approximately ~ 10^{-9} $[Al^{3+}]_{total}$ taking into account upper level of soluble Al concentration (as set by precipitation of $Al(OH)_3$ and $AlPO_4$) as well as binding to transferrin (50% saturation). At total Al concentration 1 μmol L^{-1} this means free Al concentration 10^{-15} mol L^{-1}. Thus, insoluble Al species and formation of Al – transferrin complex may not be included in calculations. Other serum proteins, notably albumin, so weakly bind to Al that these interactions could safely be neglected.[67] To take into account the formation of labile species, which are depended on level of Al concentration, we scanned the concentration range between 10^{-15} to 10^{-3} mol L^{-1}. The calculated distribution of the species in blood plasma is given in Table 5A. It appears that soluble mixed hydroxo phosphate species of aluminum is the dominating one as found in earlier works. Citrate species in the form of mixed phosphate and hydroxo complexes account for about 30% of total Al. In the broad concentration range of Al this distribution does not change significantly. Only at higher Al concentration hydroxide species gradually become more important.

Ternary complexes $AlCitPO_4$ and $AlCitPO_4OH$ are new species that occur as a result of calculation which did not appear in previous computer studies. A review of published computer simulations of the speciation of aluminum in serum shows that some studies predicted that citrate would be the main LMM ligand whereas other predicted that phosphate would be more important Al LMM binding agent. These difficulties mainly arise from the fact that aluminum speciation was calculated at pH 7.4 from the data derived at much lower pH values. Also the experimental studies use the total concentrations of Al 1000 and more times higher than the physiological ones. These difficulties were reduced in a recent study of Harris et al., on Al speciation in serum.[55] The effective binding constants for Al-citrate and Al-phosphate have been determined at pH 7.4 and total Al concentration 10 μmol L^{-1}. We added the relevant data into our database and repeated the speciation calculations excluding non-relevant Al-phosphate species. The obtained distribution of Al-species is shown in Table 5B.

It can be seen that the mixed complex $Al(PO_4)Cit$ is the dominating species, while $Al(PO_4)$ accounts for only 0.01% of LMM Al species. Thus, of the pool of LMM aluminum species, 83% of the aluminum is bound to citrate in mixed and binary complexes. The mixed hydroxo phosphate-Al species appear to be much less important than in a previous model.

In evaluating the obtained results it must be taken into account that the biological fluids are open systems which never reach true thermodynamic equilibrium. Thus, the speciation of metal ions particularly aluminum, is time dependent process. Recently, the time dependent speciation of Al was calculated using the data of pH dependence on time in solutions containing Al^{3+} ion, citrate and phosphate ligands.[68] The results of calculations indicate that the ternary mixed Al-citrate-phosphate species predominate over time until true equilibrium is reached. If phosphate is added in excess to a solution of trinuclear Al-citrate, the phosphate slowly displaces the citrate from the complex.[69] Thus, at physiological pH phosphate appears as efficient binder of Al. This agrees with Bantam et al., findings in their experiments with $Al(NO_3)_3$ spiked serum that three main Al species in serum are Al-citrate, Al-phosphate and ternary Al-citrate-phosphate complex.[70] Time dependent distribution of Al^{3+} in serum was elaborated by Beardmore and Exley.[71] Their model predicted significant role and existence of Al-hydroxide phase which is

Table 5. Calculated bio-distribution of Al(III) species in human blood plasma using different sets of LMM – Al complexes. A: Harris's model of LMM-Al complexes,[53] B: Harris et al model of LMM-Al complexes.[55]

Total Al concentration (mol L^{-1})			**1×10^{-15}**	**5×10^{-13}**	**1×10^{-11}**	**1×10^{-9}**	**1×10^{-5}**	**1×10^{-3}**
% Al-LMM Species	A	$Al(PO_4)(OH)$	88.5	88.5	88.5	88.5	88.3	37.2
		AlCit(OH)	9.1	9.1	9.1	9.1	9.2	10.3
		$Al(OH)_3$	1.3	1.3	1.3	1.3	1.3	35.6
		$Al(OH)_4$	0.5	0.5	0.5	0.5	0.5	15.2
		$AlPO_4$	0.5	0.5	0.5	0.5	0.5	0.2
	B	$Al(PO_4)Cit$	40.7	40.7	40.71	40.71	39.9	41.4
		AlCit $(PO_4)(OH)$	22.9	22.9	22.9	22.9	22.4	28.8
		AlCit(OH)	19.2	19.2	19.2	19.2	19.2	17.6
		$Al(PO_4)(OH)$	12.7	12.7	12.7	12.7	13.4	7.2
		$Al(OH)_3$	2.6	2.6	2.6	2.6	2.9	2.2
		$Al(OH)_4$	1.1	1.1	1.1	1.1	1.2	1.2

consistent with the formation of insoluble ternary Al-hydroxide-phosphate phase as found in computer models. It follows therefore, that kinetic route to equilibrium distribution of LMM species of Al in serum indicates very important role of phosphate as a competent binder of Al, forming predominantly ternary complexes.

2. 2. Gadolinium in Human Blood Plasma Model

Gadolinium complexes included in speciation are given in Table 6. Binding of Gd(III) to serum albumin was also considered. About 100 gadolinium complexes with blood plasma ligands as well as insoluble species $Gd_2(CO_3)_3$ and $Gd(PO_4)$ were included. A total concentration of gadolinium in blood plasma model was scanned from 1.2×10^{-9} to 1.0×10^{-2} mol L^{-1}, that is from normal serum level of gadolinium to much higher concentration levels.[72] High concentration levels were tried to observe trends in species formation. The results obtained with HySS2009 calculation are shown in Table 6.

Main soluble complexes in blood plasma appears to be the mixed ternary complex GdAspCit. Binding to albumin accounts for about 7.5% of total gadolinium concentration. The distribution of the Gd(III)-ion in plasma complexes has been calculated with different Gd(III)-ion con-

Table 6. The soluble Gd(III) species distribution in human blood plasma

Total Gd concentration (mol L^{-1})		1.2×10^{-9}	1×10^{-8}	1×10^{-7}	1×10^{-6}	1×10^{-5}	1×10^{-4}	1×10^{-3}	1×10^{-2}
% Gd-LMM Species	GdCitAsp	29.39	7.15	0.72	0.07	0.01	–	–	–
	$GdCit(OH)_2$	20.14	4.90	0.49	0.05	0.01	–	–	–
	GdCitLac	11.93	2.90	0.29	0.03	0.00	–	–	–
	$GdCitHisH_2$	10.47	2.55	0.25	0.03	0.00	–	–	–
	GdHSA	7.88	1.92	0.19	0.02	0.00	–	–	–
	GdCitLeu	3.04	0.74	0.07	0.01	0.00	–	–	–
	GdCit	2.78	0.68	0.07	0.01	0.00	–	–	–
	$GdCitGlnH_2$	2.42	0.59	0.06	0.01		–	–	–
	GdOxa	1.99	0.48	–	–	–	–	–	–
	GdGlyCit H_2	1.65	0.40	–	–	–	–	–	–
	GdGluCit	1.20	0.29	–	–	–	–	–	–
	$GdAlaCitH_2$	1.16	0.28	–	–	–	–	–	–
	$GdValCitH_2$	1.15	0.28	–	–	–	–	–	–
	GdCit(OH)	0.96	0.23	–	–	–	–	–	–
	$Gd(CO_3)_2$	0.69	0.17	–	–	–	–	–	–
	GdHAspCit	0.58	0.14	–	–	–	–	–	–
	GdHGlnCit	0.51	0.12	–	–	–	–	–	–
	$Gd_2(CO_3)_{2\ (s)}$	0.00	0.00	0.00	0.00	0.00	0.00	73.82	96.94
	$GdPO4_{\ (s)}$	0.00	75.67	97.57	99.76	99.98	100.00	26.18	3.06

Table 7. Stability constants of Yttrium complexes used in blood plasma model

Y-LMM species	$\log\beta_{p.q.r}$	Y-LMM species	$\log\beta_{p.q.r}$	Y-LMM	$\log\beta_{p.q.r}$	Y-LMM species	$\log\beta_{p.q.r}$
YH_{-1}	–7.80	YHis	3.00	YHCit	9.30	YAsn	5.46
YH_{-2}	–14.04	YLeu	6.09	YCit	6.80	$YAsn_2$	6.58
YH_{-2}	–17.00	$YLeu_2$	8.16	YLys	3.10	YAsp	4.75
YH_{-3}	–26.0	$YAla_2$	8.09	YH_2Cit	10.86	$YAsp_2$	8.42
Y_3H_{-5}	–33.8	YSal	8.68	$YCit_2$	10.17	YSer	5.53
Y_4H_{-6}	–32.0	YTrp	5.48	YLac	2.80	YHSer	3.50
Y(SCN)	1.60	$YH_2(PO_4)$	4.30	$YLac_2$	5.33	$Y(CO_3)$	5.71
$Y(SCN)_2$	2.90	YTyr	2.90	$YLac_3$	6.95	$Y(CO_3)_2$	10.33
$Y(SCN)_3$	3.40	YHTyr	4.43	YSal	8.68	$Y_2(CO_3)_2$	6.98
$Y(SO_4)$	3.51	YCys	4.90	YAla	5.42	YPro	5.50
$Y(SO_4)_2$	5.34	YGlu	4.82	YMet	5.72	$YPro_2$	10.21
YOxa	5.74	YGln	4.72	YPhe	3.49	$YH_{-3(s)}$	19.9
$YOxa_2$	10.09	$YGln_2$	8.05	YVal	4.79	$YPO_{4(s)}$	16.98
YGly	5.06	YTrp	3.70	$YVal_2$	9.06	$Y_2(CO_3)_{\ 3(s)}$	–31.52
YMal	4.60	YIle	6.11	YThr	3.70		
YHMal	8.14	YCys	4.90	YHypro	4.52		
$YMal_2$	7.56	YArg	3.20	$YHypro_2$	8.92		

centration ranging from 1.2×10^{-9} to 1.0×10^{-2} mol L^{-1}. Increasing the concentration of Gd(III) leads to decrease of the dominant complex concentration favoring the appeareance of insoluble species. The $GdPO_{4(s)}$ begins to form at low gadolinium concentrations and its relative percentage increases with increasing the total Gd concentration. It becomes the only species at Gd(III) concentration of 1×10^{-4} mol L^{-1} and higher. Only the solids, Gd-carbonate and Gd –phosphate are present in the system. Carbonate solid phase is competitor to phosphate only at milimolar concentrations of Gd. Thus $GdPO_{4(s)}$ and $Gd_2(CO_3)_{3(s)}$ are the dominant species in a wide range Gd(III)-ion concentration consistent with the tendency lanthanides to form insoluble complexes with phosphates and carbonates.

2. 3. Yttrium in Human Blood Plasma Model

So far there is very little results of Y(III) speciation in human blood plasma. In this work we included about 65 Y-LMM complexes with total concentration of Y(III)-ion 1×10^{-9} mol L^{-1}. Complexes used in simulation are shown in Table 7 and the results of HySS2009 calculation are shown in Table 8.

Main soluble complex in blood plasma appears to be the complex YCit. Increasing of the concentration of Y(III) leads to decrease of the dominant complex concentration favoring the appeareance of insoluble species. The distribution of the Y(III)-ion within plasma complexes has been calculated with different Y(III)-ion concentration ranging from 1.0×10^{-9} to 1.0×10^{-3} mol L^{-1}. Dominant Y(III) complexes in serum calculated by HySS2009 shown in Table 8.

Table 8. Dominant Y(III)-complexes in serum at different concentrations of yttrium

Total Y concentration (mol L^{-1})		1×10^{-9}	1×10^{-8}	1×10^{-7}	1×10^{-6}	1×10^{-5}	1×10^{-3}
% Y-LMM Species	YCit	47.63	47.63	47.62	6.27	0.49	5.04
	$Y(CO_3)_2$	32.49	32.49	32.5	4.28	0.33	5.93
	$Y(CO_3)$	10.60	10.61	10.61	1.40	0.11	2.06
	$YCit_2$	2.98	2.98	2.98	0.39	0.03	0.16
	YOxa	1.57	1.57	1.57	0.21	0.02	0.24
	$Y_2(CO_3)_{3(s)}$	0	0	0	86.84	98.98	85.64

From Table 8 it can be seen that increasing the total concentration of yttrium leads to appearance of insoluble species. $Y_2(CO_3)_{3(s)}$ becomes the dominant species at Y(III) concentration range from 1×10^{-6} to 1×10^{-3} mol L^{-1}.

3. Conclusion

The computer simulation of speciation of Al^{3+}, Gd^{3+} and Y^{3+} ions in human plasma using the program Hyss2009, indicate that the main binding plasma ligand is citrate forming binary and/or ternary complexes with metal ions. Upon increasing the total concentration of metal ions hydroxide complexes become more important. Contribution of phosphate complexes to the Al speciation strongly depends on equilibrium Al – phosphate data included in computer simulation. Phosphate does not appear to be important binder of Al^{3+} ion if set of complexes included in computer model comprised only the species relevant to physiological conditions. However, if the included set of complexes was derived from LFER approximation of equilibrium measurements made in a broader pH range, then mixed hydroxo or citrate ternary Al – phosphate complexes appear as the important species. All three metal ions show similar, behavior in blood plasma with regard to citrate binding. Citrate species are the main ones for all three metal ions forming either binary or ternary complexes. Presence of insoluble complexes is characteristic for studied metal ions and depends upon total concentration of metals.

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4. 1. Abbrevations

ICP-MS	Inductively coupled plasma
ETAAS	Electrothermal atomic absorption spectrometry
AMS	Accelerator mass spectrometry
GFC-FAAS	Graphite furnace atomic absorption spectrometry
FPLC	Fast protein liquid chromatography
MRI	Magnetic resonance imaging
ICP-OES	Inductively Coupled Plasma-Optical Emission Spectrometer
LFER	Linear free energy relationship
LMM	Low molecular mass
HMM	High molecular mass
Tfn	Transferrin
HSA	Human serum albumin

DTPA	Diethylenetriaminepentaacetic acid
DOTA	1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid
EDTMP	Ethylenediaminetetramethylphosphonic acid
Amino acid residues	Standard abbreviations

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Povzetek

Preučevali smo speciacijo Al^{3+}, Gd^{3+} in Y^{3+} ionov v človeški plazmi z računalniško simulacijo ob uporabi programa HySS2009. Posodobili smo računalniški model iz literature in zajeli 9 kovin, 43 ligandov ter preko 6100 kompleksov. K temu modelu smo dodali kritično presojene podatke za konstante Al^{3+}, Gd^{3+} in Y^{3+} z ligandi iz krvne plazme. Nizkomolekularna (LMM) speciacija Al^{3+} iona je zelo odvisna od izbranega ravnotežnega modela za sisteme kovina–fosfat in kovina–citrat. Dobljene računalniške simulacije LMM speciacijskih podatkov so: $AlPO_4Cit$ (40,7%), $AlPO_4CitOH$ (22,9%), AlCitOH (19,2%) in $AlPO_4(OH)$ (12,7%) (% skupnih LMM Al zvrsti); za Gd^{3+} ion: GdAspCit (30%) in $GdCit(OH)_2$ (20%) (% skupne [Gd]) in za Y^{3+} ion: YCit (48%), $Y(CO_3)_2$ (32%) in $Y(CO_3)$ (11%) (% skupne [Y]). Citrat se pojavlja kot pomemben ligand za vezavo in mobilizacijo preučevanih ionov, medtem ko so dominantne zvrsti ternarnega tipa.