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Interaction of UO_2^{2+} with ATP in aqueous ionic media

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Abstract

Interaction of dioxouranium(VI) (uranyl) ion with ATP was studied by ligand/proton and metal/hydroxide displacement technique, at very low ionic strength and at $I=0.15$ mol L^{-1}, in aqueous Me_4NCl and NaCl solutions, at $t=25$ °C. Measurements were carried out in the pH range 3–8.5, before the formation of precipitate. Computer analysis allowed us to find the quite stable species $UO_2(ATP)H_2^0$, $UO_2(ATP)H^-$, $UO_2(ATP)^{2-}$, $UO_2(ATP)_2^{6-}$, $UO_2(ATP)_2H_2^{4-}$ and $UO_2(ATP)(OH)^{3-}$ whose formation constants are (at $I=0$ mol L^{-1}) $\log\beta_{112}=18.21$, $\log\beta_{111}=14.70$, $\log\beta_{110}=9.14$, $\log\beta_{120}=12.84$, $\log\beta_{122}=24.82$, and $\log\beta_{11\text{-}1}=2.09$, respectively. Different values were obtained in the above ionic media at $I=0.15$ mol L^{-1} and the dependence on the ionic medium was interpreted in terms of interactions between the negatively charged complex species and cations of supporting electrolytes. The species more stable in NaCl than in Me_4NCl are those with the highest negative charge, $UO_2(ATP)_2^{6-}$ and $UO_2(ATP)_2H_2^{4-}$, and the extra stability of these species can be attributed to the interaction with Na^+. Speciation profiles show that ATP can suppress UO_2^{2+} hydrolysis, and that in the neutral to slightly alkaline range the yield of complex UO_2–ATP species is quite high. Comparison with other metal–ATP systems is also given in order to recognize the possibility of binding competition of uranyl ion in metal–ATP requiring enzymes for biochemical processes.

Keywords: ATP; Dioxouranium(VI); Complexes; Ionic media; Dependence on medium of stability constants; Speciation

1. Introduction

Metal complex formation of nucleotides is well documented, as well as its biological importance. Metal–nucleotide complexes may act as cofactor, substrate or modifier in promoting enzymatic catalysis of displacement reactions of phosphorus and in maintaining structural integrity and specificity of nucleic acids. Nucleotides bind metals through three potential binding sites: phosphate groups, sugar hydroxo groups and ring nitrogen of base. The interaction of phosphate groups determines, to a very large extent, the stability of the complexes formed with biologically important metal ions [1–3], while the selectivity and specificity of reactions involving nucleotides are largely due to nucleobase residues [4–6]. The high binding capacity of the ATP towards metal ions is, at the same time, one of the reasons for the toxicity of heavy metals which may alter the normal course of many biological processes. Adenosine 5′-triphosphate is the main biomolecule responsible for the energy transfer, usually by means of complexes of ATPase enzyme with magnesium and calcium ions. Other essential metal ions, like zinc, can participate to the enzymatic process of ATP production [7]. The presence of other metal ions, which form stable complex species with the enzyme, can compete with calcium and/or magnesium ions or with the other possible essential metal ions in ATPase binding, changing intracellular concentration of these ions and, according to the toxicity level of the external metal ion added, the function of the enzyme in the ATP production can be seriously inhibited. For this reason it is of great importance to make quantitative studies on the stability of different ATP–metal ion complexes. As examples, Boukhalfa et al. [8], in a very recent study, report results on the competition between Be^{2+} and Ca^{2+} in ATP

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binding, and Frasch [9] used VO_2^+ ion as a probe in the binding change mechanism of the ATPase–Mg^{2+} cofactor. Moreover, Ln(III)–ATP complexes have been used by Eads et al. [10] as probe in Mg^{2+}–ATP requiring enzymes. Due to the biochemical importance of the metal binding capacity of ATP, a large number of papers has been published and many data on the ATP metal complex formation are reported in some general compilations [11–15]; moreover, an IUPAC special publication [16] has been devoted to thermodynamic parameters and stability data of nucleotides protonation and metal complexes formation. In spite of this large number of papers and compilations, few studies [17,18] are reported in the literature on the coordination chemistry of nucleotides with UO_2^{2+} in aqueous solution, and never quantitative data on the stability of complex species formed were reported.

The high toxicity of uranyl ion is well known [19–25] as well as its high capacity to interact with different ligands, including phosphoric groups contained in many biological systems [26]. In some previous papers we reported the results of calorimetric and potentiometric studies on the thermodynamic parameters (log$K$, ΔH and $T\Delta S$) of UO_2^{2+} hydrolysis in several ionic media, at different ionic strengths [27–29]. Moreover, to give a further contribution to the knowledge of UO_2^{2+} speciation in natural waters, we undertook an extensive study on its ability to interact with different ligands (low and high molecular weight polycarboxylates, polyamines, amino acids) and some thermodynamic data regarding UO_2^{2+}–acetate, –glutamate and –aspartate complexes have been already published [30,31].

Here we report a potentiometric ([H^+]-glass electrode) study on the interaction of ATP with uranyl ion. The measurements were carried out in both Me_4NCl and NaCl ionic media at $I = 0.15$ mol L^{-1} and in absence of background salt, in the pH range 3–8.5, at $t = 25$ °C. Several attempts to find the right experimental conditions were made, and we found that only low concentrations of UO_2^{2+} and ATP in the concentration ratio M/L = 1:4 or 1:5 were suitable to avoid precipitation in the pH range investigated and to perform measurements in aqueous solution. The difficulty to find the best experimental conditions is, probably, the reason of the lack of thermodynamic data on UO_2^{2+}–ATP complexes for which few and only structural investigations are reported.

2. Experimental

2.1. Materials

Dioxouranium (VI) cation (UO_2^{2+}) was used as nitrate salt (Fluka). The purity of dioxouranium (VI) nitrate, checked gravimetrically after precipitation as U_3O_8 by gaseous ammonia, was >99.5%. ATP (adenosine 5′-triphosphate) was used as disodium salt hydrate (by Fluka, purity >99%). Tetramethylammonium chloride (Fluka, 97%) was re-crystallised from methanol–acetone and solvent was completely eliminated before use. Hydrochloric acid (Aldrich) and sodium or tetramethylammonium hydroxide (Merck and Fluka, respectively) were prepared by diluting concentrated solutions and were standardized against sodium carbonate and potassium hydrogen biphthalate, respectively. Tetramethylammonium hydroxide solutions were used when the background salt was the tetramethylammonium chloride. All solutions were prepared using CO_2-free freshly prepared distilled water ($R = 18$ MΩ) and grade A glassware was always employed.

2.2. Experimental equipment

Measurements were carried out using a potentiometric apparatus consisting of a Metrohm mod. 665 automatic titrant dispenser coupled with a Crison potentiometer and a combination Orion-Ross 8172 glass-electrode. The dispenser and potentiometer were connected through a personal computer and a suitable computer program allowed the acquisition of the potentiometric data. The measurement cells were thermostatted at $t = 25.0 \pm 0.1$ °C. The estimated accuracy of the system was ±0.15 mV (±0.003 pH units, ±0.6% [H^+]) and ±0.003 mL for e.m.f. and titrant volume readings, respectively.

2.3. Procedure

A volume of 25 mL of the solution containing dioxouranium(VI) nitrate (0.4–0.5 mmol L^{-1}) and ATP (1.8–2.3 mmol L^{-1}) was titrated with standard hydroxide solution (NaOH or Me_4NOH). The measurements were performed (i) with the background salts Me_4NCl or NaCl at ionic strength 0.15 mol L^{-1}, and (ii) without ionic medium, in the same range of reagent concentrations. Details of the experimental conditions used in this work are reported in Table 1. For each experiment, independent titrations of HCl with NaOH or Me_4NOH solutions were performed in the same experimental conditions of ionic strength and temperature as the systems under study in order to determine the formal electrode potential. In the titration cells pure nitrogen was bubbled through the solution in order to avoid CO_2 and O_2 inside and the solution were magnetically stirred.

2.4. Calculations

The non-linear least squares computer program ESAB2M [32] was used to determine all the parameters of acid–base potentiometric titrations (acid–base concentrations, electrode formal potential, junction potential coefficient, ionic product of water K_w). The formation constants of the dioxouranium(VI)–ATP complexes were refined using STACO and BSTAC computer programs [33]. STACO refines the formation constants by minimizing the weighted error squares sum of titrant volumes, i.e., $\sum w(v - v_{calcd})^2$, while BSTAC minimizes the weighted error

Table 1
Experimental conditions for UO_2^{2+}–ATP system, t=25 °C

I^{a}	C^{b}_{UO2}	C^{b}_{ATP}	C^{b}_{H}	pH range	n^{c}
Ionic medium Me_4NCl or NaCl					
0.150±0.005	0.00052	0.00229	0.00155	2.76–8.33	185–164
	0.00044	0.00229	0.00156	2.77–8.38	131–142
	0.00035	0.00186	0.00156	2.77–8.28	135–126
	0.00044	0.00234	0.00155	2.76–8.36	151–98
	0.00052	0.00238	0.00191	2.68–8.33	162–133
Without ionic medium					
0.010	0.00051	0.00185	0.00207	2.68–8.30	226
0.009	0.00051	0.00185	0.00207	2.68–7.22	186
0.012	0.00051	0.00231	0.00206	2.70–8.31	240
0.013	0.00051	0.00232	0.00041	3.18–8.34	185
0.011	0.00051	0.00185	0.00041	3.17–8.35	133
0.014	0.00044	0.00229	0.00165	2.75–8.42	130
0.014	0.00046	0.00229	0.00155	2.77–8.46	185
0.011	0.00041	0.00177	0.00155	2.78–8.40	162
0.014	0.00050	0.00230	0.00181	2.71–8.47	196
0.014	0.00055	0.00230	0.00181	2.71–8.50	197

[a] Mean values of ionic strength (mol L^{-1}) during the titration.
[b] mol L^{-1}.
[c] Number of experimental points (Me_4NCl–NaCl).

squares sum of potentials $\sum w(E-E_{calcd})^2$ (w is the weight used for each experimental point). Both STACO and BSTAC programs are able to perform calculations also under non-constants ionic strength conditions (in particular, at low ionic strength there may be significant ionic strength variations during the same titration) and to refine some titration parameters (such as E^0 or analytical concentrations). The ES4ECI computer program [33] was used to obtain distribution diagrams of the species in solution (speciation profiles). All the formation constants are expressed according to the equilibrium

$$pUO_2^{2+} + q\left(ATP^{4-}\right) + rH^+ = (UO_2)_p(ATP)_qH_r^{(2p-4q+r)} \qquad \beta_{pqr}$$

Preliminary analysis of potentiometric data shows that quite strong complexes are formed in the system under study. The classic proton ligand displacement technique[1] implies that $\log K_{complex} \approx \log K^{H}$ (or at least $\log K_{complex} < \log K^{H} + 1.5$, where $K_{complex}$ is the formation constant of the strongest complex species and K^{H} is the highest protonation constant). This condition is not fulfilled for the UO_2^{2+}–ATP system ($\log K^{H}_{1(ATP)}$=7.65 and $\log K_{(UO_2-ATP\ complex)}$= 9.14 at I=0 mol L^{-1}). On the other hand, if the metal undergoes strong hydrolysis, one may resort to the metal OH displacement technique[1], if $\log K_{complex} \approx \log K^{OH}$ (where K^{OH} is the UO_2OH^+ formation constant, K^{OH}= $K_{hydrolysis}/K_w$). In this case the condition is fulfilled ($\log K^{OH}_{(UO_2)}$=8.81 and $\log K_{(UO_2-ATP\ complex)}$=9.14). For the formation of less stable complex species ($p+q+r>2$), both displacement techniques are valid, and the possibility of exploiting simultaneously the H^+ and OH^- equilibrium reactions renders the calculations for this system quite robust.

2.5. Ionic strength dependence of formation constants

Dependence on ionic strength was taken into account using the Debye–Hückel type equation [34]

$$\log K = \log{}^{T}K - z^{*}I^{1/2}\left(2+3I^{1/2}\right)^{-1} + CI + DI^{3/2} \qquad (1)$$

where $C=c_0p^{*}+c_1z^{*}$, $D=d_0p^{*}+d_1z^{*}$, $p^{*}=\sum(\text{moles})_{\text{reactants}}-\sum(\text{moles})_{\text{products}}$ and $z^{*}=\sum(\text{charges})^2_{\text{reactants}}-\sum(\text{charges})^2_{\text{products}}$.
K is the formation constant, ${}^{T}K$ the formation constant at infinite dilution, and C and D empirical parameters. According to previous results [34], when all interactions are taken into account, the values c_0=0.11, c_1=0.20, d_0=0 and d_1=−0.075 can be used. In a paper [35] on the protonation of ATP in several supporting electrolytes, including tetraalkylammonium salts, other C and D values have been reported, and in this work the appropriate values according to the used medium were considered in the calculations. For the hydrolysis of uranyl ion the parameters C and D reported in Ref. [27] were used.

3. Results and discussion

3.1. Hydrolysis of dioxouranium(VI) and protonation constants of ATP

To make a correct speciation model of the system UO_2^{2+}–ATP we took into account the protonated species of ATP (and in some cases also the formation of weak ion pairs with Na^+) and the hydrolytic species of uranyl ion whose equilibrium constants we have already determined in NaCl medium, at different ionic strengths. In particular, ATP protonation constants and Na^+–ATP weak complex species formation constants (referred to the reaction: $pNa^+ + qATP^{4-} + rH^+ = Na_p(ATP)_qH_r^{(p-4q+r)}\beta_{pqr}$) at I=0 mol L^{-1} and t=25 °C are [35]:

$$\log\beta_{011} = 7.65$$

$$\log\beta_{012} = 12.18$$

$$\log\beta_{110} = 2.06$$

$$\log\beta_{111} = 7.86$$

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[1] In other words, the effective displacement reactions that occur into solution

$$UO_2^{2+} + H_jATP^{(4-j)-} = UO_2ATPH_{j-1}^{(2-4+j-1)} + H^+$$

$$UO_2(OH)_i + ATP^{4-} = UO_2ATP(OH)_{i-1}^{(2-4-i+1)} + OH^-$$

must be true equilibrium reactions.

Table 3
Equilibrium constants for the formation of UO_2^{2+}–ATP complexes, at $t=25$ °C

Reaction	log*K*		
	$I=0.15$ mol L^{-1} (Me_4NCl)	$I=0.15$ mol L^{-1} (NaCl)	$I=0$ mol L^{-1}
$UO_2^{2+}H_2(ATP)^{2-}=(UO_2)(ATP)H_2^0$	4.94	3.75	6.03
$UO_2^{2+}H(ATP)^{3-}=(UO_2)(ATP)H^-$	5.88	4.82	7.05
$UO_2^{2+}ATP^{4-}=(UO_2)(ATP)^{2-}$	7.49	7.42	9.14
$(UO_2)(ATP)^{2-}+ATP^{4-}=(UO_2)(ATP)_2^{6-}$	4.68	5.05	3.70
$(UO_2)(ATP)H^-+H(ATP)^{3-}=(UO_2)\ (ATP)_2H_2^{4-}$	2.91	3.97	2.47
$UO_2(OH)^+ATP^{4-}=(UO_2)(ATP)(OH)^{3-}$	6.55	6.27	7.28

and the hydrolysis constants of uranyl ion, referred to the following equilibrium reactions,

$$pUO_2^{2+}+rH_2O=(UO_2)_p(OH)_r^{(2p-r)}+rH^+ \qquad \log\beta_{p0r}$$

$$pUO_2^{2+}+rOH^-=(UO_2)_p(OH)_r^{(2p-r)} \qquad \log\beta_{p0r}^{OH}$$

are ($I=0$ mol L^{-1} and $t=25$ °C) [27]:

$$\begin{array}{ll} \log\beta_{10-1}=-5.19 & \log\beta_{10-1}^{OH}=8.81 \\ \log\beta_{20-2}=-5.76 & \log\beta_{20-2}^{OH}=22.24 \\ \log\beta_{30-4}=-11.82 & \log\beta_{30-4}^{OH}=30.18 \\ \log\beta_{30-5}=-15.89 & \log\beta_{30-5}^{OH}=54.11 \\ \log\beta_{30-7}=-29.26 & \log\beta_{30-7}^{OH}=68.74 \end{array}$$

3.2. Formation and stability of UO_2^{2+}–ATP species

Experimental measurements were fitted to the complexation model including, in the pH range investigated, the following six species: $UO_2(ATP)H_2^0$, $UO_2(ATP)H^-$, $UO_2(ATP)^{2-}$, $UO_2(ATP)_2^{6-}$, $UO_2(ATP)_2H_2^{4-}$ and $UO_2(ATP)(OH)^{3-}$. The values of their formation constants (calculated, using both STACO and BSTAC computer programs [33], without allowing for the formation of weak ATP–Na^+ ion pairs) are reported in Table 2. The formation constants at $I=0$ mol L^{-1} reported in the Tables 2 and 3 are not extrapolated values, but they are semi-empirical values calculated from measurements at low ionic strengths by using Eq. (1) and the empirical parameters values already reported for similar systems [27,28,34,35]. This correction procedure introduced a negligible additional error, ≈0.1 *I*, i.e.,<0.002, being $I<0.015$ mol L^{-1}. The values reported in Table 2 show that UO_2^{2+}–ATP complexes are very stable and this can be better seen by considering the partial equilibria, as reported in Table 3. As expected, equilibrium constants in NaCl and in Me_4NCl are significantly different, according to the different interacting capabilities of Na^+ and Me_4N^+ towards anions. Generally, for complexes of metal ions with negatively charged ligands, the stability follows the order $Me_4N^+>Na^+$, since Na^+ forms more stable ion pairs with anions (this competition can be described by the equilibrium $ML+Na^+=NaL+M^{z+}$). As can be seen from the values of formation constants reported in Table 3, for the two species with higher charge, $(UO_2)(ATP)_2^{6-}$ and $(UO_2)(ATP)_2H_2^{4-}$, we have an opposite trend. In fact, in addition to the interaction between ATP^{4-} and Na^+, we must consider the interaction of the anionic complex species with Na^+ that implies a stabilization effect. Weakening or stabilizing effects are the results of two equilibria:

$$(ATP)H_r^{(4-r)-}+Na^+=Na(ATP)H_r^{(4r-1)-} \qquad (r=0,1)$$

$$(UO_2)_p(ATP)_qH_r^{(2p-4q+r)}+Na^+ = Na(UO_2)_p(ATP)_qH_r^{(4q-2p-r-1)-} \qquad (r=0,2)$$

and for highly negatively charged anions, such as ATP, the second effect may be the predominant one. The trend for the stabilized species is $(UO_2)(ATP)_2^{6-}<(UO_2)(ATP)_2H_2^{4-}$ (see the relative formation equilibria in Table 3) since the interaction of ATP^{4-} with Na^+ is more relevant than that of $H(ATP)^{3-}$ with the same cation.

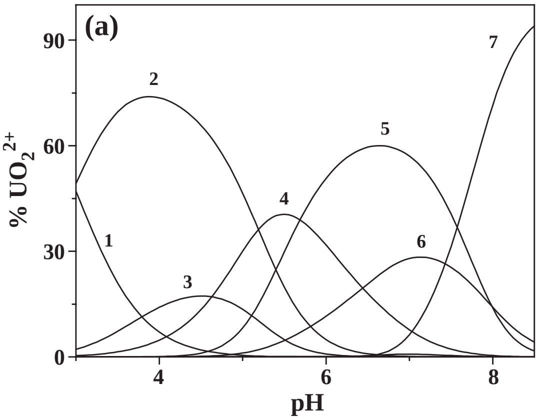
In Figs. 1 and 2 the distribution diagrams for the UO_2^{2+}–ATP system in NaCl and Me_4NCl ionic media ($I=0.15$ mol L^{-1}, $t=25$ °C) are reported. In order to check species formation percentages in different conditions, the diagrams were drawn using 1:2 and 1:100 UO_2^{2+}:ATP concentration ratios. In the pH range investigated (pH=3–8.5) all species formed in the uranyl–ATP system are present in a

Table 2
Overall formation constants for the UO_2^{2+}–ATP complexes[a], in NaCl and Me_4NCl, at $t=25$ °C

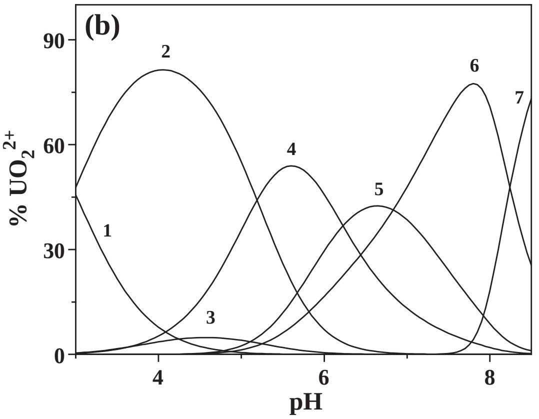
I/mol L^{-1}	$\log\beta_{112}\pm s$[b]	$\log\beta_{111}\pm s$	$\log\beta_{110}\pm s$	$\log\beta_{120}\pm s$	$\log\beta_{122}\pm s$	$\log\beta_{11-1}\pm s$
0	18.21±0.02	14.70±0.02	9.14±0.02	12.836±0.008	24.82±0.01	2.09±0.02
0.15 (Me_4NCl)	15.66±0.01	12.68±0.03	7.49±0.02	12.173±0.004	22.394±0.008	1.05±0.01
0.15 (NaCl)	14.00±0.03	11.13±0.02	7.42±0.01	12.473±0.008	21.41±0.02	0.77±0.01

[a] Referred to the reaction: $pUO_2^{2+}+q(ATP^{4-})+rH^+=(UO_2^{2+})_p(ATP^{4-})_qH_r^{(2p-4q+r)}$.

[b] s=standard deviation on the parameters.



C_{Me_4NCl} = 0.15 mol L^{-1}; C_{UO_2} = 0.5 mmol L^{-1}; C_{ATP} = 1 mmol L^{-1}

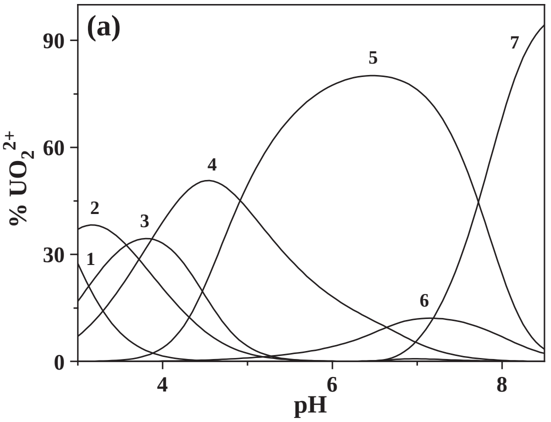


C_{Me_4NCl} = 0.15 mol L^{-1}; C_{UO_2} = 10^{-3} mmol L^{-1}; C_{ATP} = 0.1 mmol L^{-1}

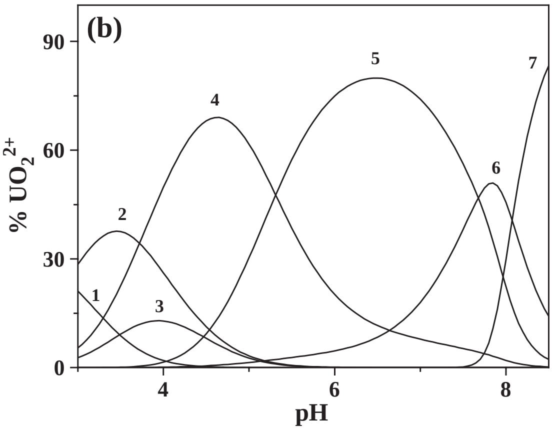
Fig. 1. Distribution of species vs. pH in the system H^+–Me_4N^+–UO_2^{2+}–ATP^{4-}–Cl^- at different values of ligand and metal concentrations, at $t=25$ °C. Species: (1) $(UO_2)(ATP)H_2^0$; (2) $(UO_2)(ATP)H^-$; (3) $(UO_2)(ATP)_2H_2^{4-}$; (4) $(UO_2)(ATP)^{2-}$; (5) $(UO_2)(ATP)_2^{6-}$; (6) $(UO_2)(ATP)(OH)^{3-}$; (7) $(UO_2)_3(OH)_7^-$.

significant yield. Moreover, some observations can be made on the speciation profiles of Figs. 1 and 2. First of all, formation percentages of the various species are quite different in the two supporting electrolytes (for some species this difference is greater than 50%), and this strongly indicates the importance of ionic medium, in addition to the ionic strength.

As can be seen, the main species at pH 7 is the $(UO_2)(ATP)_2^{6-}$ reaching over 70% formation in NaCl medium, also for lower uranyl and ligand concentration (curve 5, Fig. 2a,b). Worth of mention is also the very high yield of the hydroxo species $UO_2(ATP)(OH)^{3-}$ at low reagents concentration (curve 6, Figs. 1b and 2b), in the two considered ionic media: the formation percentage of this species at pH = 7 is over 15% (NaCl) and 45% (Me_4NCl), and it increases noticeably at pH = 7.5–8 (≈ 75% in Me_4NCl and ≈ 50% in NaCl, respectively). In the pH range of physiological interest (7–7.5), the total amount of UO_2^{2+} is present as $UO_2(ATP)^{2-}$, $UO_2(ATP)_2^{6-}$ and $UO_2(ATP)(OH)^{3-}$ species (Figs. 1b and 2b, curves 4, 5 and 6, respectively). The formation of these highly stable soluble complex species



C_{NaCl} = 0.15 mol L^{-1}; C_{UO_2} = 0.5 mmol L^{-1}; C_{ATP} = 1 mmol L^{-1}



C_{NaCl} = 0.15 mol L^{-1}; C_{UO_2} = 10^{-3} mmol L^{-1}; C_{ATP} = 0.1 mmol L^{-1}

Fig. 2. Distribution of species vs. pH in the system H^+–Na^+–UO_2^{2+}–ATP^{4-}–Cl^- at different values of ligand and metal concentrations, at $t=25$ °C. Species: (1) $(UO_2)(ATP)H_2^0$; (2) $(UO_2)(ATP)H^-$; (3) $(UO_2)(ATP)_2H_2^{4-}$; (4) $(UO_2)(ATP)^{2-}$; (5) $(UO_2)(ATP)_2^{6-}$; (6) $(UO_2)(ATP)(OH)^{3-}$; (7) $(UO_2)_3(OH)_7^-$.

allows us to emphasize the strong suppressing effect on the hydrolysis of uranyl that also avoids the formation of U(VI) insoluble oxide species. Less stable species of uranyl (for instance, uranyl–acetate [30] or UO_2^{2+}–gluta-

Table 4
Literature stability constants of some metal–ATP systems at $t=25$ °C

Metal ion	I/mol L^{-1}	M(II)–ATP complex species		Ref.
		logβ_{110}	logK_{111}[a]	
Mg^{2+}	0.10 (Me_4NCl)	4.55	2.32	[16]
	0.12 (NaCl)	4.01	–	[36]
Ca^{2+}	0.10 (Me_4NCl)	4.24	2.16	[16]
	0.12 (NaCl)	3.7	–	[36]
Zn^{2+}	0.10 (Me_4NCl)	5.16	2.69	[16]
	0.12 (NaCl)	4.1	–	[36]
Cu^{2+}	0.10 (Me_4NCl)	6.42	3.35	[16]
	0.12 (NaCl)	6.0	–	[36]
Ni^{2+}	0.10 (Me_4NCl)	5.21	2.79	[16]
	0.12 (NaCl)	4.54	–	[36]
Cd^{2+}	0.10 (Me_4NCl)	5.68	3.0	[16]
Co^{2+}	0.10 (Me_4NCl)	5.1	2.66	[16]
	0.12 (NaCl)	4.58	–	[36]

[a] Equilibrium constant refers to the reaction: $M^{2+}+HATP^{3-}=M(ATP)H^-$.

mate and –aspartate [31] species) are not able to suppress the formation of insoluble hydrolytic species of the uranyl at pH>6–6.5. The formation of so highly stable soluble UO_2^{2+}–ATP species is also the main reason of hazard for the enzyme metabolism.

3.3. Comparison with the stability of other metal complexes of ATP

From literature analysis we can assert that this is the first contribution to the quantitative speciation study of the system UO_2^{2+}–ATP, and, therefore, no comparison can be made with previous results. Nevertheless, an interesting comparison can be made with the stability of other ATP–divalent metal ion systems, also in order to individualize the competitive ability of uranyl ion and, therefore, to assign to it a potential hazard in modifying the normal enzyme metabolism. Many data are reported in the literature on the M(II)–ATP complex formation and stability. In Table 4 we report some literature data (in Me_4NCl [16] and NaCl [36] media, for omparison with data here reported) on the stability of complex species of ATP with some divalent cations. To this end, we chose Mg^{2+}, Ca^{2+}, Zn^{2+}, Cu^{2+}, Co^{2+} and Ni^{2+}, often involved in the metal enzyme complexes [7], and Cd^{2+} which is known to be able to alter the activity of ATPase enzyme and, therefore, can influence the ATP concentration [37]. By comparing data reported in Table 4 with the stability constants of UO_2^{2+}–ATP complex species (see Table 2) it is evident that UO_2^{2+} ion is able to replace all the above metal ions in an eventual interaction with ATP in its protonated and/or deprotonated form.

4. Conclusions

The reported quantitative data on the interaction between UO_2^{2+} and ATP show that this interaction is quite relevant and leads to very high yields in terms of complex species concentrations. Different types of complexes are found at different pH values and this must be carefully taken into account when considering the speciation in different natural waters and biological fluids. Also the dependence on medium needs thorough consideration since the stabilization (or weakening) effects due to the ions of supporting electrolyte can be quite relevant. A rough estimate of the strength of these interactions can be obtained using the equation [38]

$$\Delta \log K(\mathrm{Na}^+, \mathrm{Me_4N}^+) = -\log\left(1 + K_{(\mathrm{NaATP})}[\mathrm{Na}^+]\right) + \log\left(1 + K_{\left(\mathrm{NaUO_2(ATP)}_q\mathrm{H}_r\right)}[\mathrm{Na}^+]\right).$$

By suitable calculations we have $\log K_{(\mathrm{NaUO_2(ATP)_2})} = 1.1$ and $\log K_{(\mathrm{NaUO_2(ATP)_2H_2})} = 2.4$: these fairly high formation constants account for the unusual high stability of 1:2 complexes.

A comparison of the stability of metal ion–ATP complexes shows higher stability of the UO_2^{2+}–ATP species with respect to other ATP systems containing essential and toxic metal ions, and let us to affirm that the presence of dioxouranium (VI) ion reduces significantly the availability of ATP in the metabolic enzyme processes.

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