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Development of an *in vitro* test for screening of chelators of uranium

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Abstract. This work deals with an *in vitro* spectrophotometric method to evaluate the chelating ability of various organic ligands for uranyl ion. In this way, an uranium complex formed with a peculiar ligand is chosen as a reference. Any modification of the UV-Visible absorbance of the U(VI) reference complex, owing to its dissociation upon the addition of a new ligand, permits to compare the affinity of the latter ligand for U(VI) with that of the reference ligand. This test allows to screen easily a lot of ligands before to evaluate their *in vivo* uranium chelating property.

Key words. Uranium (VI) titrimetry – uranium ligands – Chromotrope 2R complexes.

Introduction

Uranium under several chemical forms is widely used in the nuclear fuel cycle facilities. After an accidental human contamination, which may be due to a dust inhalation or an uranium salt ingestion, an appropriate treatment could be necessary to accelerate uranium excretion and to reduce its deposition in affected organs, therefore preventing manifestation of related adverse effects. At the present time, the therapy proposed after uranium contamination is still an intravenous injection with a 250 mL sodium bicarbonate solution (1.4%) [1] However, because of the limited efficiency of bicarbonate treatment, the development of more effective substances therefore remains an important aspect of radioprotection [2].

The screening of new compounds able to chelate uranyl ion and to permit its body elimination involves a lot of *in vivo* tests. Studies were conducted using rats and mice on

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multidentate catecholate and hydroxypyridonate [3,4], phosphonic acid derivatives [5], phenolic agent like Tiron [6], biphosphonates [7] and tripode phosphorylated [8] compounds. These studies are rather fastidious and expensive. At this step of screening of new compounds, an *in vitro* test should be sufficient to determine its ability to complex uranium with a higher affinity than bicarbonate, for example.

The aim of this study was to settle an *in vitro* test for screening the affinity for uranium of new compounds. The accuracy of the test has been checked in measuring the chelating affinity of different molecules considered as references and chosen according to their known ability in chelating uranium *in vivo*.

This technique allowed a very fast and inexpensive determination of the chelating ability of various compounds.

Method

Principle

The complexation property of these compounds with uranium was evaluated in an aqueous buffered solution (pH = 7), using a technique based on competitive complexation [9] and spectrophotometry.

When a metallic ion is complexed with a chromophore chosen as a reference, a measurable modification of its UV-visible absorption spectrum occurs. The absorption difference between the free form of the chromophore and the cation complexed form can be used to specify a chelating property. Indeed, the addition of another complexing agent, which does not absorb in UV at the same wavelength as the reference chromophore, should induce a significant modification of the UV absorption intensity of the chromophore related to their respective chelating ability. In these conditions, the complexation ability of a compound can easily and quickly be compared to that of the reference molecule. Moreover, the result obtained with this chosen reference could be referred to those measured with another one.

The choice of this reference molecule has to comply with a few constraints: solubility and stability in water at physiological pH, high complexation strength of the metal ion and reaction kinetic as fast as possible, great intensity of the absorption spectrum in the UV-visible field and above all measurable modification of the spectrum associated to the complexation in a wavelength field different from those of the chosen metal ion.

In the case of the uranyl ion, the reference compound is derived from chromotropic acid, which was described to have a strong affinity for uranium (VI) [6]. The absorption spectrum of this compound lies in the UV-visible field [6].

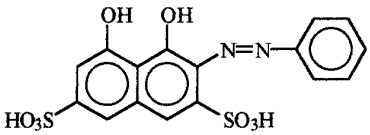
When the affinity of the ligand for uranium (VI) is substantially higher than that of the reference ligand Chromotrope 2R, the absorbance measured after addition of a stoechiometric amount of ligand must be that of the pure Chromotrope 2R. In this case, the complex between uranyl and Chromotrope 2R is destroyed by the new ligand.

When the affinity of the ligand for uranium is in the same level than that of the Chromotrope 2R, the absorbance should range from the value of the pure Chromotrope 2R (0.25) to the absorbance of the complexed Chromotrope 2R (0.5). If this variation is considered as linear and if the stoechiometry of the U(VI)/Ligand complex is the same for the new ligand as for the Chromotrope 2R, the complexation strength (i.e. the conditional stability constant) of the ligand could be estimated, compared to the reference molecule.

When the affinity of the ligand for uranium is clearly weaker than that of the Chromotrope 2R, the equilibrium must remain undisturbed and the absorbance-measured (0.5) corresponds to that of the complexed Chromotrope 2R.

When the ligand tested displaces the Chromotrope 2R complex, it can be assumed that the new complex formed is stronger and that it would be interesting to test this compound *in vivo.*

Such a method could be generalised to various metal ions if a chromophore compound well-known for its chelating properties can be used as a reference.



Chromotrope 2R

Protocol

Complexation between uranium and Chromotrope 2R:

A 0.1 mM stock solution of the Chromotrope 2R was prepared in saline and the pH was adjusted at 7. The absorption spectrum of the solution was measured between 200 and 800 nm. When a solution of uranyl nitrate ($UO_2(NO_3)_2$) was added, a modification of the colour of the solution due to the formation of the complex was observed. The difference between the absorption spectrum of the Chromotrope 2R and the spectrum of the complex was measured. The resulting spectrum exhibited a maximum wave at 580 nm (Fig. 1). The pH was adjusted with 20 mM HEPES buffer which did not complex uranium and did not absorb at 580 nm. The intensity of the absorbance peak was correlated to the degree of complexation of the Chromotrope 2R.

It is noteworthy that a neutral solution of uranyl nitrate does not present any absorption at 580 nm.

The reaction and the measure were made in saline (NaCl 0.9% in Milli-Q distilled water). This also set the ionic strength of the medium (I = 0.15). The spectra were measured with Beckman DU 7500 using single use cells at room temperature.

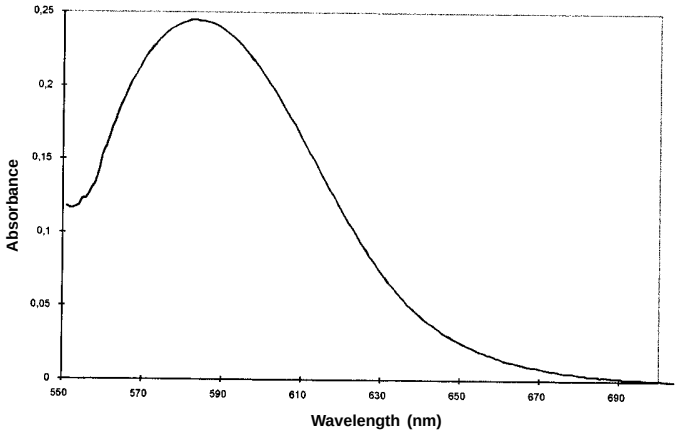


Fig. 1. Difference spectrum of Chromotrope 2R complexed to U(VI) at 20 °C between: – sample: Chromotrope 2R: 0.166 mM, U(VI): 0.083 mM, Hepes: 3.3 mM, pH = 7 – reference: Chromotrope 2R: 0.166 mM, Hepes: 3.3 mM, pH = 7.

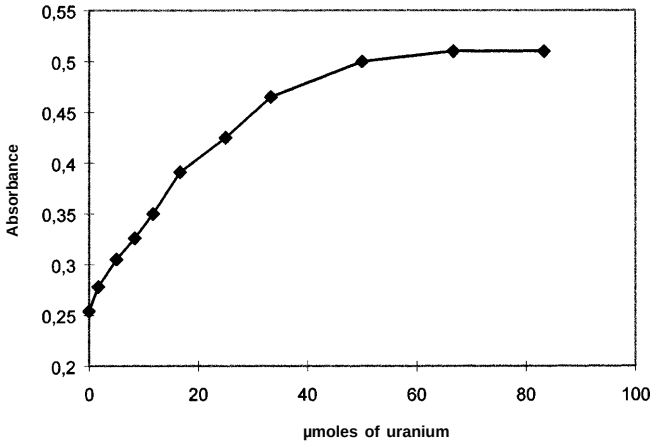


Fig. 2. Variation of the absorbance at 580 nm of Chromotrope 2R *versus* increasing concentrations of uranium (VI) (pH = 7) at 20 °C. Chromotrope 2R: 0.166 mM, U(VI): ranging from 0 to 100 µM, pH = 7; Hepes: 3.3 mM.

Destruction of the complex between uranium and Chromotrope 2R by a ligand:

The following reagents were added in this order: 1 mL of a 1 mM solution of Chromotrope 2R (Aldrich), 3 mL of 20 mM HEPES buffer (Aldrich) at pH 7, 1 mL 0.5 mM uranyl nitrate solution (Prolabo). The solution was mixed, then protected from direct sunlight and left for 15 min. One mL of the compound to assay was added; its final concentrations in the vial was ranging from 10^{-2} to 10^{-7} mM. The absorbance of the solution was measured at 580 nm 30 minutes later.

Results and discussion

The Chromotrope 2R is a dye derived from the chromotropic acid. It presents a chelating power for U(VI) [6]. This complexation results in a change of the colour of the solution. The highest difference between the spectra of the free Chromotrope and the U(VI) complex is observed at 580 nm (Fig. 1).

When the variation of the absorbance of a 0.166 mM Chromotrope 2R solution versus the amount of added uranium is plotted, the maximum of absorbance is reached after the addition of 0.0833 mM of uranium (Fig. *2*). This result indicates that 1 uranyl ion is complexed with 2 Chromophore 2R molecules. This concentration of 0.0833 mM has been retained for the test in order to be certain that the reaction was complete and that there was no free uranium in the solution. As the values of absorbance remain stable during the time, one admits that the equilibrium is reached in all cases.

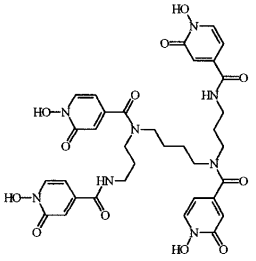
At the concentration used, uranyl nitrate may precipitate at a pH higher than 5. It is therefore necessary to respect the order of addition of the reactant in the solution: Chromotrope, HEPES, $UO_2(NO_3)_2$ and at last the ligand. Moreover, at this pH and for U(VI) concentrations higher than 0.1 mM, the appearance of uranyl microprecipitates should disturb the measure of absorbance [10,11].

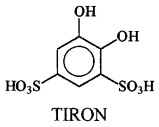
When a ligand L^2 is added into a solution containing an other ligand L^1 (Chromotrope 2R) and the cation UO_2^{2+}, the following reaction takes place:

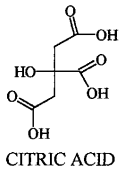
$$L^2 + L^1\ UO_2^{2+} \Leftrightarrow L^1 + L^2UO_2^{2+}.$$

The protocol described here was applied to some molecules known for their *in vivo* complexation ability: bicarbonate, Tiron, citric acid and 3,4,3-Li-(1,2-HOPO) [4]:

The results are presented in figures 2 and 3 and call for the following comments:







– our results show that the bicarbonate ion, which is known as *in vivo* strong complexing compound, exhibits a rather weak chelating power. The shift of the complex is only obtained with a bicarbonate concentration (10^{-2} M) 100 times higher than that of the Chromotrope 2R (Fig. 3). The complexing strength observed *in vivo* could be explained through the mass effect of bicarbonates: indeed the level of bicarbonate administered is very high towards the uranium amount.

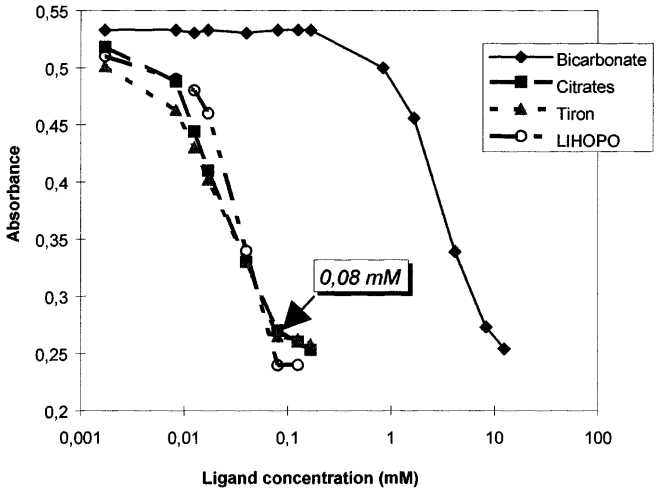


Fig. 3. Variation of the absorbance at 580 nm of Chromotrope 2R at 20 °C. Chromotrope 2R: 0.166 mM.; uranyl nitrate: 0.0833 mM; Hepes: 3.3 mM and Tiron 3,4,3-LI(1,2-HOPO), sodium bicarbonate or citrate at various concentrations (pH = 7).

– Citric acid, Tiron and 3,4,3-Li-(1,2-HOPO) exhibit complexing strengths in the same range of magnitude than Chromotrope 2R (10^{-4} M) (Fig. 3). Our results strongly suggest that 1 uranyl complexes with 1 molecule of 3,4,3-Li-(1,2-HOPO). Actually, the complex between uranium and the Chromotrope 2R is totaly destroyed (absorbance = 0.23) when the concentration of 0.083 mM of 3,4,3-Li-(1,2-HOPO) is achieved in the medium, which is also the concentration of uranium.

– It is underlined that Tiron and 3,4,3-Li-(1,2-HOPO) were tested *in vivo*. Results obtained showed that both compounds are able to complex the uranyl ion [4].

Conclusion

After the complexation of uranium with a chosen chromophore, herein the Chromotrope 2R, any modification of the absorption spectrum observed in the solution will be due to a dissociation of the complex. This will be obtained by the addition of a compound having an affinity for uranium at least equivalent to that of the reference ligand. In these conditions, the complexation ability of the added compound can easily and quickly be compared to that of the reference molecule.

The aim of this work was to design a simple test in order to perform a screening of potential uranyl ligands while limiting, as far as possible, the *in vivo* studies. The idea was to find a good complexing agent of uranium like the Chromotrope 2R, with relevant spectrophotometric properties and to test different ligands already known for their *in vivo* properties. When the ligand tested displaces the complex, it can be assumed that the new complex formed is stronger and that it is interesting to test that compound *in vivo*. In most of the cases, this test does not allow the direct determination of the stoechiometry of the complexation, and the comparison between the complexation power of ligands has to be done very carefully.

However, the ranking of potential chelating agents would need the use of a set of different reference compounds having a complexation constant closer to that of the tested ligands.

Finally, it is obvious that this test could be used with other metals since the formation of the complex with a ligand chosen as a reference disturbs the absorption spectrum.

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