XAS applied to pharmaceuticals: drug administration and bioavailability

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We present selected XAS applications, focused towards practical hospital questions of drug administration and bioavailability, where the technique is driven up to its limits of sensitivity. i) XAS was used to study the interactions between the components of parenteral nutrition solutions, in particular zinc and aminoacids, possibly modifying their bioavailability. ii) We studied by EXAFS a series of binary and ternary copper-aminoacid complexes, in view of the development of an efficient oral drug against copper deficiencies in Menkes disease. iii) EXAFS and XANES analysis allowed us to characterise the solution form of a new arsenic containing drug against leukaemia. In parallel to the XAS measurements, we analysed trace elements levels along patients' hairs, using X-ray fluorescence excited by synchrotron radiation. The measurements along the hair allow for a monitoring of essential trace elements during therapy.

Keywords: XAS, pharmaceutical, parenteral nutrition, Menkes disease, leukaemia, zinc, copper, arsenic

1. Introduction

Metal ions are present in the form of various bioinorganic complexes in biological fluids. Deficiencies of essential trace elements are known disease causes, an artificial supplementation being then necessary. Moreover several drugs contain metal ions as active components. Exact characterisation of the drug before administration is necessary, as well as monitoring of the drug metabolism and speciation in the organism during and after therapy.

X-ray absorption spectroscopy (XAS) reflects the local environment of the metal ion regardless of the physical state (crystal, gel or solution) and is thus particularly suited for structural characterisation of the trace element containing pharmaceuticals. On the other hand, the high sensitivity of synchrotron radiation excited X-ray fluorescence (XRF) spectroscopy, allows us to perform multi-elemental analysis on very small biological samples. Three different examples will demonstrate the information we can obtain by applying XAS and XRF spectroscopies to pharmaceutical problems.

2. Materials and methods

X-ray absorption data were collected at the LURE synchrotron storage ring (positron currents ranging from 260 to 300 mA) in Orsay, France. All spectra were recorded at room temperature, using a Si(111) double crystal monochromator for EXAFS and Si(311) for XANES spectra. Platinum, zinc and copper foil spectra have been recorded for energy calibration on the As, Zn and Cu edge respectively. Solid state and concentrated solutions (over 10 mM) were recorded in transmission mode, while diluted solutions were recorded in fluorescence mode, using a 7 element Canberra detector.

All spectra were analysed using the LASE software (Curis, 2000) either on Alpha workstation, or Macintosh and PC computers running the linux operating system. For each sample, at least two spectra were normalised and averaged, extraction and Fourier transform filtering of the EXAFS signal being subsequently performed on the averaged spectra with error propagation (Curis & Bénazeth, 2000) across the different stages. All injectable solutions and their models were prepared in 0.9% NaCl aqueous solution.

X-ray fluorescence spectra of hair were also recorded at the LURE synchrotron using a 14 keV excitation energy and a focal spot of 1 mm, achieving ppm detection limits on ng quantities of hair. Thus, analysing single spots along the hair, a kinetic study is possible (Nicolis, 2000). Before analysis, hair were washed according to the IAEA procedure (Borella, 1996). Control hair spectra from healthy people were recorded under the same conditions as reference.

3. Results and discussion

3.1 Zinc complexation in parenteral nutrition solution

Zinc is the second most abundant trace element in human body following iron. Over 200 enzymes activity relies on zinc complexation. (Kaim & Schwederski, 1994) Whilst normal feeding is sufficient for correct zinc absorption, an artificial supplementation can become necessary during severe digestive pathologies. This supplementation is carried on by parenteral administration of nutritional solutions containing a mixture of sugars, aminoacids, vitamins, electrolytes and trace elements (Anglade, 1990).

Interactions between solution components can lead to decreased bioavailability or even precipitation. We were particularly interested to the complexation of zinc to cysteine and histidine, possibly reducing the bioavailability of both zinc and aminoacids.

Both histidine and cysteine deprotonation (and consequently ability of metal chelation) rises with pH. We recorded Zn/histidine and Zn/cysteine solution XAS spectra at pH 7, under fluorescence mode because of the low solubility of Zn as $Zn(OH)_2$ precipitates at this pH value. The spectrum of a Zn acetate solution was recorded at the same conditions as a reference of free hexahydrated Zn. Comparison of the near edge features of the spectra (Figure 1) indicates a lowering of the intensity of the white line going from free zinc to complexed, the effect being more pronounced with the cysteine system. This change is typical of a change from octahedral to tetrahedral coordination, in accordance with the crystal structures of both complexes. Indeed, in the solid state $Zn(cysteine)_2$ complex, zinc is coordinated to 2 S and 2 N atoms in a tetrahedral geometry. In the $Zn(histidine)_2$ crystal (Chen, 1994), Zn atoms are surrounded

by 4 near neighbours (2 N and 2 O atoms) and 2 distant O atoms in a deformed octahedral coordination.

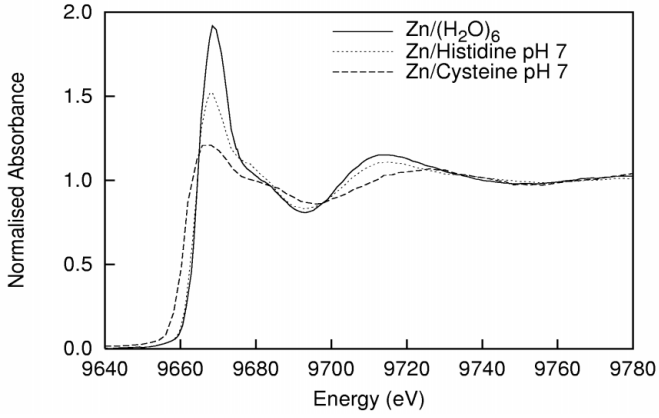

Figure 1
XANES spectra of free and complexed zinc

Fourier transforms of the cysteine complex EXAFS spectrum demonstrate an elongation of the first shell compared to free or complexed to histidine zinc (as is expected if sulphur coordination occurs). A series of peaks at longer distances present in the Fourier transform of the histidine complex spectrum is typical of the imidazole ring (Figure 2).

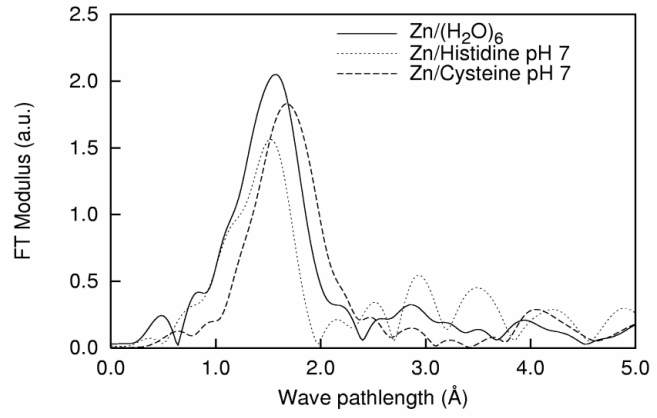

Figure 2
Fourier transforms of free and complexed zinc

Fitting of the first shell Fourier transform filtered EXAFS spectra, using a series of solid state model spectra, results to a tetrahedral coordination for both histidine and cysteine complexes. In the cysteine system, Zn is coordinated to 2 S and 2 N atoms (at 2.259 and 2.099 Å respectively), while in the histidine system Zn is coordinated to 4 N atoms at 2.032 Å. The very low Zn concentration (1 mM) results to noisy spectra, not allowing a precise fit of the second shell in order to identify the two distant O atoms capping the crystal state Zn(histidine)$_2$ octahedron.

3.2 Copper histidine complex in treatment of Menkes disease

The Menkes disease is a rare genetic disorder resulting in lethal Cu deficiencies. The Cu deficiency is due to the lack of an ATPase responsible for Cu cellular efflux. The only currently used treatment is the subcutaneous administration of a Cu/histidine complex. Indeed, copper aminoacid complexes are considered to be the transport form of Cu(II) in blood (Sarkar, 1966). In a view of developing better supplementation complexes and possibly an orally administered form, we applied XAS to the characterisation of the Cu/histidine complex in the injectable solution. The same considerations as for the zinc complexation apply to the pH of the solutions.

Fitting of the non-filtered EXAFS spectrum has been carried on using the crystal structure of Cu(histidine)$_2$ as a starting model and taking into account multiple scattering. Results indicate a square planar coordination of the Cu to 2 N atoms at 1.974 Å and 2 O atoms at 2.033 Å, the square being capped by 2 further O atoms at 2.48 Å (Figure 3), in accordance with the crystal structure (Camerman, 1978).

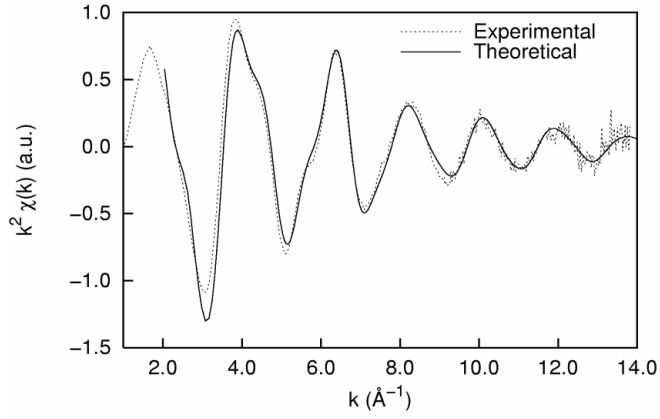

Figure 3
Fitting of the Cu/histidine solution EXAFS

3.3 An arsenic containing antileukaemic drug

Acute promyelocytic leukemias are treated by chemotherapy, nevertheless 25% of patients have a relapse after the first treatment. For these cases a new drug is tested, injectable solubilised arsenic trioxyde (Chen, 1997, Shen, 1997, Soignet, 1998, Niu, 1999). Two different techniques based on X-ray absorption were applied to the study of this treatment.

Two questions arising about the arsenic form in the injectable solution are the valence of arsenic and its coordination. We plan on a second stage of the study, to characterise the drug and its metabolites in biological fluids. In order to characterise the drug solution and prepare the biological samples speciation experiments, we recorded XAS spectra of the drug and of a series of reference arsenic compounds of valence III and V, both in solid and solution form: arsenic trioxyde, arsenious, arsenic and dimethylarsenic (a possible metabolite) acids. XANES spectra (Figure 4) are very well distinguished and indicate the arsenic being in valence (III) in the injectable solution.

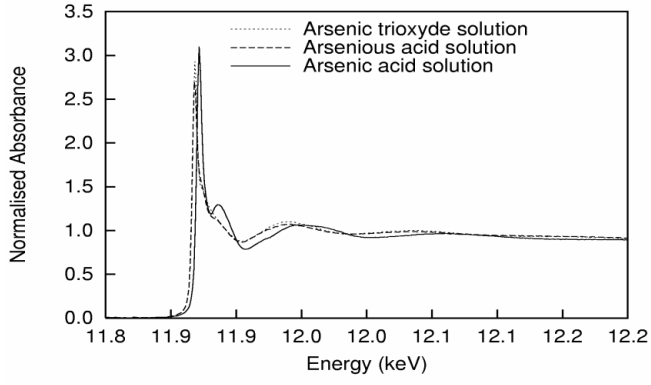

Figure 4
XANES spectra clearly differentiate As(III) from As(V)

Fitting of the non-filtered EXAFS spectra (Figure 5) yields a coordination to three O atoms at 1.779 Å, as in an arsenious acid solution.

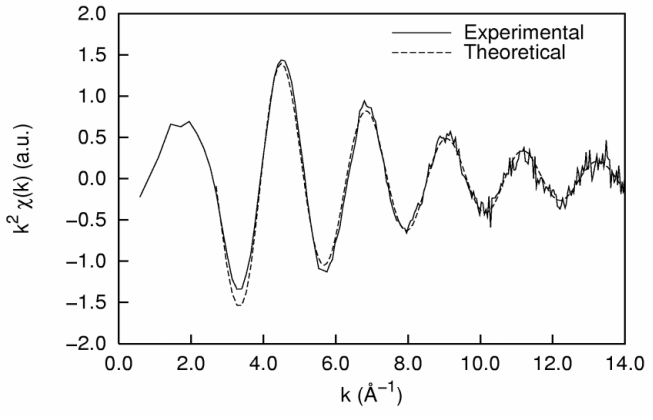

Figure 5
Fitting of the As injectable solution EXAFS

In the second part of the study, we recorded X-ray fluorescence spectra of hairs of patients having received the arsenic treatment. Recording spectra on successive points of single hairs allowed us to monitor the arsenic content in patient's hairs for a period up to 5 months (taking into account a growth rate of 1 mm/3 days). The spectra were recorded for at least 3 hairs per patient in order to average fluctuations.

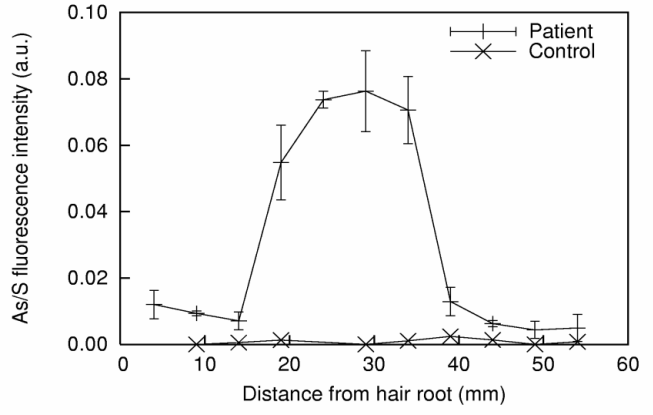

Figure 6
Arsenic content in hairs of a patient after As_2O_3 therapy.

The results (Figure 6) show a steep rise in arsenic content with the beginning of therapy and a fall to normal levels in a month after the end of the treatment.

4. Conclusion

Common difficulties raised in all cases of pharmaceutical XAS applications are the complexity of the systems involved in several simultaneous pH dependent equilibria and the low concentration of the trace element in solution. In our approach, we demonstrated that suitable model system selection and precise control of experimental conditions, combined with the use of sensitive detectors, can lead us to useful insight about pharmaceutical problems, possibly yielding to solutions for such practical problems as drug stability and biovailability or supplementation optimisation. Moreover, XRF excited by Synchrotron radiation is sensitive enough to allow for a kinetic study along hair and thus trace element assimilation monitoring during and after therapy.

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