

An attempt at kidney stone analysis with the application of synchrotron radiation

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Received 29 November 2007

Accepted 25 January 2008

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X-ray absorption near-edge spectroscopy (XANES) is a spectroscopic technique using synchrotron light to determine the valence state of excited atoms as well as the electronegativity of their neighbouring atoms. XANES spectra can provide information about the chemical bond in the second coordination shell of the excited atom. In this study, XANES spectra of unknown compounds from human kidney stones were recorded around the *K*-edges of sulfur, phosphorus and calcium. The XANES results agree well with the diffractogram data of the same stones obtained through an X-ray powder diffraction (XRPD) technique. By comparing the measurement techniques presented here, it is shown that XANES requires a smaller amount of each sample than XRPD for analysis.

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Keywords: X-ray absorption spectroscopy; X-ray powder diffraction; X-ray absorption near-edge spectroscopy; kidney stones.

1. Introduction

Kidney stones are formed in the urinary tract by substances present in urine. The salts that form these stones are combinations of various minerals and chemicals including those that are derived from personal diet and drugs. The presence of these stones is called nephrolithiasis, and is an important health problem all over the world, although the incidence rates vary (Tanthanuch *et al.*, 2005). In Thailand, the endemic area of nephrolithiasis is in the north-eastern part (Yanagawa *et al.*, 1997). Kidney stone analysis is recommended during the evaluation of patients with nephrolithiasis. Knowledge of the composition of stones will be useful for the treatment plan and removal. For instance, calcium and struvite stones are more amenable to disintegration by extracorporeal shock-wave lithotripsy than those composed of cystine (Pak *et al.*, 2003). The identification of the stone constituent may reveal the underlying metabolic disturbances, such as the association between cystine stones and cystinuria (Kourambas *et al.*, 2001). Consequently, kidney stone analysis will assist in the development of proper rehabilitation, treatment plan and prevention of further recurrence.

Synchrotron radiation techniques for trace-element analysis in biological samples, especially in the field of kidney stone analysis, have already been established and developed. Different analytical methods based on synchrotron light application, for instance X-ray fluorescence (XRF), wide-angle X-ray scattering and X-ray absorption spectroscopy, were reviewed by Bazin *et al.* (2006). Synchrotron X-ray diffraction was used to observe the intracrystalline proteins and amino acids associated with calcium oxalate monohydrate

stone (Fleming *et al.*, 2003). The presence of intracrystalline proteins and amino acids had implications on the destruction of calcium oxalate crystals formed in the urinary tract and the prevention of kidney stones. Estimation opportunities and model experiments on the direct analysis of phase-structure kidney stones directly in the patient with the use of synchrotron X-ray diffraction have been reported (Ancharov *et al.*, 2005, 2007). In addition, the distributions of trace heavy metals in urinary stones were investigated by synchrotron X-ray microfluorescence (Bazin *et al.*, 2007). Knowledge of the distributions of heavy elements in urinary stones can give access to understanding the formation of the metals in stones. Another excellent tool based on synchrotron radiation is X-ray absorption spectroscopy (XAS).

XAS spectra can be divided into two important regimes, as shown in Fig. 1. The first part is X-ray absorption near-edge spectroscopy (XANES), which extends to the first 30 eV past the absorption edge, and the second part is extended X-ray absorption fine spectroscopy (EXAFS), which covers photon energies from ~50 eV to some 1000 eV past the edge (Brown *et al.*, 1995). XANES provides information on the local electronic structure, while EXAFS provides information on the local structural environment (Rosolen & Abbate, 2001).

XANES is expected to form a new way of extracting kidney stone compositions with better accuracy and sensitivity to support nephrolithiasis research. XANES spectroscopy allows the valence determination of an excited atom as well as the electronegativity of neighbouring atoms (Prange *et al.*, 2002). Furthermore, XANES spectra can yield information on the type of chemical bond in the second coordination shell of the excited atom (Prange *et al.*, 2002).

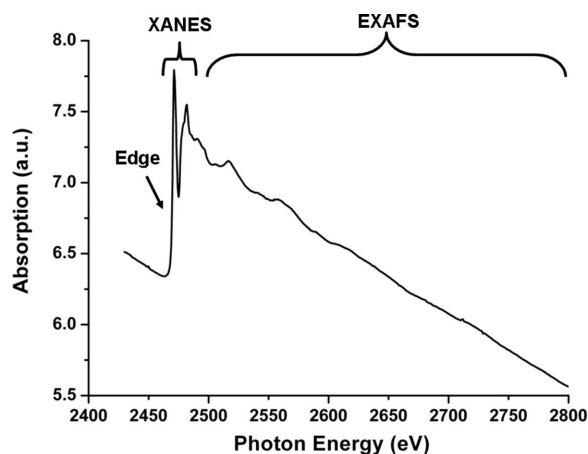


Figure 1
Example of an XAS spectrum of sulfur contained within the ferric disulfide (FeS_2) standard compound. This spectrum was recorded in transmission mode at BL-8 of the NSRC, showing the XANES and EXAFS regions (modified from Pattanasiriwisawa *et al.*, 2007).

Since 2006, the XAS beamline (BL-8) of the National Synchrotron Research Center (NSRC) has been available to users. The commissioning results show a reasonably good performance of the beamline and the XAS station for transmission-mode measurements (Klysubun *et al.*, 2007). Consequently, determination of the chemical compositions of kidney stones in this study can be carried out using a fingerprinting method (XANES) for comparison with spectra of reference chemical compounds at BL-8 of NSRC. Moreover, the XAS data will be compared with the results obtained using a routine method for kidney analysis, X-ray powder diffraction (XRPD).

2. Experimental

2.1. Sample preparation

Fifteen kidney stones were collected and randomly selected after their removal from 15 patients at Sappasithiprasong Hospital, Ubon Ratchathani Province, Thailand. The stones were washed with water to remove any blood, and dried on filter paper. Then the dry stones were ground until they were well blended for analysis by XRPD and XANES.

2.2. XRPD measurements

The powdered samples were sieved using a $63\ \mu\text{m}$ mesh sieve to ensure a uniform size and sifted onto a vaseline-coated zero-background plate to reduce the preferred orientation of the grains. The X-ray diffraction patterns were collected using a Philips X'Pert MPD (Philips Analytical, The Netherlands), using a $\text{Cu K}\alpha$ radiation source ($\lambda = 1.54\ \text{nm}$) at 40 kV and 35 mA. Each sample was measured in the range $10\text{--}100^\circ\ 2\theta$ with a step width of $0.02^\circ\ 2\theta$ and counting time of 10 s per step. A xenon proportional counter was used as the detector. The data were analyzed using Philips X'Pert Graphics and Identify software.

Table 1

Mineral structure of the kidney stones specified by XRPD.

Chemical compositions	Structure	Number of kidney stones
L-Cystine	$\text{C}_6\text{H}_{12}\text{N}_2\text{O}_4\text{S}_2$	1
Struvite	$\text{NH}_4\text{MgPO}_4\cdot 4\text{H}_2\text{O}$	5
Whewellite	$\text{CaC}_2\text{O}_4\cdot\text{H}_2\text{O}$	9

2.3. XANES measurements

The powdered samples were ground and filled into a sample holder. The sample holder was a rectangular frame with Kapton tape on one side. The sample holder size was $12\ \text{mm} \times 6\ \text{mm}$, covering the beam size of the XANES station. XANES measurements of all stone samples and reference compounds were performed at BL-8 at the NSRC using synchrotron radiation from a storage ring operated at an energy of 1.2 GeV (Klysubun *et al.*, 2007). The synchrotron radiation was monochromated by a double-crystal X-ray monochromator equipped with $\text{Si}(111)$ crystals for K -edge measurements of sulfur and calcium, and an $\text{InSb}(111)$ crystal for K -edge measurements of phosphorus. All measurements were carried out in transmission mode (Klysubun *et al.*, 2007). The absorption spectrum as a function of energy was recorded using two ionization chambers located before and after the sample. The ionization chambers were filled with 400 mbar of N_2 gas for the Ca K -edge, 60 mbar of air for the S K -edge and 40 mbar of air for the phosphorus K -edge measurements.

3. Experimental data and discussion

The chemical compositions of all 15 kidney stones were initially identified using XRPD by comparing the measured diffractograms with the standard compounds, as summarized in Table 1. XRPD is used routinely for analysis of the chemical compositions of kidney stones and its results are used in comparison with our XANES results.

XANES was used as a fingerprint method to probe and analyze the human kidney stones. The experiments were performed by measuring the K -edge spectra of sulfur, phosphorus and calcium. The K -edge measurement of sulfur has a maximum at the location of the first peak, for which an electron is excited to the valence state (white line) for standard L-cystine located at 2471.39 eV (Fig. 2). Fig. 2 shows the fingerprint of XANES spectra of kidney stone and reference standards (L-cystine and L-methionine). The location of the first peak which described the excitation of electrons to the valence state (white line) for standard L-cystine is located at 2471.39 eV (Fig. 2). The sample stone has a similar pattern to the reference spectrum of L-cystine. Consequently, this stone is of the L-cystine type. The result also shows that sulfur in the two compounds (L-cystine and L-methionine) of the same oxidation number (-2) could be specifically distinguished by its individual spectra (Fig. 2). The ferric phosphate dihydrate standard also coincides with the phosphorus K -edge located at 2152 eV (Fig. 3). The phosphorus XANES spectra of the

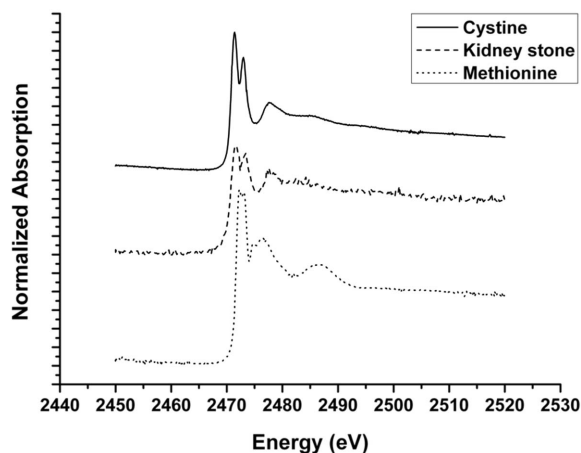


Figure 2 Sulfur *K*-edge XANES spectra of reference compounds used for fitting the kidney spectrum. L-cystine, kidney stone and L-methionine are shown as the solid line, broken line and dotted line, respectively.

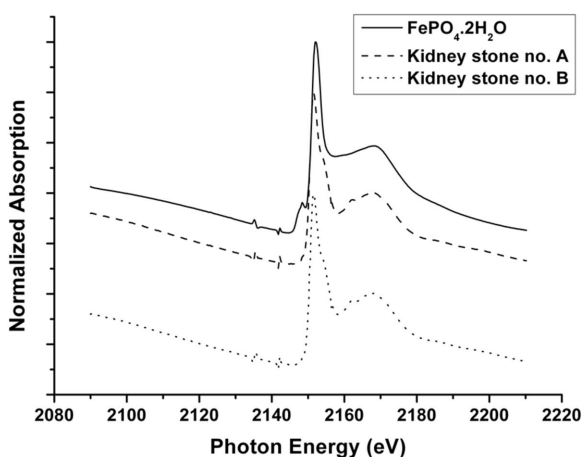


Figure 3 Phosphorus *K*-edge XANES spectra of a reference compound (ferric phosphate dihydrate; $\text{FePO}_4 \cdot 2\text{H}_2\text{O}$) and two struvite ($\text{NH}_4\text{MgPO}_4 \cdot 4\text{H}_2\text{O}$) kidney stones (*A* and *B*). $\text{FePO}_4 \cdot 2\text{H}_2\text{O}$, stone *A* and stone *B* are shown as the solid line, broken line and dotted line, respectively.

two examples of struvite kidney stones (*A* and *B*) match the reference compound (ferric phosphate dihydrate; $\text{FePO}_4 \cdot 2\text{H}_2\text{O}$) as shown in Fig. 3. The result exhibits the highly specific element pattern of XANES when the neighbouring atoms of the absorbent atom are different. There is a small peak at 2148.23 eV of the P *K*-edge XANES spectrum contained within $\text{FePO}_4 \cdot 2\text{H}_2\text{O}$ (solid line) in contrast with the spectra of the struvite ($\text{NH}_4\text{MgPO}_4 \cdot 4\text{H}_2\text{O}$) kidney stone. Finally, the maximum of the first peak of calcium oxalate monohydrate ($\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$, whewellite) agrees until the first peak of the Ca *K*-edge absorption measurement located at 4052.09 eV (Fig. 4). The calcium-type kidney stones were also analyzed by XANES and compared with the Ca *K*-edge spectra of reference compounds calcium carbonate (CaCO_3) and calcium oxalate ($\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$) as shown in Fig. 4. The spectra of calcium oxalate in kidney stone fits very well with the standard calcium oxalate compound. All measured XANES spectra were normalized to the maximum of the white line for better comparison of energy positions in the

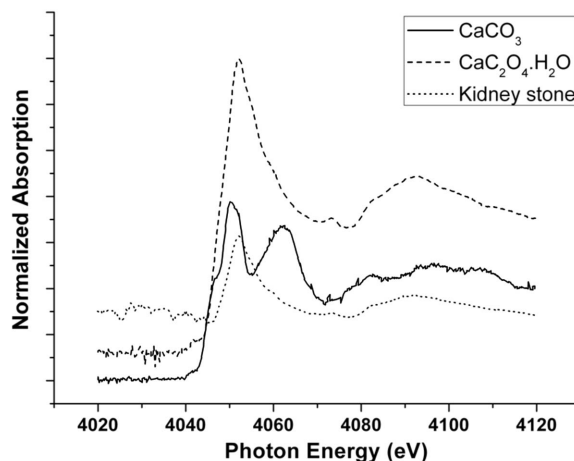


Figure 4 Calcium *K*-edge spectra of a kidney stone (dotted line), calcium carbonate (CaCO_3) (solid line) and calcium oxalate ($\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$) (broken line).

fingerprinting method between kidney stones and standard compounds.

All XANES data agree with the XRPD data. The application of XANES presented here shows the wide opportunity to use synchrotron light in medical research, particularly in the field of kidney stone analysis (Fleming *et al.*, 2003; Ancharov *et al.*, 2005, 2007; Bazin *et al.*, 2006, 2007). XANES is also a high-specificity technique which provides information on the near environment of a specific element (Nicolis *et al.*, 2003) from complex mixtures of crystals and amorphous solids (Eichert *et al.*, 2005). Additionally, XANES can be used as a qualitative and quantitative method by controlling the thickness of the samples in order to avoid possible thickness effects and pinhole effects (Prange *et al.*, 2002).

4. Conclusion

This work shows the facility and possibility of XAS with synchrotron radiation at the National Synchrotron Research Center as a tool for identifying elements contained in biological specimens, such as kidney stones. In addition, XANES can be element specific in any compound of elements in a mixed sample.

In further work, we will apply the XANES and EXAFS technique to measure other elements in both inorganic and organic compounds contained within various kidney stones. Moreover, the XAS technique will also be employed to analyze or determine drug metabolisms and drug-causing urinary stones.

We gratefully thank the National Synchrotron Research Center (grant no. 2-2549/LS06) for supporting this research. Valuable comments on the manuscript from Professor Helmut Wiedemann are gratefully acknowledged.

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