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Measurement of Chemical Composition of Bone by X-ray Absorption Fine Structure

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ABSTRACT: Bone composition has an important role in the human and animal body. However, of inherent difficulties information on characterization of bone structure and composition as biomedicine materials is limited. Therefore, further investigations are needed. In this study, X-ray Absorption Fine Structure (XAFS) in Fluorescence mode was used to investigate the bone composition and the biomedicine materials as standard samples. The obtained results were compared with the published data. Good agreement between experimental results and published data was obtained. This investigation confirmed that XAFS is a useful technique for bone structure and composition. In this work, details of sample preparation procedures, experimental setups and data analysis, are presented and discussed.

KEYWORDS: *X-ray absorption fine structure (XAFS); Fluorescence; Bone mineral; Hydroxyapatite*

INTRODUCTION

Absorption of x-rays by matter varies smoothly with photon energy except at certain energies where abrupt increases occur [(1)]. These energies, known as absorption edges correspond to the x-ray photon attaining sufficient energy to excite additional electron energy levels in the material. [(2)]The absorption around an absorption edge does not vary mono-tonically in condensed material like bone, but displays a complex structure which extends few electronvolts above the edge **[(3)]**.

Isolated atoms do not exhibit this behavior, which clearly illustrates the fact that the structure is a result of the effect of the surrounding atoms (i. e., oscillatory structure) [(4)]. The oscillatory structure, collectively known as X-ray Absorption Fine Structure [(4,5)] has been known to exist for many years, but it was not until the early seventies that a comprehensive theory of its origin became accepted. The phenomenon of X-ray Absorption Fine Structure extending from the near edge to well beyond the edge is now denoted by XAFS**[(5)]**. The XAFS is the modulation of the x-ray absorption coefficient at energies near and above an x-ray absorption edge **[(5,6)]**. XAFS is also referred to as X-ray Absorption Spectroscopy (XAS) [(6)] and is divided into the near edge (XANES)**[(7)]** and extended (EXAFS) **[(8)]** regions. XANES and EXAFS regions contain related, but slightly different information about an element's local coordination and chemical state. The spectrum structure within 40 eV of the edge is known as X-ray Absorption Near-Edge Structure (XANES) **[(7,8)]**. More than ~ 40 eV above the edge, the photoelectron can be

thought of as being free and the oscillations in this region are known as extended x-ray absorption fine structure (EXAFS) [9]. For a detailed description of XAFS, the overview given by Koningsberger et al., [8] is recommended. However, XAFS is predominantly caused by interference between the wave function of the outgoing photoelectron and that small amount of itself which is back-scattered from the surrounding atoms. One of the key attractions of XAFS is that, it depends only on the local structural environment of the absorber and does not require the presence of long-range order.

The advantage of the XAFS is the small sample quantity required for measurements, and a flexible sample environment. This means that XAFS can be used to obtain structural information for non-crystalline samples, and has led to an explosion of interest in the applications of XAFS to biology, medicine and nanocomposite. The term nanocomposite was first used in the early 1980s [10]. The most general definition of a nanocomposite is a multi-phase compound in which one of the phases has a length scale in the nanometer range. In principle this nanocomposite includes a wide range of biological systems, such as bones. Successful technological application of nanocomposite clearly requires a detailed understanding of the underlying physics and chemistry, which in turn relies on a thorough knowledge of the structure at the atomic scale. XAFS is one of the many techniques that can provide information on the microstructure of materials and it has some specific advantages in the study of composites. The XAFS method is used as a powerful tool to study the valance state and coordinate structure of bone [11-13].

The biomedicine materials such as, calcium carbonate (calcite) $CaCO_3$, Tri-Calcium Phosphate [$(Ca_3\ [PO_4]_2)$; TCP], Calcium Hydrogen Phosphate [$(CaHPO_4)$; CHP] and Hydroxyl Apatite [$(Ca_{10}\ [PO_4]_6(OH)_2)$; HA] are widely known and have received great interest.

HA is a biologically important mineral which has been studied extensively, including investigations of its microscopic and crystal lattice structure [2,4]. HA is an inorganic compound whose chemical composition is similar to the composition of the bone. The study of the Ca structure in rat bones as biomedicine materials has received less attention, and still more information is unknown, because of the inherent difficulties in the characterization of their structure and composition [14,15] . In this study, XAFS method was also applied to obtain information on calcium (Ca) and phosphorous (P) structure in rat bone. The overall aim of this study was to achieve a better understanding of the characterization of Ca and P structure in bone. XAFS experiments were carried out by using healthy male and female Sprague–Dawley (SD) rat bones, and biomedicine materials as standard samples.

MATERIALS and METHODS

Preparation of Chemical Composition of Bone

In this study, the calcite, TCP, CHP and HA as standard samples were purchased from Shanghai Pure Chemicals, China. The healthy Male Rats (MR) and Female Rats (FR) were obtained from the Institute of bone metabolism, Fudan University. The MR and FR were obtained at ages of 9 and 7 months, respectively, and each age group has more than 10 individuals. Before killing, they lived under the same conditions, including the same temperature (room

temperature), the same light and dark cycles, and fed with the same food at the same time. When proceeding experimental sample, rats were killed and lumbar were taken out. Then, each bone was cleaned with distilled water and dried. For XAFS experiment, samples were grinded into fine powder with agate pestle. Powder could be smeared on tape (often in transmission method) or compressed into slice (often in fluorescence method).

The Basic Principles of XAFS Technique

In XAFS technique, an x-ray is absorbed by an atom when the energy of the x-ray is transferred to a core-level electron (K, L, or M shell) which is ejected from the atom. The atom is left in an excited state with an empty electronic level (a core hole). Any excess energy from the x-ray is given to the ejected photo-electron. In fact, X-rays (light with wavelength $0.06 < \lambda < 12$ A° or energy $1< E < 200$ keV) are absorbed by all matter through the photo-electric effect.

The light intensity of an x-ray beam passing through a material of thickness t is given by the absorption coefficient μ:

$$I = I_0 e^{-\mu t} \quad \cdots\cdots\cdots\cdots\cdots\cdots (1)$$

Where I_0 is the x-ray intensity hitting the material, and I is the intensity transmitted through the material. The absorption coefficient μ depends strongly on x-ray energy E and atomic number Z, and on the material density ρ (g/cm3) and Atomic mass A:

$$\mu = \rho Z^4 / AE^3 \quad \cdots\cdots\cdots\cdots\cdots\cdots (2)$$

Where, mass absorption coefficient of elements is given as $\mu_m = \mu/\rho$ (cm^2/g). In addition, μ has sharp Absorption Edges corresponding to the characteristic core-level energies of the atom.

XAFS Measurements

In this experiment measurements, the matrix Ca and P in bone sample were selected as elements of interest. The absorption edge of Ca (4.05 keV) or P (2.15 keV) was chosen to be detected in XAFS method. Considering the lower energy photon requirement, XAFS measurements were carried out in the fluorescence mode at 4B7A station in Beijing Synchrotron Radiation Facility (BSRF). To imply fluorescence method, samples were diluted with ZnO. The storage ring was operated at 2.2 GeV with an electron current of 50~100 m A. The synchrotron radiation from a bending magnet was monochromatized with a Si (111) double-crystal monochromator. A small ionization chamber filled with air was placed between the end of the beamline and the sample chamber.

The beam size (FWHM) at the sample position was approximately 25 mm (H) ×25mm (V). The typical photon flux was (10^7 ~10^9) photons/s.mA.0.1 BW) for 4 keV X-rays. The energy resolution ($\Delta E/E$) was about 10^{-4} at 10 KeV. The energy range adjustable was 4~22 KeV. The set up of a typical XAFS experimen t is shown in Figure. 1.

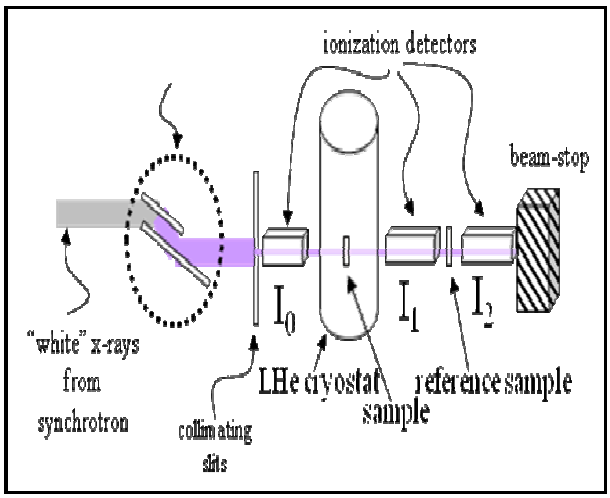

Fig. 1: The set -up of a typical XAFS experiment

The sample chamber was filled with He, and energetic Auger electrons from the

ionized sample surrounding He atoms. The sample was mounted on a copper holder connected to a current amplifier, and a biased plane electrode was placed 3 mm apart from the sample surface.

The electrode was a copper film of 100 nm deposited onto a polycarbonate film of 6 mm thickness, and the electrode was biased at 50 V. The sample holder was a copper plate of 0.2 mm thickness, and it had a central hole of 15 mm diameter. A powder of the sample was supported on a peace of adhesive tape attached to the central hole of the holder.

Experimental X-ray Absorption Measurements

From equation (2) x-rays with a small energy spread or bandwidth is given as:

ΔE = 1 eV at 10keV (3)

For concentrated samples, XAFS is best measured in transmission. To do this, enough transmission through the sample to get a decent signal for ***I*** is needed.

By using the given equation,

$\mu(E)t = -\ln(I/I_0)$ (4)

The sample thickness t can be adjusted so that μ (E) t ~ 2.5 above the absorption edge and / or the edge step Δ μ (E) t ~ 1 for Fe foil t ~7μm. The conditions are that, the sample must be uniform, and free of pinholes.

bigger that an absorption length. In this study, intensities of the incident beam and that transmitted through calcite, TCP, CHP, For a powder, the grain size cannot be much

HA, MR and FR samples were measured by XAFS. Fluorescence method was implied to get XAFS spectra of P absorption edge.

XANES part of spectra were detected to compare their valance state and coordinate structure. Spectra recorded above the Ca K absorption edge have been used to provide information on the local environment of Ca in a variety of biological calcium phosphates and model compounds [16,17].

RESULTS and DISCUSSION

The absorption coefficient μ values of the materials; calcite, TCP, CHP, HA, MR and FR were measured by XAFS technique in fluorescence mode at selected energies. In this study, the standard samples were selected supposing the possible coordinate structure of Ca in bone. The absorption coefficient μ values of the calcite, TCP, CHP, HA, MR and FR were plotted against the energy (eV) in Figs. 2-7. The Ca XAFS spectra of the MR and FR Lumbar comparisons were made with reference spectra of $Ca_{10}(PO_4)_6(OH)_2$ or $CaHPO_4$, and $CaCO_3$ as shown in Figs. 2-7. Different shapes in spectra indicate different structures in molecular level.

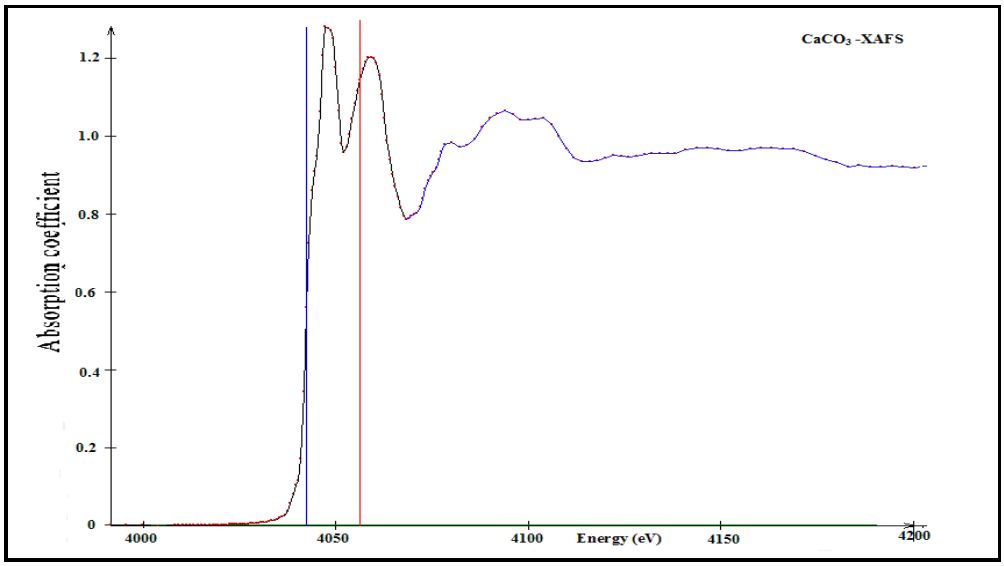

Figure. 2: XAFS Spectrum of Calcium carbonate (calcite), $CaCO_3$

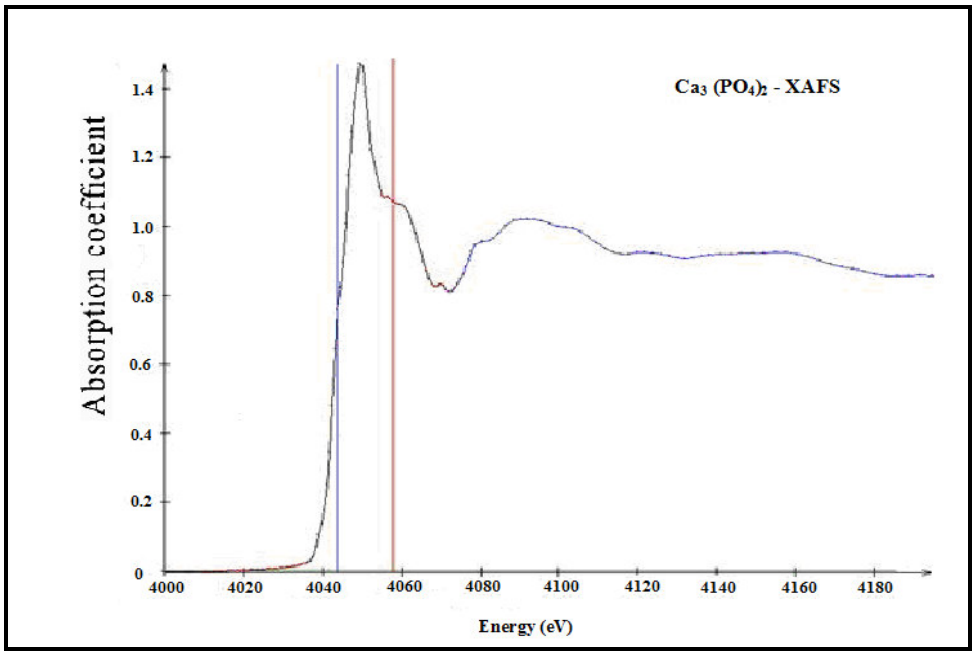

Figure. 3: XAFS Spectrum of Tri-calcium phosphate, $Ca_3(PO_4)_2$

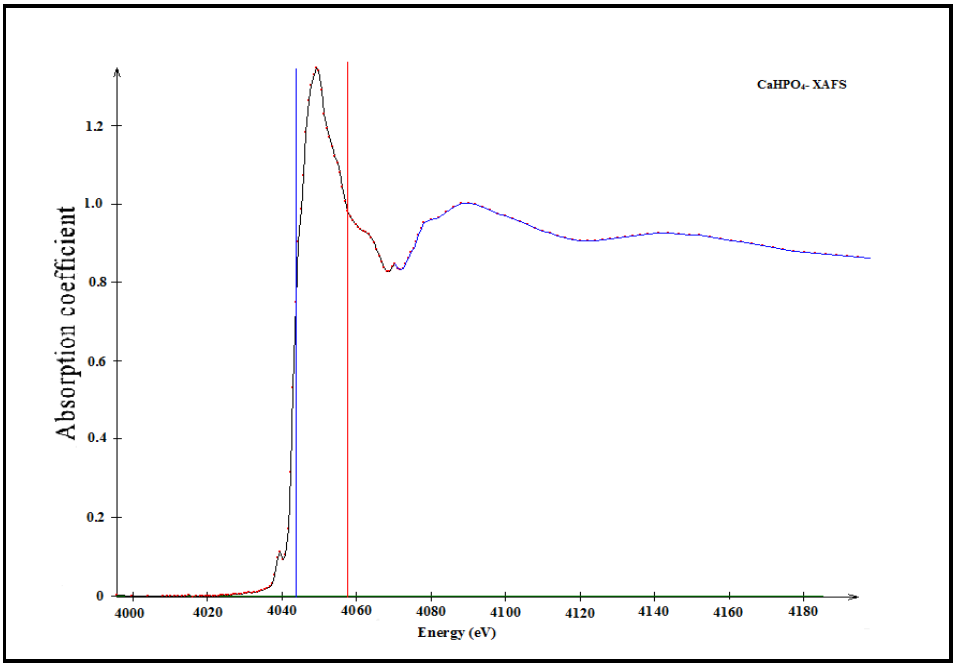

Fig. 4: XAFS Spectrum of Calcium hydrogen phosphate, $CaHPO_4$

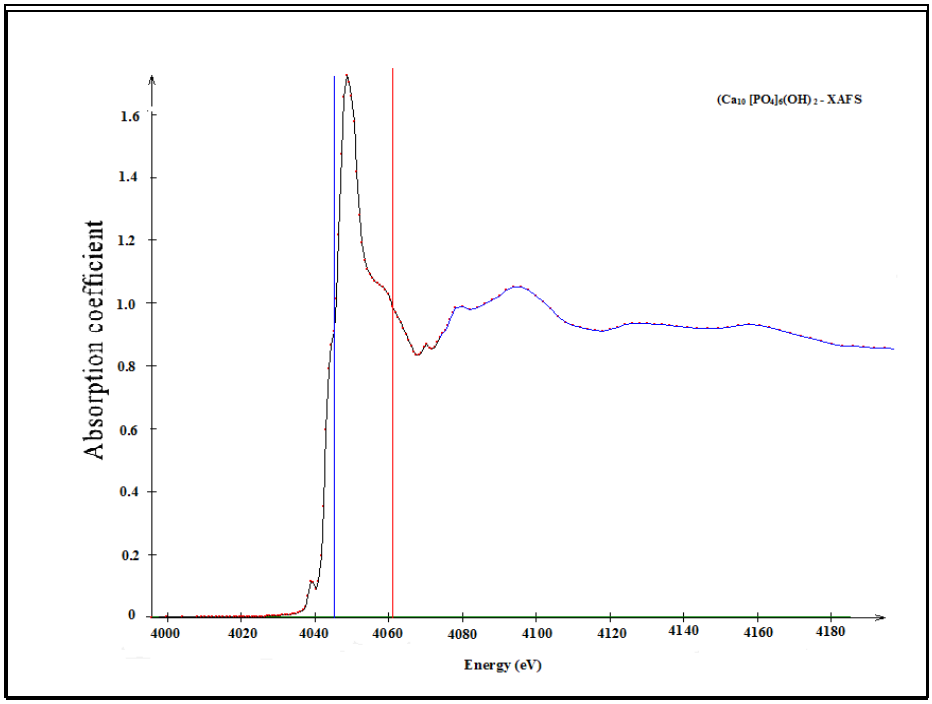

Fig. 5: XAFS Spectrum of Hydroxyl apatite, $Ca_{10}(PO_4)_6(OH)_2$

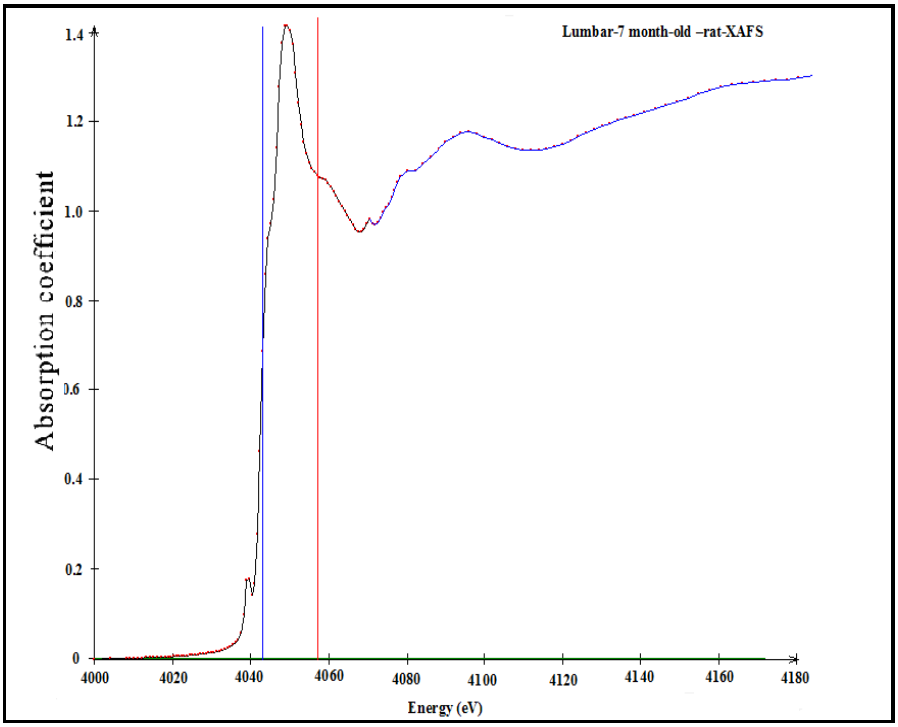

Fig. 6: XAFS Spectrum of Female-Lumbar 7 month-old rat

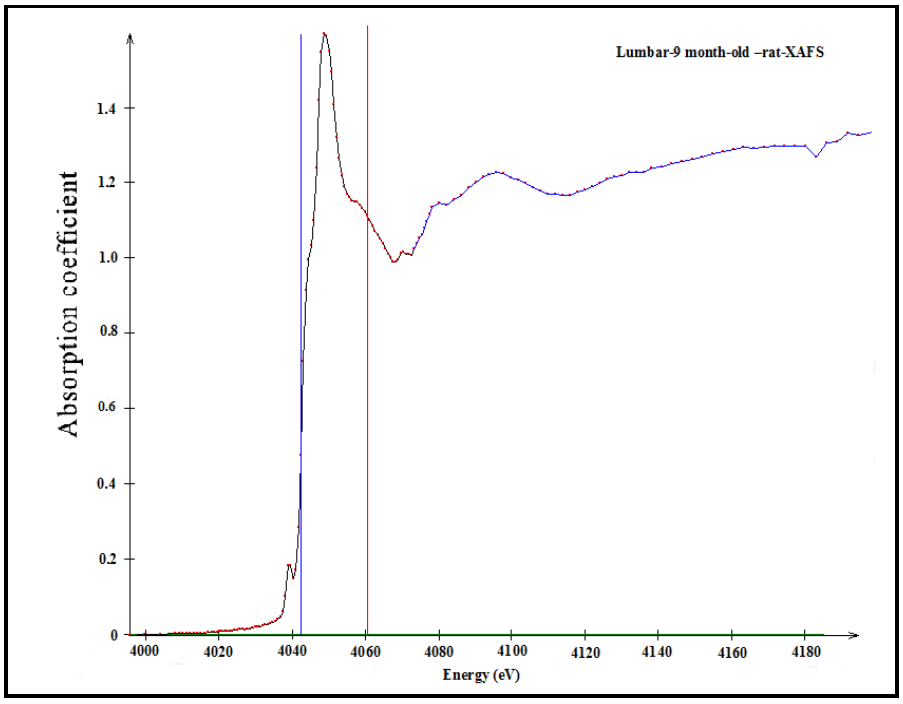

Fig. 7: XAFS Spectrum of Male-Lumbar 9 month-old Rat

By comparing with standard samples in near edge structure, the structure of Ca in lumbar should be close to Ca_{10} (PO_4) $_6$ (OH) $_2$ or $CaHPO_4$, distinct different from $CaCO_3$. The structure of Ca in bone is still an open problem, compared to $CaCO_3$ but the principal XAFS pattern shown in Figs. 4 and 5, is easily

understood that the XAFS spectra show characteristic features, and that these spectra can be utilized as a finger print of each phase. The pre-edge of $Ca_3 (PO_4)_2$ is also different from bone samples as shown in Figures. 3, 6 and 7. From Figures. 6 and 7, it is clear that the structure of MR and FR samples didn't show any difference. In order to get a clear picture of Ca structure in bone with age, it will be very helpful if we could extract information from expanded fine structure. Unfortunately the beam time was too limited to allow improvement of the signal.

CONCLUSIONS

The X-ray absorption fine structure (XAFS) measured with the X-ray fluorescence yield strongly depends on the concentration, thickness and detection geometry resulting from the selfabsorption effect. A correction procedure for the self-absorption effect is presented using a simple theory of X-ray fluorescence yield and applied to X-ray absorption near-edge structure (XANES) of a thin iron foil and an iron compound. The advantage of the XAFS is the small sample quantity required for measurements, and a flexible sample environment.

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