Application of X-ray Spectrometry at X-ray Absorption Edges for Investigation of Human Albumin

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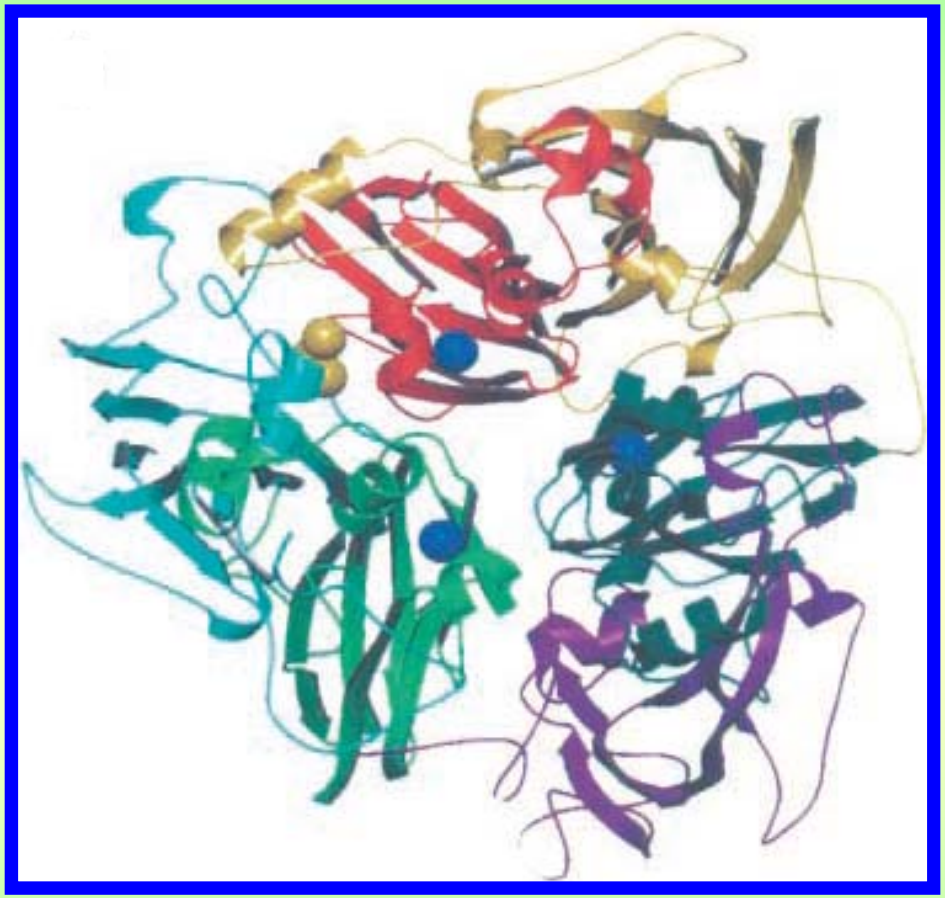
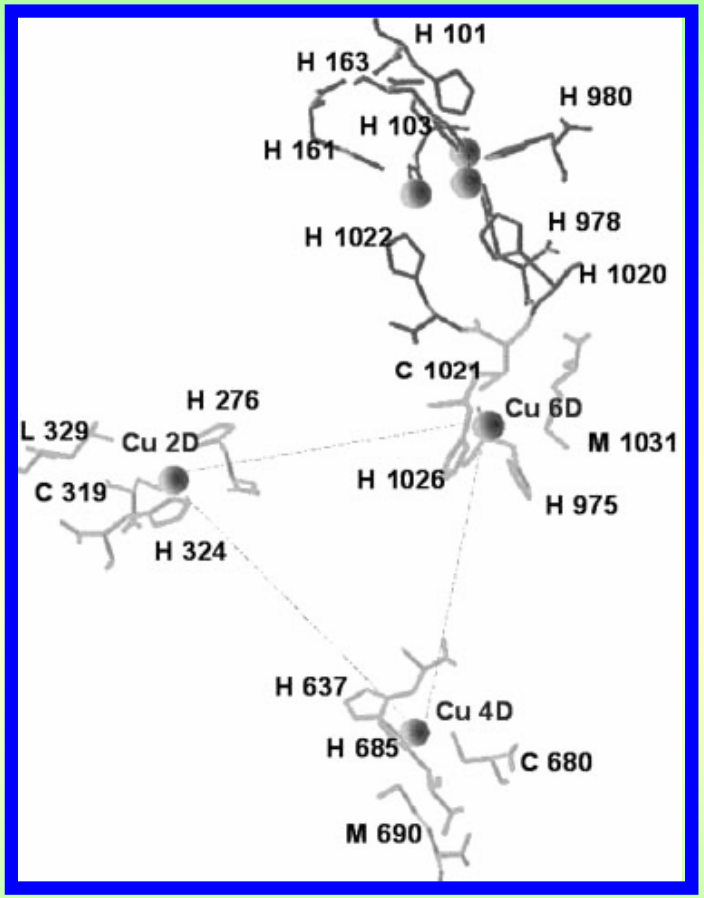
Investigation Object



Ceruloplasmin in a human albumin containing **thousands** of atoms…



…including **only six Cu** atoms.

The figures are taken from:

Bielli P., Calabrese L. – Cell Mol. Life Sci. 59 (2002), pp. 1413–1427.

Our equipment:

STAR (Samsung Electronics, Suwon, South Korea), General View

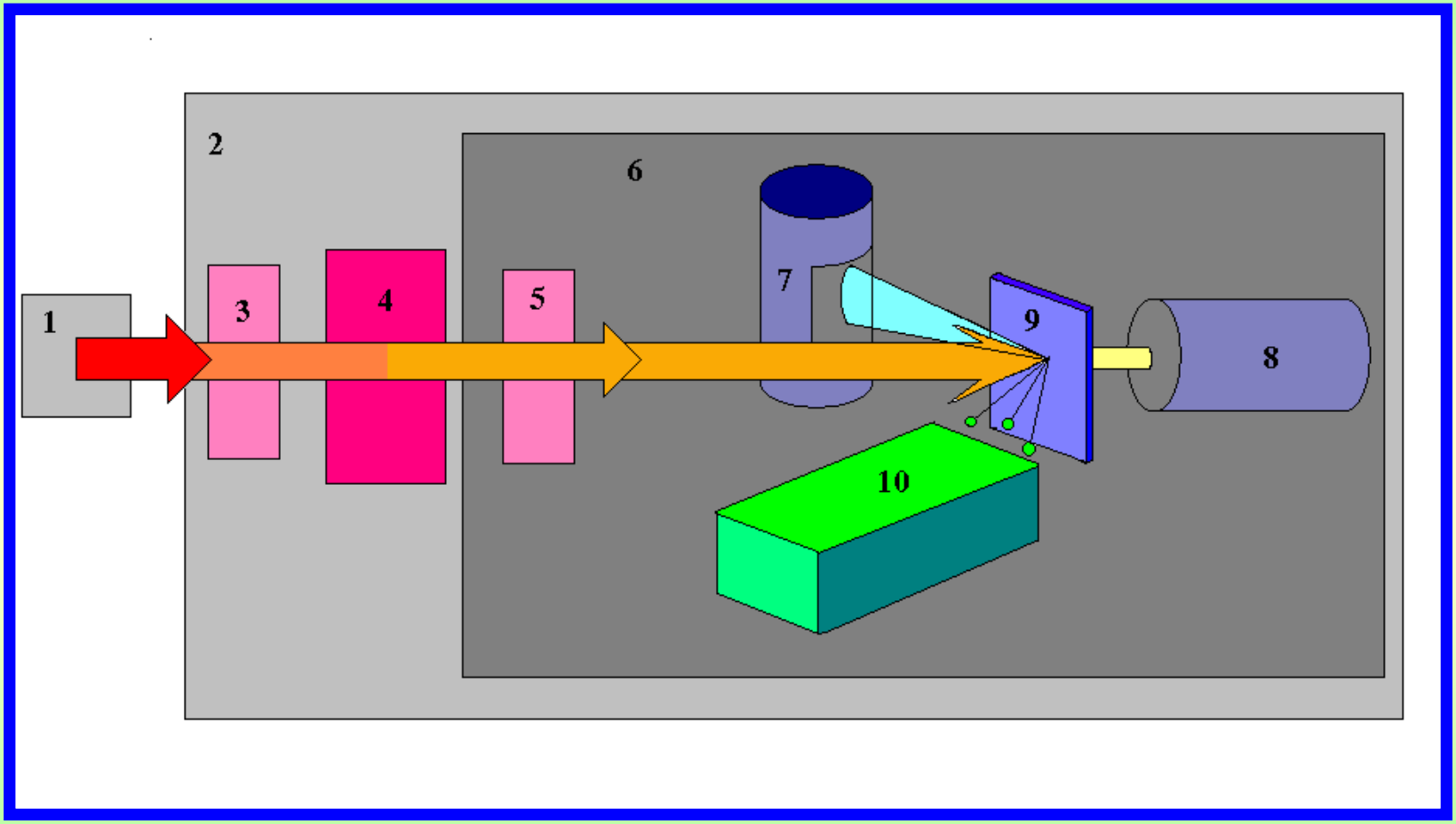
1 is the vacuum spectrometer chamber (dia. 1300 mm), 2 is the cap hung on telfer, 3 is the registration system, 4 is the x-ray rotating anode source. The total device weight is about 2 tons.

Our equipment:

MAXIM (KRISS, Daejon, South Korea), General View

1 is the vacuum chamber exposed to the atmosphere, 2 is the wall of vacuum chamber with technological flanges, 3 is the turbomolecular vacuum pump STP 1003, 4 is the x-ray rotating anode source with the power up to 60 kW. The chamber diameter is 1300 mm, the total device weight is about 1.5 tons.

X-Ray Absorption Measurement: Functional Schematics



1 - X-ray generator, 2 - spectrometer vacuum volume, 3 - entrance slit, 4 - monochromator, 5 - exit slit, 6 - sample shield, 7 - x-ray detector, 8 - shoot through x - ray detector, 9 - sample, 10 - channeltron.

X-ray Absorption: Technical Characteristics

Here the technical characteristics of substance investigation methods based on x-ray and electron spectrometry at absorption edges are enumerated.

1. Chemical composition detection: **sensitivity up to 10^{18} atoms / cm^3**.

2. Compound concentration detection: **accuracy up to 10^{18} atoms / cm^3**.

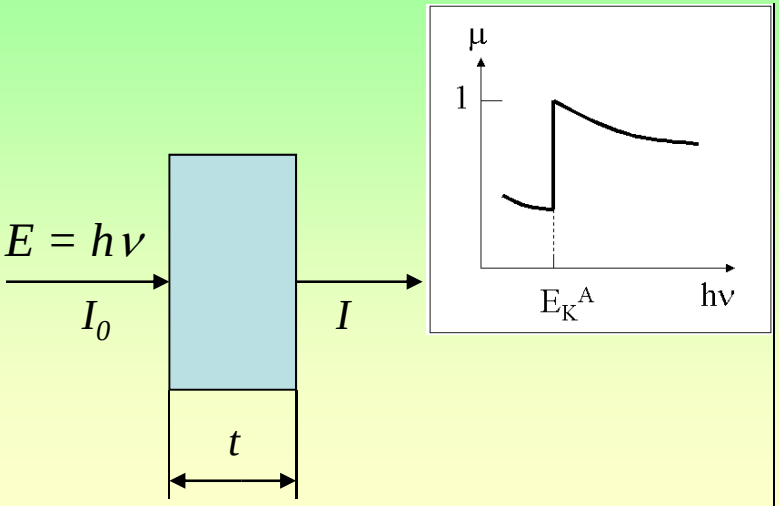
3. Non-destructive depth profiling: **resolution up to 1 nm**.

4. Atomic structure determination: **resolution up to 10^{-3} nm**.

5. Electron structure determination: **resolving force up to 6000**.

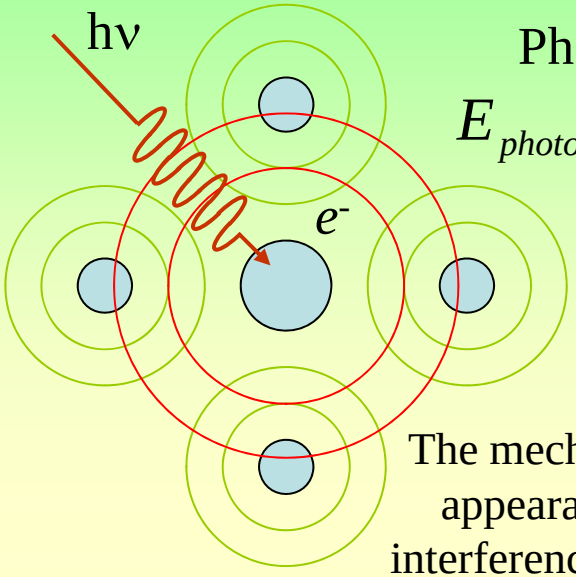
6. Absorbing elements: **oxygen (O) and heavier.**

EXAFS Essence



$\mu = t^{-1} \ln(I/I_0)$

Measuring of a spectrum as $\mu(h\nu)$.



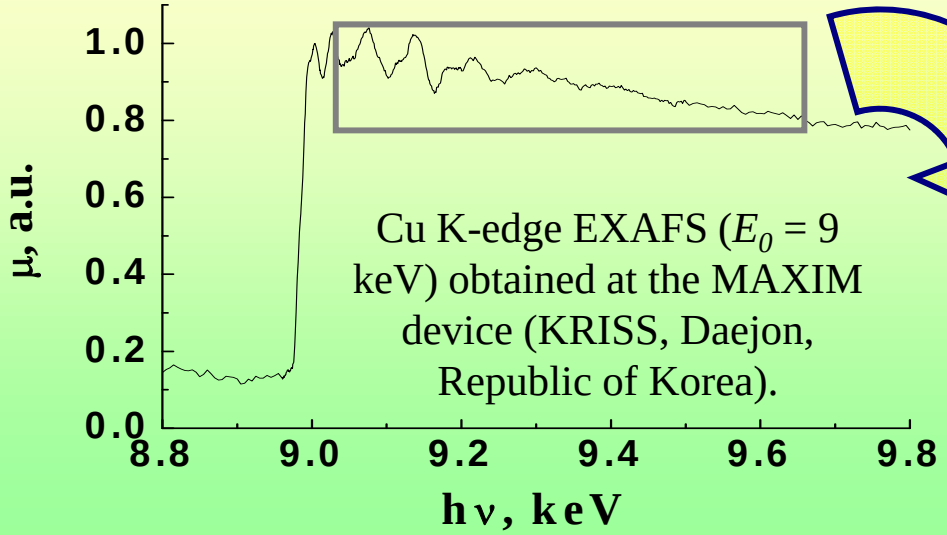
Photoelectron energy:

$$E_{photoelectron} = h\nu - E_{bond}.$$

X-ray absorption coefficient:

$$\mu \propto \left\langle \Psi_f \left| \hat{z} \right| \Psi_i \right\rangle^2$$

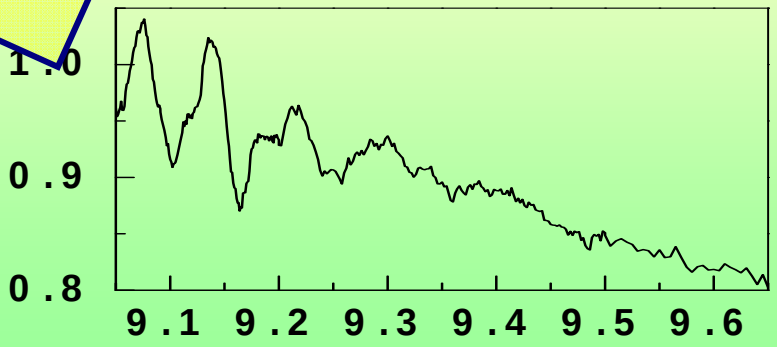
The mechanism of EXAFS signal appearance as the result of the interference of primary (red circles) and scattered (green circles) waves.



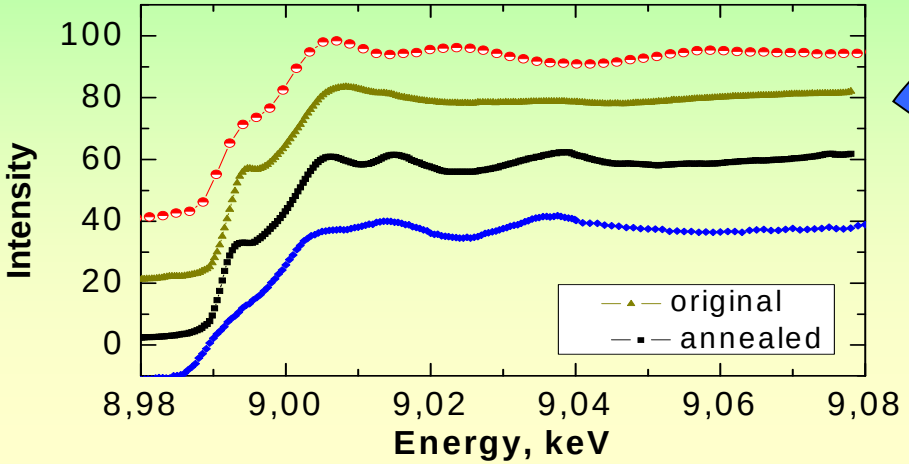
Cu K-edge EXAFS (E_0 = 9 keV) obtained at the MAXIM device (KRISS, Daejon, Republic of Korea).

EXAFS oscillations:

$$\chi(k) \propto \sum_j K_j \sin\left(2kR_j + \delta_j(k)\right)$$

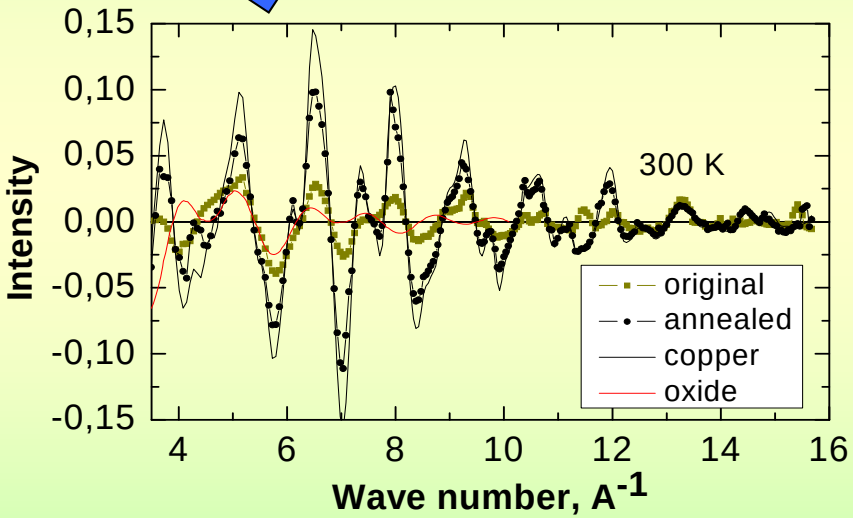


EXAFS Spectrometry: Determination of Substance Atomic Structure

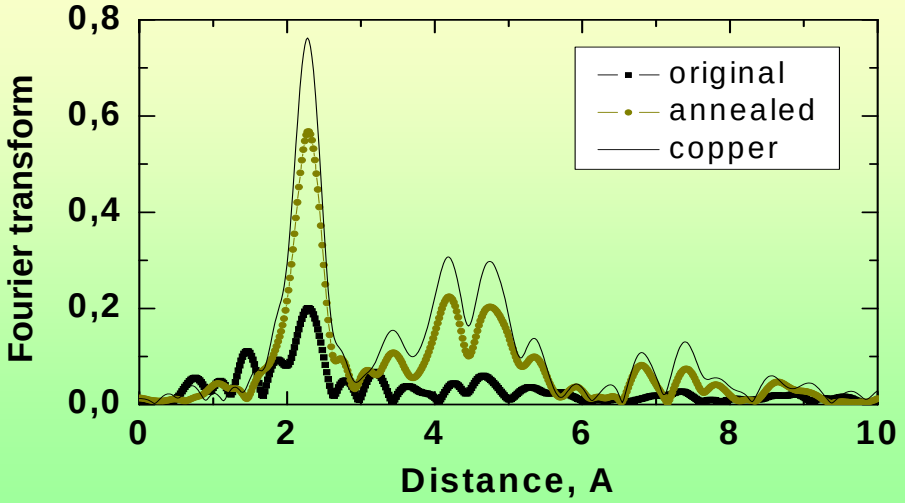


Cu K-edge XAFS spectra

EXAFS = Extended X-ray Absorption Fine Structure



EXAFS oscillations



EXAFS oscillation Fourier transform

EXAFS Spectrometry: Innovations in EXAFS Spectrum Analysis

1. EXAFS oscillations are separated via the variation principle. That is, a functional to be minimized by the sought oscillation function is suggested.

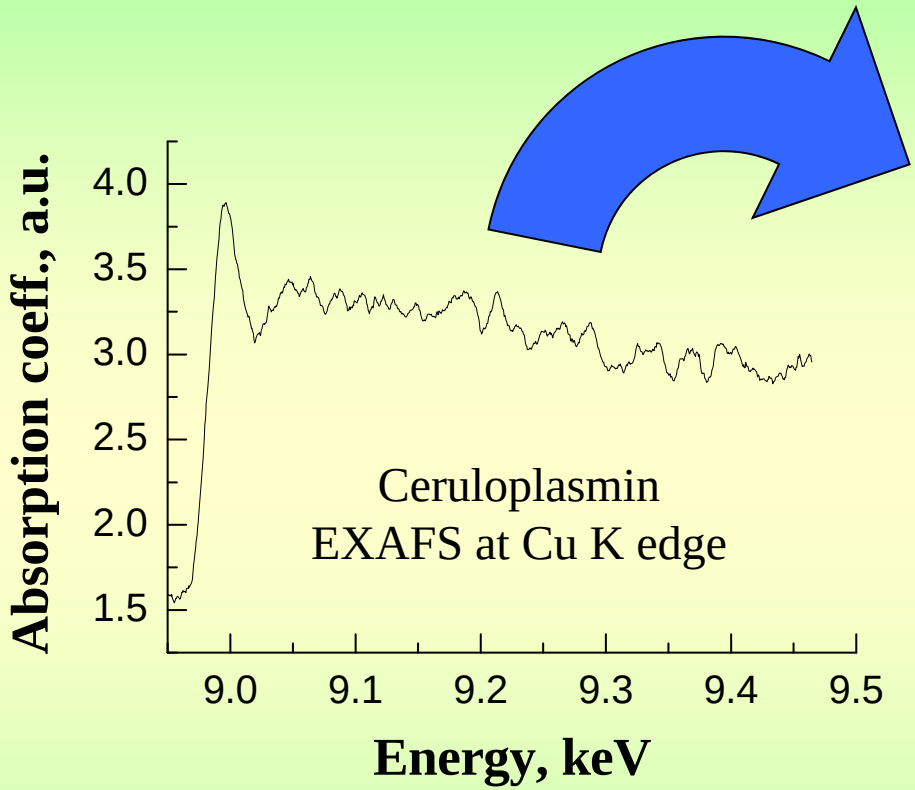
2. A well-known method is used to determine the edge energy value. It is evaluated in such way that the spectrum first derivative is maximal.

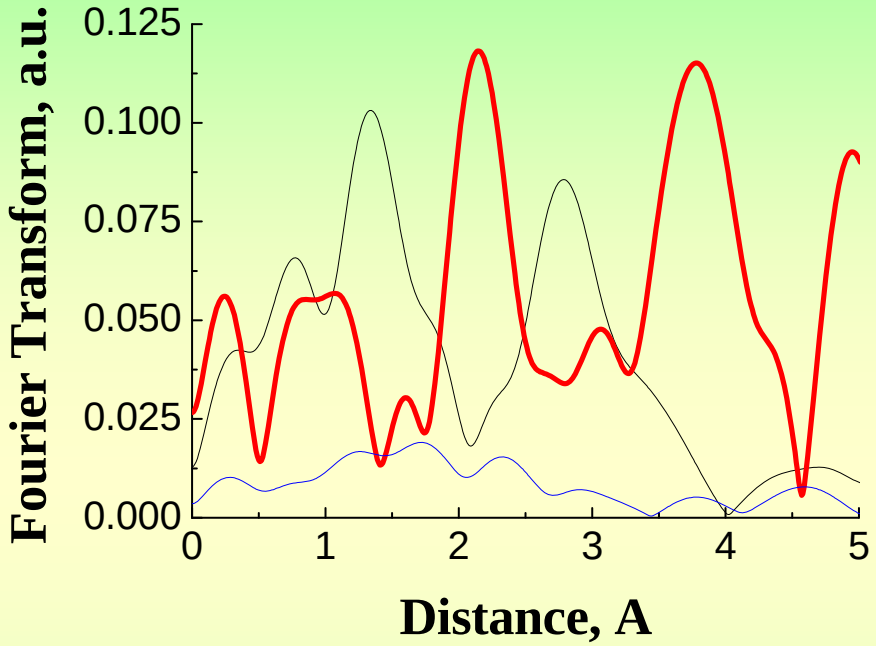
3. The EXAFS oscillation Fourier transform dependence on Fourier window limits is analyzed. The range within which the Fourier window limits provide the most correct analysis results is determined.

The new EXAFS analysis method was applied to several metal spectra and gave rise to valid results.

EXAFS Spectrometry:

Ceruloplasmin Investigation Performed by New Method



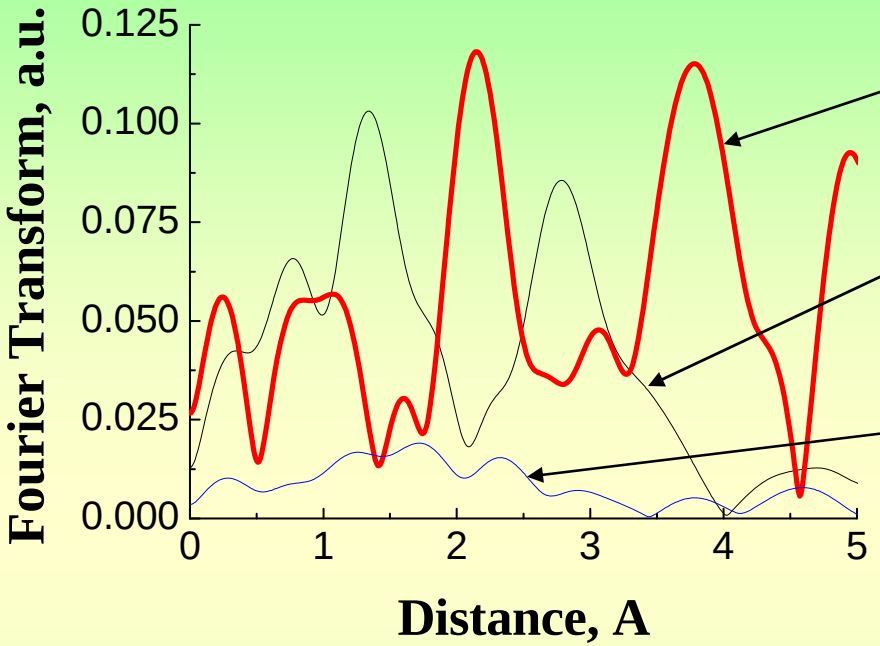


The bold red curve is related to ceruloplasmin, the black curve describes Cu_2O, and the blue curve corresponds to $Cu(NO_3)_2 \cdot 3H_2O$.

The peak at 4 A may correspond to the **triangle of Cu atoms** in the ceruloplasmin molecule.

The peak nearly at 2 A describes the closest surrounding of Cu atoms. This sphere is supposed to consist of **anions with fully occupied electron shells (like O^{2-})**.

Ceruloplasmin and Other Substances



ceruloplasmin

Cu_2O: the closest Cu atom neighbors are two O atoms in the state close to O^{2-}

$Cu(NO_3)_2 \cdot 3H_2O$: the Cu atoms are known to be surrounded by four O atoms with the charge greater than –2

Anions like O^{2-} seem to backscatter electrons better than similar atoms with a greater charge (like O, O^-, etc.) due to

1. Rutherford scattering of photoelectrons in a repulsive Coulomb field,
2. scattering properties of completely filled electron shells.

Conclusions.

System approach for (S)XAFS is developed and demonstrated corresponding hardware and experimental setup and treatment of obtained spectra considering the nanodimensions of objects under investigation.

The new EXAFS treatment method managed to perform EXAFS analysis of ceruloplasmin.

The assumption of compact Cu atom triangle existence in the ceruloplasmin molecule was confirmed.

Ceruloplasmin EXAFS analysis allows to suggest the first coordination shell of Cu atoms in ceruloplasmin to consist of anions with completely occupied electron shells.