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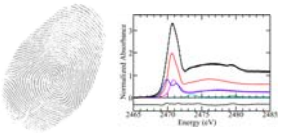
Speciation of mixtures using near-edge spectra



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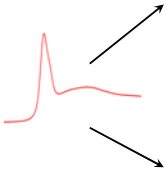
Overview

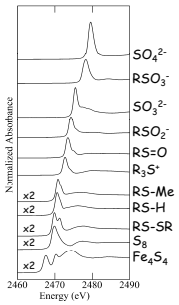
- Least Squares Analysis
- Example: Least-Squares Fitting Analysis of Sulfur K-edge Spectra of Living Mammalian Cells
- Principal component analysis
- Example: A photo-chemical reaction
- Choice of Model Spectra
- Summary

Near-Edge Spectra as Tools



- Deduce local information:
  - Electronic, geometric, etc
  - Empirically or using calculations
- Identify chemical forms present in "unknown" sample
  - "Speciation"
  - Spectra used as fingerprint
  - Compare with library of standards

X-ray Absorption Near Edge spectra



Near-edge spectra provide a "fingerprint" of chemical type.

Need a comprehensive library of "model compound" spectra.

More sensitive than EXAFS (low concentrations are OK)

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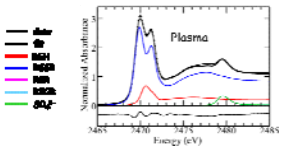
Least Squares Analysis



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Near-Edge Spectra in Speciation

- Increasingly used to determine chemical forms in complex, heterogeneous samples
  - Environmental sciences
    - e.g. sediments, soil, organisms
  - Life and health sciences
    - e.g. tissue samples
- Often such samples are difficult to analyze using other methods, which involve extractions, drying, etc. during which chemical form may change

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Near-Edge Spectra in Speciation

- XAS can analyze all of the element in the sample, no matter how it is present:
  - Aqueous solution
  - Crystalline or amorphous solid
  - Species adsorbed on a surface
  - Trapped gas
  - …
- X-ray absorption spectroscopy (XAS) can analyze a material essentially without pretreatment
  - Although:
    - Usually freeze the sample
    - May grind it to give a more representative sample

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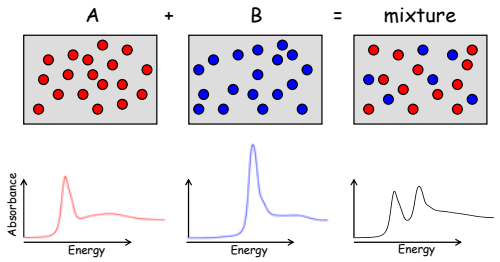
Analysis of mixtures



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Near-edge Spectra of Mixtures

- The spectrum of a mixture of more than one chemical forms of an element appears as the sum of the spectra of the individual components
- The height of the edge jump of each component is proportional to the quantity of the element in that form

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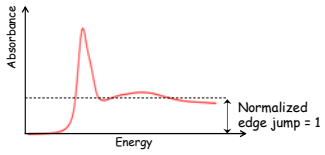
Near-edge Spectra of Mixtures

- Generally spectra are normalized so that their edge jump is unity



- Model spectra are normalized to the same amount of the element
- The height of the edge jump of each component is proportional to the fraction of the element present in that form

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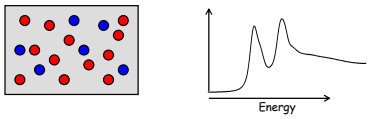
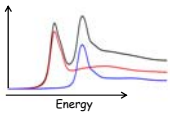
Fitting can yield Quantitative Analysis



Model spectrum of mixture with a sum of model compounds, and use least-squares fitting to quantitatively analyze how much of each is present.



Black curve is sum of red plus blue curves

Edge jump of curves proportional to fraction in fit

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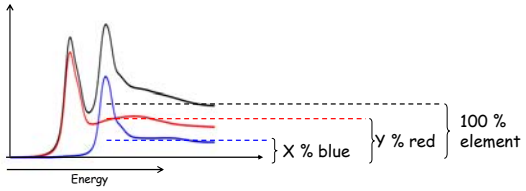
Fitting can yield Quantitative Analysis

Composition = X% blue + Y% red



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Least Squares Fitting

- In least-squares fitting we minimize a function F:

$$F = \frac{1}{N}\sum_{j=1}^{N}\left(y_{j,obs} - y_{j,calc}\right)^2$$

- Where the calculated intensity is given by:

$$y_{j,calc} = \sum_{i=1}^{m} x_i I_{i,j}$$

$y_{j,obs}$ – observed intensity of mixture spectrum at energy j

$y_{j,calc}$ – intensity of calculated spectrum at energy j

j – energy point number

N – total number of energy points

i – component number

m – number of components

$I_{i,j}$ – normalized intensity of spectrum of component i at energy j

x_i – proportion (fraction) of element as component i

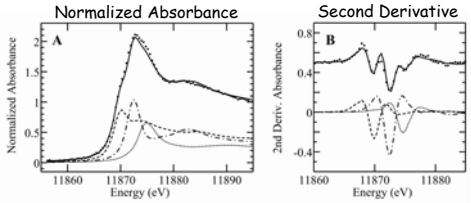
Methods of Deconvolution – Least-Squares Fitting.

Can be applied to limited data sets – a single spectrum can be analyzed (that's often all we have).

Pro – can yield quantitative estimate of chemical composition.
Con – difficult to judge whether small contributions are significant.
Con - cannot detect small fractions if something is there in excess
Con – absolutely require model spectra.

Fitting of Derivatives

- Derivatives of the spectra can also be fit
- Near-edge and derivative fits should agree



Least squares fit of the seaweed Ulva: Points: experimental seaweed spectrum, solid lines: best fits. Components (---) R_3As, (-.-.-) R_3AsO, (…) arsenate.
George et al. Mol. Nutr. Food Res. 2009, 53, in the press

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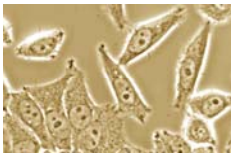
Example: Least-Squares Fitting Analysis of Sulfur K-edge Spectra of Living Mammalian Cells



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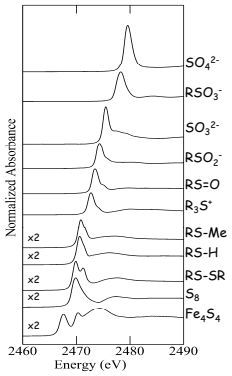
Sulfur K-edge X-ray Absorption Spectra

Sulfur K Near-edge spectra of biological model compounds.

The spectra are very sensitive to the chemical form of sulfur.



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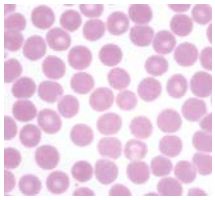
Curve-fitting example – Sulfur forms in whole blood



Whole blood can be easily separated into plasma and erythrocytes by centrifugation.

Use sulfur K-edge XAS to investigate the chemical forms of sulfur in horse erythrocytes and plasma.

Pickering, I. J., Prince, R. C., Divers, T. C. and George, G. N. "Sulfur K-edge X-ray Absorption Spectroscopy for Determining the Chemical Speciation of Sulfur in Biological Systems" *FEBS Letters* 1998, **441**, 11-14.

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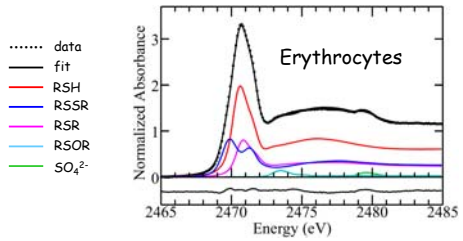
Curve-fitting example – Sulfur forms in whole blood



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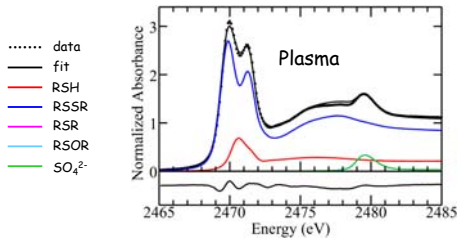

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Curve-fitting example – Sulfur forms in whole blood

chemical type (%)	RSSR	RSH	RSMe	RSOR	SO_4^{2-}
Erythrocytes	21	55	21	2	1
Plasma	76	21	0	0	3

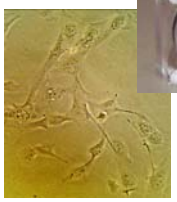
Erythrocytes show a predominance of reduced sulfur forms (i.e. thiols) while plasma shows oxidized sulfur forms (i.e. disulfides).

This confirms the well known idea that the inside of cells is much more reducing than the outside.

Pickering, I. J., Prince, R. C., Divers, T. C. and George, G. N. "Sulfur K-edge X-ray Absorption Spectroscopy for Determining the Chemical Speciation of Sulfur in Biological Systems" *FEBS Letters* 1998, **441**, 11-14.

Sulfur K-edge Spectra of Living Mammalian Cells – MDCK cell cultures

Culture of Madin-Darby Canine Kidney Cells



Grow cells on Transwell™ plates – on a 10μm thick polycarbonate film.

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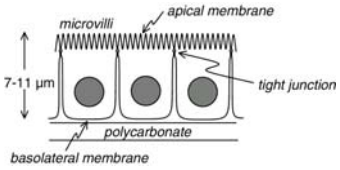
Sulfur Spectroscopy of Living Mammalian Cells

Culture of Madin-Darby Canine Kidney (MDCK) Cells



MDCK cells are considered to be prototypical polarized epithelial cells.

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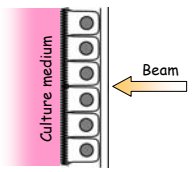
Sulfur Spectroscopy of Living Mammalian Cells

Cells are mounted in the X-ray beam (with minimal possible disturbance, but rotated through 90°) still growing on their polycarbonate substrate.



Cells cultures are kept in an incubator at beamline until loading.

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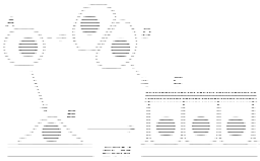
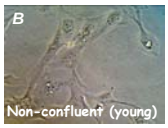
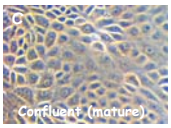
Stages in Cellular Development







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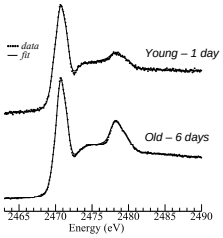
Effects of Cellular Developmental Status



% sulfur	young	old
RSSR	22.4	11.7
RSH	37.7	41.7
RSMe	33.3	37.1
RSOR	3.2	0.9
RSO_2^-	0.8	1.0
RSO_3^-	2.1	6.7
SO_4^{2-}	0.5	0.9

Sulfur X-ray absorption spectra reveal changes in sulfur biochemistry as a function of cellular developmental status.
*Gnida et al. Biochemistry **46**(51), 14735-14741 (2007)*

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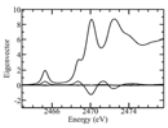
Principal Component Analysis



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Methods of Deconvolution – Principal Component Analysis

This is a technique that can be applied to extended data sets –

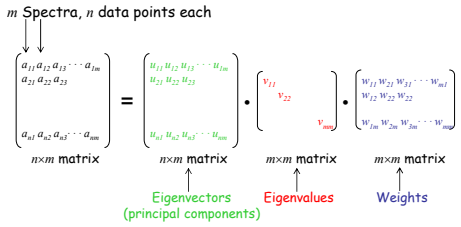
m spectra of a mixture of $< m$ components.

1. Principal Component Analysis – can (in principal) count the number of components.
2. Target Transformation – can identify components of mixtures.

Principal Component Analysis

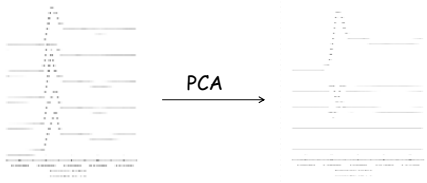
Based on a mathematical technique called Single Value Decomposition

$$\mathbf{A} = \mathbf{U}\cdot\mathbf{V}\cdot\mathbf{W}^t$$



Principal Component Analysis

$$\mathbf{A} = \mathbf{U}\cdot\mathbf{V}\cdot\mathbf{W}^t$$



Spectra **A** $\quad$ **U•V**

Principal components are NOT the component spectra

In this example 3 components could be used to reconstitute the data

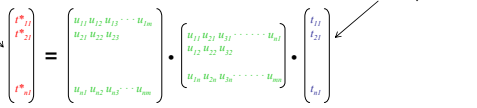
Target Transformation

Essentially asks the question "is this model spectrum a member of our set of spectra?". Target transformation can thus be used to test whether candidate component spectra are present.

$$\mathbf{T^*} = \mathbf{U \cdot U^t \cdot T}$$

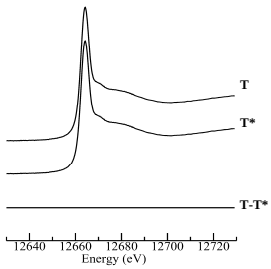
Transformed spectrum

Candidate spectrum



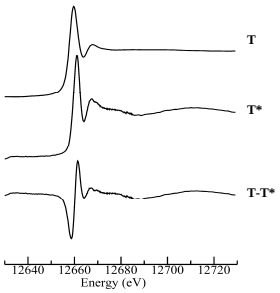
- **U** can be restricted to include only the significant principal components
- If **T** and **T*** are (essentially) identical then candidate spectrum is part of the set and the candidate compound is a part of the mixture.

Target Transformation



Target transformation for aqueous selenite – **T** and **T*** are essentially identical so selenite is present in the mixture.

Target Transformation



Target transformation for α-selenium – **T** and **T*** are different so α-Se is not present in the mixture.

Principal Component Analysis

Principal Component Analysis will not work when you have fewer spectra than components in the mixture.

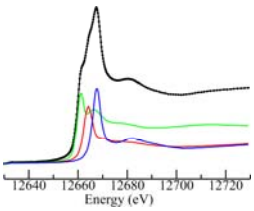
All spectra must have common abscissae.

Target transformation absolutely requires accurate model spectra.

Note that a "component" could itself be a complex mixture of species that has invariant composition amongst the spectra in the set.

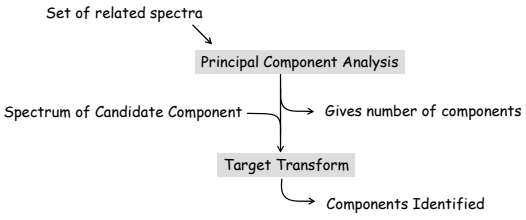
Quantitative analysis

The conclusions of principal component analysis and target transformation can be used with least-squares fitting to obtain a quantitative analysis.



Results of a three-component least-squares fit

Principal Component Analysis and Target Transformation



Quantitative Analysis

Set of related spectra → number and identities of components from Principal Component Analysis and Target Transform

↓

Least Squares fitting of each spectrum in set

↓

Quantitative analysis of whole set

Quantitative Analysis

Least Squares fitting of each spectrum in set

Two alternative approaches are available in using the least-squares analysis of the data to obtain a quantitative analysis of the set:

1. Use model compound spectra to do least squares fitting
2. Use target transforms of model compound spectra to do least squares fitting.

The second method is actually rather like fitting the data with itself – we consider fitting with target transforms to be undesirable, although numerically correct.

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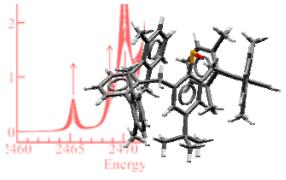
Example: A photo-chemical reaction



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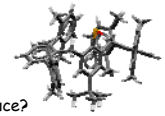
Example – A photo-chemical reaction

R-S· is an elusive free-radical chemical intermediate of biochemical importance.

From considerations of the expected electronic structure we expect R-S· to have a very low-lying transition in the sulfur K near-edge spectrum.

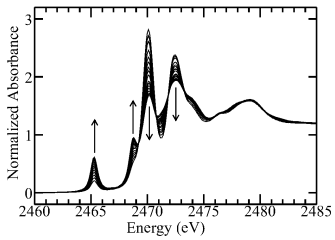
Observation – prolonged exposure to beam of a stable sulfenic acid (RSOH) produces a transition in exactly the region expected for R-S·

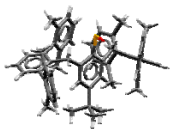
$$RSOH \xrightarrow{h\nu} RS\cdot + OH\cdot$$



Could this be R-S· ?
How many components does the reaction produce?
Use Principal Component Analysis to find out.

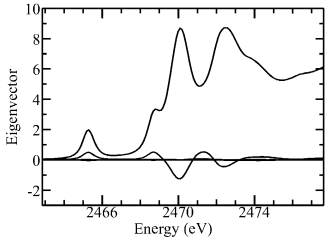
Spectral Time Course





Spectra taken with increasing time show increasing levels of low-energy peaks.

Principal Component Analysis



Twenty spectra at different times; only two components are indicated.

Example – A Chemical Reaction

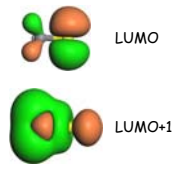
$$RSOH \xrightarrow{h\nu} RS\cdot + OH\cdot$$

Principal component analysis indicates only two components in all spectra.

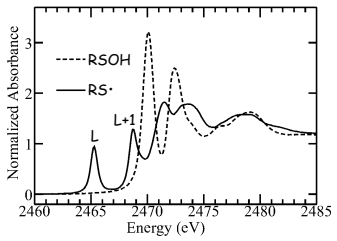
Use kinetic analysis with the spectrum of the starting material, and difference spectra to compute spectra of product.

Spectrum of RS·

$$RSOH \xrightarrow{h\nu} RS\cdot + OH\cdot$$

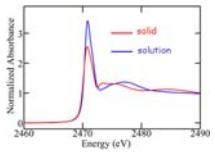


Computed energy difference ~4eV



The 2465 eV peak can be used as a probe of RS· in biological systems

Choice of Model Spectra



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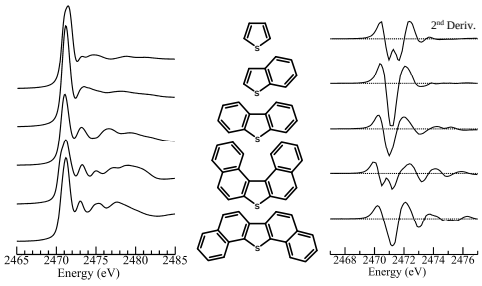
The Choice of models is critical to a good analysis

Spectra depend on the chemical and physical environment.

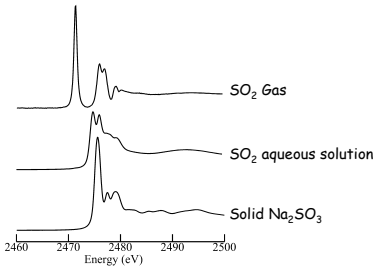
It is critical that model spectra are collected under appropriate conditions.

Sulfur K-edge XAS spectra of substituted thiophenes.



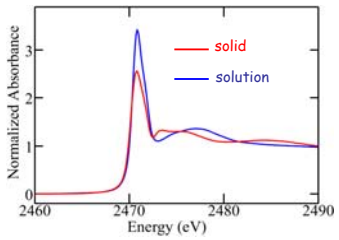
Remote structure can have significant affects …

Physical form can be important



Solids vs. Solutions…

solid vs. aqueous solution spectra of methionine



In general peaks of solution spectra are more pronounced than with solids. The reason for this is that crystal packing forces in the solid distort the molecule lifting orbital degeneracy and cause bound state transitions to be more spread out in energy.

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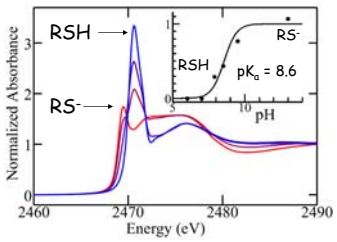
pH can be important

Spectra of an aqueous solution of cysteine taken at different pH values.



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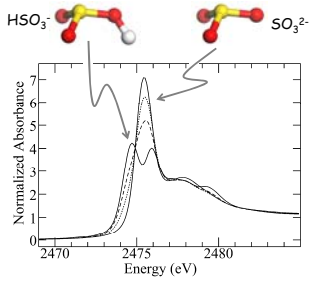
pH can be important



Sulfite has a pK_a of 6.2

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Summary

One spectrum – use Least-squares fitting.

Set of spectra – use PCA, Target & Least-squares.

PCA · · · · · · · · · · · · · · · · · · Gives number of components.
Target · · · · · · · · · · · · · · · · Gives identities of components.
Least-Squares Fitting · · · Gives quantitative estimation.

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