Metallomics, Toxicology and Trace Elemental Analysis – Use of TXRF for Clinical Applications

Bruker AXS
Madison, WI
Welcome

Today’s Topics

- TXRF – how does it work?
- Trace elemental analysis in clinical chemistry
- Tissue analysis
- Biological liquid applications
- Interactive Q & A

Speakers

Michael Beauchaine
Business Development Scientist TXRF
Madison, WI  USA

Dr. Armin Gross
Global Product Manager TXRF
Berlin, Germany
TXRF – How does it work?
Principles of total reflection X-ray fluorescence (TXRF) spectroscopy

Total reflection X-ray fluorescence spectroscopy

- Samples must be prepared on a reflective media
- Polished quartz glass or polyacrylic glass disc
- Dried to a thin layer, or as a thin film or microparticle

Beam angle: 0° / 90°
Principles of total reflection X-ray fluorescence spectroscopy

Samples for TXRF

- Powders: Direct preparation or as suspension
- Liquids: Direct preparation
- Always as a thin film, micro fragment or suspension of a powder
- Necessary sample amount: Low µg respectively µl range

Simple quantification

- Matrix effects are negligible due to thin layer
- Quantification is possible by internal standardization
Principles of total reflection X-ray fluorescence spectroscopy

In TXRF the samples are prepared as thin films or layers

- Matrix effects are negligible
- Quantification is possible
Principles of total reflection X-ray fluorescence spectroscopy

In TXRF the samples are prepared as thin films or layers

- Matrix effects are negligible
- Quantification is possible

- TXRF detects elements from Na(11) to U(92)
- The element sensitivities depend on the atomic number
- The sensitivity factors are calibrated ex works
- Quantification requires the addition of one standard element
The instrument S2 PICOFOX

**Benchtop TXRF spectrometer**

**S2 PICOFOX**

- Metal-ceramic X-ray tube
  - Mo anode
  - Air-cooled
  - Optionally other tubes available
- Multilayer monochromator
- XFlash® silicon drift detector
  - Electro-thermally cooled
  - $\leq 149$ eV @ MnKα 100 kcps
- Automatic version
  - 25 sample cassette
Introduction to Trace Elements in Clinical Chemistry
Metallomics

New frontier within interdisciplinary science investigating trace elements and the role of metals in biological, environmental and clinical systems.

* Metallomics Journal – RSC Publishing
Metallomics

**Research topics include**

- Regulation of the uptake, accumulation and metabolism of metals and other trace elements in biological systems
- The interaction of metallo-drugs, incl. chemotherapy agents
- Elemental distribution and concentrations linked to genomics
- Chemical speciation, dynamics, and kinetics of trace elements in biological systems.
- Physiological and pathological mechanisms related to trace elements in human health and disease
- Metal exchange between biota and the environment
- Biosensing of metals, including diagnostic and therapeutic radioactive metals
- Instrumentation and methods demonstrating solutions in are of metallomics
Trace Essential Elements
Impact on Clinical Chemistry

• Metals are increasingly used in dietary supplements for health and prevention of disease

• Essential trace elements or micro-nutrients are those with concentrations below 50 ppm in humans

• Classified as “essential” when a deficiency causes a medical symptom and a nutritional supplementation will avoid or relieve such symptom

• Excessive uptake can lead to intoxication (Se, Cu, Mo, and Cr)
Elemental analysis in medicine

Standard method in clinical chemistry
Atomic absorption spectrometry (AAS)

Toxicology, forensic medicine
Oncology, Endocrinology Research

Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) / Mass Spectrometry (ICP-MS)
### Trace Essential Elements

#### Biological Function

<table>
<thead>
<tr>
<th>Element</th>
<th>Good nutrition sources</th>
<th>Metabolic function</th>
<th>RDA*</th>
<th>Deficiency symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromium</td>
<td>meat, whole grain, vegetable oil, beer</td>
<td>compound of Glucose Tolerance Factor (sugar metabolism)</td>
<td>35 µg</td>
<td>depression</td>
</tr>
<tr>
<td>Cobalt</td>
<td>meat, shellfish, milk, eggs</td>
<td>compound of Cobalamin (Vit B-12)</td>
<td>2 - 3 µg</td>
<td>fatigue, depression</td>
</tr>
<tr>
<td>Iron</td>
<td>meat, green vegetables, fish, ogge, whole grain</td>
<td>compound of many enzymes, e.g. P450 monooxygenase</td>
<td>8 mg</td>
<td>iron deficiency anemia</td>
</tr>
<tr>
<td>Iodine</td>
<td>seafish, shellfish</td>
<td>compound of thyroid hormones</td>
<td>150 µg</td>
<td>goitre, cretinism</td>
</tr>
<tr>
<td>Copper</td>
<td>whole grain, nuts, cocoa, green vegetables, fish, shellfish</td>
<td>compound of many redox enzymes, e.g. cytochrome c oxidase</td>
<td>900 µg</td>
<td>anemia-like symptoms, risk factor for cancer</td>
</tr>
<tr>
<td>Manganese</td>
<td>black tea, nuts, whole grain, green vegetables</td>
<td>activator of many enzymes -&gt; antioxidant metabolism, bone synthesis, gluconeogenesis</td>
<td>2.3 mg</td>
<td>immune deficiency, blood coagulation disorder</td>
</tr>
<tr>
<td>Molybdenum</td>
<td>ubiquitous</td>
<td>compound of the universal molybdenum cofactor</td>
<td>45 µg</td>
<td>risk factor for cancer, immune deficiency</td>
</tr>
<tr>
<td>Nickel</td>
<td>nuts, vegetables, cereals</td>
<td>compound of many enzymes, e.g. urease or hydrogenases</td>
<td>not det.</td>
<td>not fully clarified</td>
</tr>
<tr>
<td>Selenium</td>
<td>meat, nuts, fish</td>
<td>compound of 30-50 selenoproteins, e.g. glutathione peroxidase</td>
<td>55 µg</td>
<td>risk factor for cancer, immune deficiency</td>
</tr>
<tr>
<td>Zinc</td>
<td>animal food, cheese, fish, shellfish, whole grain, seeds</td>
<td>zinc dependent enzymes are involved in almost all metabolic and cell signaling functions, e.g. alcohol dehydrogenase, carbonic anhydrase</td>
<td>11 mg</td>
<td>dermatitis, risk factor for cancer, immune deficiency</td>
</tr>
</tbody>
</table>

*) Recommended Dietary Allowance, US Department of Agriculture
# Trace Essential Elements

## Biological Matrices Analyzed with TXRF

<table>
<thead>
<tr>
<th>Biological matrix</th>
<th>Typical volume</th>
<th>Sample preparation for TXRF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood - whole blood $^1$</td>
<td>500 µl</td>
<td>1:1 dilution with H$_2$O, addition of internal Ga standard</td>
</tr>
<tr>
<td>Blood - serum $^1$</td>
<td>500 µl</td>
<td>1:10 dilution with H$_2$O, addition of internal Ga standard</td>
</tr>
<tr>
<td>Blood - serum, small volumes</td>
<td>&lt;10 µl</td>
<td>1:2 dilution with H$_2$O, pipetting on carrier, addition of 1 µl Ga standard solution</td>
</tr>
<tr>
<td>Urine</td>
<td>ml</td>
<td>direct addition of internal standard, fume off chlorine with HNO$_3$</td>
</tr>
<tr>
<td>Tissue homogenates from mice</td>
<td>15 µl</td>
<td>1:1 dilution with Y standard solution or digestion in 65% HNO$_3$, 1 h, 70°C</td>
</tr>
<tr>
<td>Seminal fluid</td>
<td>µl</td>
<td>direct addition of internal standard</td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td>µl</td>
<td>direct addition of internal standard</td>
</tr>
<tr>
<td>Mother’s milk</td>
<td>ml</td>
<td>direct addition of internal standard</td>
</tr>
<tr>
<td>Tear fluid</td>
<td>µl to ml</td>
<td>direct addition of internal standard</td>
</tr>
</tbody>
</table>

$^1$) for details see Lab Report XRF 77, Trace Element Analysis of Blood Samples
Trace Essential Elements Measurement Techniques

Common techniques for trace element analysis

- ICP-OES (inductively coupled plasma optical emission spectroscopy)
- AAS (atomic absorption spectroscopy)

Both techniques require

- Time consuming sample preparation
- Use and digestion of harmful acids
- Larger amount of sample
- Dilution of sample
Tissue Analysis by TXRF Spectroscopy
Application studies
Rapid screening of fish samples

Fish as nutrient
- Nutrition relevant elements like Cu, Zn and Se in food are regularly monitored

Fish as bioindicator
- Accumulation of heavy metals varies with route of uptake and species of fish
- Use as biomonitors for assessment of bioaccumulation of contaminants within ecosystems

Zebrafish Danio rerio
Application studies
Rapid screening of fish samples

Fish as nutrient

• Nutrition relevant elements like Cu, Zn and Se in food are regularly monitored

Fish as bioindicator

• Accumulation of heavy metals varies with route of uptake and species of fish

• Use as biomonitors for assessment of bioaccumulation of contaminants within ecosystems

Task

• Analysis of metal content in fish standard DORM-3*

*) Fish protein, Canadian National Research Council Preparation by removal of bones and oil, subsequent enzyme hydrolysis; protein hydrolysate was spray dried, sieved (297 µm screen), blended and bottled.
Sample preparation of plants, tissues, grains

Solid materials are ground to fine particle size and resuspended for direct analysis without digestion.

1. Fill powder in mortar
2. Grind carefully
3. Weigh about 20-50 mg
4. Transfer to tube
Application studies
Sample preparation for rapid screening

suspend in detergent solution

add standard

homogenize

pipette on carrier
Application studies
Sample preparation for rapid screening

Dry through heat/vacuum → Load the instrument → Start data acquisition

30.03.2011
Application studies
Rapid screening of fish samples

Recovery

- Cd, Sn and Hg could not be determined due to line overlaps with Molybdenum unit
- All other element concentrations are in good concordance with reference values
- Only informational values are available for Al, Mn and Se

n = 10
Application studies
Rapid screening of fish samples

Detection limits

- Detection limits are improved by a factor of 2 by sample digestion
- Digestion causes partial loss of Cl, Se and Br
- Reproducibility remains unchanged
Application studies
Analysis of Se

The analysis of Se (and other elements) in biological matrices is not a trivial task!
Sample preparation required for Se analysis in blood samples by ICP-OES

- Digestion with a “cocktail” of 80% H$_2$SO$_4$, 12% HClO$_4$ and 8% HNO$_3$
- Operation of a hydride generator required separates elements like Se from matrix
- Achievable detection limit is 5 µg/l

Application studies
Analysis of Se

**Conclusion**
- Se analysis with ICP-MS or ICP-OES is laborious and costly
- AAS is not suitable for multi-element analysis

**Opportunities**
- TXRF enables fully quantitative results for extremely small sample amounts
- Required detection limits accomplished (10 – 200 µg/L)
Application studies
Analysis of Se

Known facts

- Se status is related to heavy metal metabolism, accumulation, excretion
- SePP form stable adducts with heavy metals in vitro
- SePP receptors were identified, mainly expressed in kidney, brain and thyroid glands, where heavy metals accumulate at physiological conditions

Hypothesis

- SePP binds heavy metals and triggers biological inert deposition of heavy metals in target tissues
Application studies
Analysis of Se

- Diet with high Se content (3 weeks)
- Diet with low Se content

Effect of dietary Se content

Organ specificity

Suitability of TXRF

30.03.2011
Application studies
Analysis of Se

Setup
- Mice were treated through drinking water for 12 days with 5 mg/L HgCl₂
- Genotypes represent the wildtype situation (+/+), as well as the homozygous knockout (-/-) of the Se-transport protein SePP

First trends
- 2 mice each
- Without SePP, the transport of heavy metal species (i.e. Hg) to classical SePP-target tissues might be reduced
Trace Elemental Analysis in Biological Liquids
Liquid samples
Urine

Introduction
• The trace element content of urine is an indicator for the human health status

Task
• Participation in round robin test for urine samples

Sample preparation and measurement
• Direct application after addition of a Ga standard
• Measurement time 1000 s
• After treatment with HNO₃ directly on the carrier, samples were measured again
Liquid samples
Urine

Challenges of urine samples testing:
• Detection limits in the low ppb range required
• High amount of chlorine and calcium disturb TXRF measurements with sum peaks of matrix elements

Solution
• Vaporize Cl with HNO₃
• Perform second measurement of same sample

TXRF spectrum of urine sample
grey = before, blue = after HNO₃ treatment
Liquid samples
Urine

Results
- TXRF applications lab received certification for the elements shown below

<table>
<thead>
<tr>
<th>Element</th>
<th>TXRF (µg/l)</th>
<th>Ref. (µg/l)</th>
<th>Recov. (%)</th>
<th>TXRF (µg/l)</th>
<th>Ref. (µg/l)</th>
<th>Recov. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>As</td>
<td>154,0</td>
<td>144,0</td>
<td>107</td>
<td>195,0</td>
<td>194,0</td>
<td>101</td>
</tr>
<tr>
<td>Cr</td>
<td>20,0</td>
<td>14,9</td>
<td>134</td>
<td>12,0</td>
<td>10,3</td>
<td>117</td>
</tr>
<tr>
<td>Fe</td>
<td>760,0</td>
<td>800,0</td>
<td>95</td>
<td>573,0</td>
<td>605,0</td>
<td>95</td>
</tr>
<tr>
<td>Ni</td>
<td>14,0</td>
<td>15,5</td>
<td>90</td>
<td>7,8</td>
<td>10,3</td>
<td>76</td>
</tr>
<tr>
<td>Se</td>
<td>11,0</td>
<td>13,4</td>
<td>82</td>
<td>91,0</td>
<td>91,0</td>
<td>100</td>
</tr>
<tr>
<td>Zn</td>
<td>1160,0</td>
<td>1220,0</td>
<td>95</td>
<td>797,0</td>
<td>838,0</td>
<td>95</td>
</tr>
</tbody>
</table>

Conclusion
TXRF allows precise and accurate urine analysis after direct sample preparation.
Liquid samples
Platinum in Blood

**Application** - Platinum analysis of Cancer patients during Chemotherapy treatment

- Platinum is highly toxic – narrow therapeutic window
- Need to monitor individual patients as there is a variability in kidney functions and treatment must be adapted
- Variety of Platinum based cancer drugs and new ones being developed
- Study of Pharmacokinetics: absorption, distribution, and elimination
- Tolerable dosage levels must be established
Liquid Samples
Platinum in Blood

**Measurements and sample preparation**

1 cm³ Whole Blood

Centrifuged at 10,000 rpm

Remove supernatent containing serum

Freeze at -4 °C

TXRF
Liquid Samples
Platinum in Blood

- Serum samples spiked with different Pt standards
- Level of detection calculated at 67 µg/l
- Compton Peak at 15 keV was used as Internal Standard
Liquid Samples
Platinum in Blood

- Pt monitoring over a 4 day period for toxicological control of medication
- Slow decrease of Pt after application indicates kidney function

* Prof. Eduardo D. Greaves, Universidad Simon Bolivar, Caracas, Venezuela
Liquid Samples
Trace Element Analysis in Serum & Blood

Measurements and sample preparation

Blood Serum
  Dilution, Ultrapure water
  Int. Standard, Ga
  TXRF

Whole Blood
  Digestion, 10% tetramethylammonium hydroxide @ 1 hr
  Dilute in 2% HCl solution
  TXRF
Liquid Samples
Trace Element Analysis in Serum & Blood

• Comparison of TXRF to ICP-MS reference values for trace elements in whole blood
• Good concordance of TXRF with reference values for essential elements
• Other elements (P, S, Cl, K, Ca, Br, Rb, Sr) could also be determined during one measurement
• Samples analyzed for 600s

<table>
<thead>
<tr>
<th>Whole blood standard</th>
<th>TXRF</th>
<th>Std. dev.</th>
<th>ICP-MS</th>
<th>Std. dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe</td>
<td>440000</td>
<td>900</td>
<td>435000</td>
<td>12000</td>
</tr>
<tr>
<td>Cu</td>
<td>662</td>
<td>43</td>
<td>623</td>
<td>21</td>
</tr>
<tr>
<td>Zn</td>
<td>5010</td>
<td>118</td>
<td>5038</td>
<td>69</td>
</tr>
<tr>
<td>Se</td>
<td>123</td>
<td>18</td>
<td>123</td>
<td>10</td>
</tr>
<tr>
<td>Pb</td>
<td>399</td>
<td>6.5</td>
<td>396</td>
<td>100</td>
</tr>
</tbody>
</table>

All values in µg/l
Liquid Samples  
Trace Element Analysis in Serum & Blood

<table>
<thead>
<tr>
<th>Serum standard</th>
<th>TXRF</th>
<th>Std. dev.</th>
<th>AAS</th>
<th>Std. dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe</td>
<td>2920</td>
<td>87</td>
<td>1964</td>
<td>196</td>
</tr>
<tr>
<td>Cu</td>
<td>1690</td>
<td>43</td>
<td>1562</td>
<td>312</td>
</tr>
<tr>
<td>Zn</td>
<td>2190</td>
<td>118</td>
<td>2225</td>
<td>334</td>
</tr>
<tr>
<td>Se</td>
<td>97</td>
<td>18</td>
<td>102</td>
<td>26</td>
</tr>
<tr>
<td>Au</td>
<td>1343</td>
<td>13</td>
<td>1965</td>
<td>393</td>
</tr>
<tr>
<td>Pb</td>
<td>11</td>
<td>5.8</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

All values in µg/l

- Comparison of TXRF and AAS reference values in blood serum
- TXRF has better standard deviations compared to AAS
- **No Digestion** procedure was applied
Liquid Samples
Se in Mother’s Milk

- Se concentration during lactation period was monitored with whole milk
- **No Pretreatment** of milk was done, only addition of Internal Standard
- Detection limits were calculated to **3 µg/l**
- Good concordance to milk powder standards (NIST BCR 150)
Liquid Samples
Conclusion

Accuracies and Sensitivities comparable to AAS or ICP without the need for complex and time-consuming sample preparation and instrument calibration

- Ability to analyze minute samples

- Allows for monitoring of trace metal profiles or metabolic interactions for detection of unsuspected nutritional deficiencies

- Element determinations in distinct fractions of blood (lipids, proteins, etc.) is possible

- Elements Na to U within one measurement

- Detection limits of 1 to 100 ppb for most elements
## TXRF vs. AAS/ICP-OES

<table>
<thead>
<tr>
<th>TXRF</th>
<th>AAS/ICP-OES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small footprint/portable</td>
<td>Large, fixed installation</td>
</tr>
<tr>
<td>Microgram sample size</td>
<td>Milligram sample size</td>
</tr>
<tr>
<td>%-ppb levels</td>
<td>ppm – sub ppb levels</td>
</tr>
<tr>
<td>Single std. calibration</td>
<td>Multi-standard calibration</td>
</tr>
<tr>
<td>Low maintenance</td>
<td>High maintenance</td>
</tr>
<tr>
<td>Non-destructive</td>
<td>Destructive</td>
</tr>
<tr>
<td>Fast learning curve</td>
<td>Long learning curve</td>
</tr>
<tr>
<td>Fast sample prep</td>
<td>Laborious sample prep</td>
</tr>
</tbody>
</table>

- **TXRF**
  - Small footprint/portable
  - Microgram sample size
  - %-ppb levels
  - Single std. calibration
  - Low maintenance
  - Non-destructive
  - Fast learning curve
  - Fast sample prep

- **AAS/ICP-OES**
  - Large, fixed installation
  - Milligram sample size
  - ppm – sub ppb levels
  - Multi-standard calibration
  - High maintenance
  - Destructive
  - Long learning curve
  - Laborious sample prep
Any Questions?

Please type any questions you may have for our speakers in the Q&A panel and click Send.
Thank you for your attention!

Upcoming:

14th International Conference on Total Reflection X-ray Fluorescence and Related Methods
06–10 June 2011, Dortmund, Germany

3rd International Symposium on Metallomics
15-18 June, 2011, Muenster, Germany

www.bruker.com
www.s2picofox.com