HEAVY ELEMENT DETERMINATION IN HEALTH-RELATED ORGANIC MATRIX MATERIALS BY TOTAL REFLECTION X-RAY FLUORESCENCE.

E. D. Greaves
Universidad Simón Bolívar, Apartado 89000, Caracas 11080A, Venezuela. egreaves@usb.ve

ABSTRACT
Recent developments in TXRF techniques make the method appropriate for simple, accurate and precise analysis of heavy elements in organic materials in health-related and pollution problems. Special advantages are derived by the use of monochromatized beams of X-rays: i) The reduction in the spectrum background allows direct irradiation of organic matter specimens. Hence human tissue and body fluids are prepared by simple procedures involving dilution, homogenization and standard addition avoiding the need for specimen digestion. This results in faster, cheaper methods that decrease sample contamination problems. ii) The presence of a large Compton scattered signal in the spectrum and its use as an internal standard reference allows further simplification by avoiding the need to add an element as internal standard in test specimens. Quantification methods rely on determination of spectrometer sensitivity relative to the Compton peak. Required use of standard reference materials or matrix matching for this purpose may be substituted by a semiempirical reference model or by the use of a standardized specimen made from the unknown by spiking with elements absent in the matrix. Applications include trace element determination in amniotic fluid in fetus malformation studies, analysis of brain specimens and cerebrospinal fluid in central nervous system disorders, the influence of trace elements in cataract genesis and blood, body fluids and tissue drug-level monitoring. Accuracy, precision and detection limits attained in the analysis of platinum in minute amounts of serum and urine from oncological pediatric patients undergoing chemotherapy with Pt-containing drugs shows TXRF to be the most appropriate technique for routine monitoring of platinum blood levels in cancer patients.

INTRODUCTION
The importance of heavy elements in human health studies is well established. Normal healthy organism contains a number of essential heavy elements, which play an important role in the human physiology. Monitoring of body fluids for disease diagnosis or assaying for the presence or absence of toxic elements is part of the modern arsenal of the physician. The introduction in the human body of foreign heavy elements for diagnosis or therapeutic reasons has become common practice. However, all invasive procedures, which involve the introduction in the body of heavy elements either as contrast media, as essential constituents of radiopharmaceutical compounds or as integral parts of drugs must consider the risk-benefit balance of their use. The development of simple accurate and precise procedures of heavy element monitoring is important to reduce the risk and to maximize the benefits.

A number of modern analytical techniques are appropriate for trace element analysis of the human body. Techniques such as Graphite Furnace Electrothermal Atomization Atomic Absorption Spectroscopy (GF-ETA-AAS), Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES), Neutron Activation Analysis (NAA) and Total Reflection X Ray Fluorescence (TXRF) are appropriate for determination of metals at very low concentration levels. Organic samples of human origin have been analyzed previously by TXRF using various sample preparation techniques: by sample chemical digestion, by cold plasma ashing.
This document was presented at the Denver X-ray Conference (DXC) on Applications of X-ray Analysis.

Sponsored by the International Centre for Diffraction Data (ICDD).

This document is provided by ICDD in cooperation with the authors and presenters of the DXC for the express purpose of educating the scientific community.

All copyrights for the document are retained by ICDD.

Usage is restricted for the purposes of education and scientific research.

DXC Website  ICDD Website
direct irradiation of microtome sectioned tissue\textsuperscript{6,7}. ICP and AAS procedures as well as TXRF reports\textsuperscript{8,9} have traditionally relied on methods of sample digestion and comparison with inorganic standards in order to quantify the analytes. However, new methods in recent development such as flow injection AAS and graphite furnace conditioning in GFAAS attempt to simplify or eliminate the digestion procedure and to analyze the sample directly. This has the advantages of minimizing the possibility of sample contamination and reducing the time and cost of the analysis. In this paper we show developments in this direction: in the use of TXRF for assay of undigested body fluids and tissue.

Energy dispersive X-ray fluorescence techniques have the advantage of being a simultaneous and multielemental technique. Total reflection methods have increased the sensitivity and lowered the detection limits of XRF two to three orders of magnitude to the level of picogram quantities in standard instruments and to the level of femtogram quantities with synchrotron-excited measurements.

Direct organic sample irradiation with polychromatic X-ray sources has the disadvantage of the elastic and Compton inelastic interaction that takes place between the X-rays and the light elements in the organic matrix. The resulting polychromatic scattered radiation reaching the detector produces a large background in the spectrum, which increases the detection limits and decreases the precision of the analysis. The use of monochromatic X-rays avoids this shortcoming of polychromatic sources with a dramatic reduction of the background noise in the region of the analytical lines. Another advantage is that the Compton inelastic interaction of the excitation radiation with the sample produces a large, usually isolated, peak in the spectrum. This peak has been shown to be proportional to the specimen mass on the reflector. Being that TXRF is a non-absolute technique, a reference or internal standard is needed in order to obtain a relationship between the net count rate observed by the detector and the analyte concentration. Addition of Co, V, Ga or any element not present in the specimen is usually done to fulfill the requirements of standardization.\textsuperscript{10,11,12,13} However, the Compton peak is a signal which may be used as an internal standard, allowing quantification without the need to add an element as internal standard to the test specimen\textsuperscript{14}. The use of the Compton peak as internal standard requires measuring the spectrometer relative sensitivity for the particular matrix material. In this paper we review the relations used for quantification using the Compton peak as internal standard and show the alternative procedures we have developed, in lieu of a standard reference material, for spectrometer sensitivity measurement.

**QUANTIFICATION PROCEDURE. THEORY**

For a thin sample, neglecting absorption and enhancement, the fluorescent count rate $N_{il}$ of the $i$th peak of element $t$ is obtained by integrating the production factor $Q_{il}(E)$ over the excitation intensity of the source $I(E)$ as a function of energy from the absorption edge $E_{ed}$ to the maximum source energy $E_{max}$ as follows:\textsuperscript{14}:

$$N_{il} = GC_i \varepsilon(E_{il}) \int_{E_{ed}}^{E_{max}} Q_{il}(E)I(E)dE$$

where $G$ is a geometrical factor, $C_i$ is the concentration of the $i$th analyzed element in the sample, $\varepsilon(E_{il})$ is the efficiency of the detector at the excitation energy $E_{il}$.
The production factor is given by the expression:

\[ Q_{i,E} = \tau(E) \left( 1 - \frac{1}{f_k} \right) W_i k \]

where \( \tau(E) \) is the photoelectric mass absorption coefficient of element \( i \), \( W_i \) is the fluorescence yield, \( f_k \) is the fraction of excited atoms that decay by photon emission of the series corresponding to the quantified line and \( J \) is the jump factor.

When an internal standard element is added to the sample, a similar relation to Eq. (1) gives its net count rate \( N_s \). Hence the count rate relative to the standard is given by:

\[ \frac{N_i}{N_s} = \frac{e(E_i) \int_{E_{abs}}^{E_{em}} Q_i(E) I(E) dE}{e(E_s) \int_{E_{abs}}^{E_{em}} Q_s(E) I(E) dE} \times \frac{C_i}{C_s} \]

In principle it is possible to theoretically evaluate expression (3) and to deduce from it the unknown concentration \( C_i \). However, that procedure is hampered by:

i) Our imprecise knowledge of the incident X-ray spectrum, \( I(E) \), which depends on a number of experimental factors such as: the spectrum produced by the X-ray tube used; the cut-off filter, or monochromator crystal fitted in the spectrometer and the distortion of the beam produced at the reflector surface.

ii) The imprecise value of the efficiency of the particular detector used and

iii) Lack of accurate values for some of the fundamental parameter that enter into Eq. (2).

If the spectrometer uses a monochromator set at \( E_{ex} \) the integrals in (3) may be eliminated and the relation simplified to:

\[ \frac{N_i}{N_s} = \frac{e(E_i) Q_i(E_{ex})}{e(E_s) Q_s(E_{ex})} \times \frac{C_i}{C_s} \]

If the relative sensitivity of the analyte with respect to that of the internal standard is defined as:

\[ R_{is} = \frac{e(E_i) Q_i(E_{ex})}{e(E_s) Q_s(E_{ex})} = \frac{S_i}{S_s} \]

This leads to:

\[ \frac{N_i}{N_s} = \frac{S_i C_i}{S_s C_s} \]

Where, \( N_i \) and \( N_s \) are the net peak intensities of the element and the internal standard; \( S_i \) and \( S_s \) are the sensitivities of the analyte and internal standard and \( C_i \) and \( C_s \) are the concentrations of the analyte and internal standard, respectively. The sensitivity ratio \( R_{is} \) is the parameter calculated using the calibration curve obtained from the standards. Since all the terms in Eq. (6) are known for a standard, except the sensitivity ratio, this ratio can be obtained from Eq. (6) so that the unknown concentration is obtained using:
In the case where the incoherent or Compton peak is used as internal standard an equation that relates the net count rate of element $i$, $N_i$, to the net count rate of the Compton scattered radiation, $N_C$, can be written in the same way as Eq. (4). Neglecting the scattering contribution of the reflector the ratio between $N_i$ and $N_C$ can be written as:

$$\frac{N_i}{N_C} = \frac{e(E_i)Q_i(E_{ex})}{e(E_C)\sigma_c(E_{ex}, \psi)\Delta\Omega} \times C_i$$

(8)

where $\sigma_c$ is the differential incoherent scattering cross section at the excitation energy $E_{ex}$ and $\psi$ is the scattering angle, which is 90 degrees in TXRF, and $\Delta\Omega$ is the solid angle with which the detector looks at the sample. The fraction in the right hand side of Eq. (8) may be defined as the relative sensitivity of element $i$ to the Compton scattered peak $R_{ic}$, so that the concentration of the unknown element $i$ can be expressed as:

$$C_i = \frac{N_i}{N_C} \frac{1}{R_{ic}}$$

(9)

The similarity of Eq. (9) and Eq. (6) and (7) allows the use of computer codes designed for calculation of concentrations with elements as internal standards. For quantification using the Compton peak as internal standard it suffices to set the internal standard concentration $C_i$ to unity and to replace the relative sensitivity to the internal standard element $R_{is}$ by the relative sensitivity to the Compton scattered peak $R_{ic}$ in Eq. (6). The Compton peak reflects geometrical effects on the reflector observed during the analysis in the same way as an internal standard does.

**QUANTIFICATION PROCEDURE, EXPERIMENTAL**

**Instrumentation and spectrum analysis**

Measurements were performed with an energy dispersive XRF Spectrometer (Canberra) which included a Si(Li) detector 3 mm thick and 30 mm$^2$ area of nominal 180 eV resolution, modular nuclear electronics and a PC based S100 multichannel analyzer. Irradiation was performed in a modified TXRF module designed by the Atominstitut of Vienna, Austria. The excitation source was a Mo anode X-ray tube operated at 40/45 kV and 20 mA. Several different set-ups have been used in the TXRF module as spectrum modifiers. A set-up which uses a TiAP$\text{\textsuperscript{1}}$ crystal Bragg monochromator$\text{\textsuperscript{16}}$, a set-up which uses a flat quartz plate as a high energy filter or cut-off, and a multilayer monochromator.

Data processing of spectra raw data were done using the QXAS package$\text{\textsuperscript{17}}$. Signal intensities were derived with the spectrum fitting routine AXIL and concentrations calculated with the “Regression of count rate “ routine provided in the package. In those instances in which the Compton peak is used as the internal standard, a carefully defined range of channels, spanning the incoherent Compton peak in the spectrum, is also quantified in both unknowns and standard spectra. Detection limits were calculated using the following relationship, with a counting time of 1000 seconds$\text{\textsuperscript{18}}$:

$$L.D_i = \frac{3\sqrt{N_i}}{N_i} C_i$$

(10)

$\text{\textsuperscript{1}}$ TiAP. Organo-metallic single crystal of thallium-containing salt of phthalic acid with interplanlar d-spacing of 25.750 Angstroms. Supplied for crystal spectrometers.
Where $i$ represents the analyte, $N_b$ is the number of counts of the background under the peak of the analyte, $N_i$ is the number of counts or net peak area for the analyte and $C_i$ is its concentration.

**Sensitivity calibration**

The spectrometer sensitivity calibration for a particular organic matrix material may be done relative to an element as internal standard or relative to the Compton peak as internal standard. In both cases it is usually necessary to have a sample of the particular matrix with known element concentrations. To fulfill this requirement the following alternatives can be followed:

i) Acquisition of a certified standard reference material of the same matrix or of a reasonably closely-matched matrix (i.e., Bovine blood serum for human blood serum),

ii) Prepare a standardized sample. This can be accomplished by adding known amounts of various elements to a quantity of the unknown material. (These elements must span the range of atomic weights of interest and must be below detection limits in the matrix), and

iii) Construct a semiempirical model of the standard. This is accomplished by obtaining two spectra: a spectrum of a multi-elemental aqueous standard solution and a spectrum of the organic sample in question that has been spiked with a known amount of one of the elements, absent in the matrix, and present in the multi-elemental solution. With this data, a virtual standard material spectrum is constructed which is used to derive the relative sensitivity. Details are given in reference 14.

**Samples and sample preparation.**

A variety of corporal fluids and some tissue samples have been analyzed in various projects. These include the following: blood, urine, amniotic fluid, cerebrospinal fluid, semen, brain tissue and crystalline (eye lens). In almost all cases sample manipulation and treatment were performed in a laminar flow cabinet.

Amniotic fluid samples were taken by amniocentesis from pregnant women in the Instituto Medico-docente La Trinidad in Caracas, Venezuela. Samples were kept frozen at -5°C until analysis. Samples were centrifuged to precipitate cells and particles and 500 µl of the clear fluid transferred to an Eppendorf capsule. 10 µl of 1000 ppm Co solution was added as internal standard and homogenized by vibration (Vortex).

Cerebrospinal fluid samples were taken by spinal tap from patients suffering from epidemic neuropathy in Cuba. Samples were kept frozen at -5°C until analysis. Sample treatment was the same as for amniotic fluid.

Semen samples were collected from volunteer donors in a study of lead pollution and semen quality. Samples were kept frozen at -20°C until analysis. Analysis was done in whole semen and in digested samples. Frozen samples were liquefied at room temperature and digested in covered beakers in a fume cupboard with 1:1 solution of ultrapure HNO$_3$ under moderate heating conditions (~85°C). With digested semen gallium was added as internal standard: 10 µl of 1000 ppm Ga standard solution was added to 90 µl of the sample. The same procedure was used in one whole semen sample to determine the sensitivity relative to the Compton peak.

Brain tissue samples from healthy, male individuals, who suffered accidental and instantaneous death were taken at the morgue of the Hospital Central of Barquisimeto, Edo. Lara, Venezuela. Brains were dissected, not more than 24 hours after death, and frozen at -50°C until sample preparation. Different brain sections as cerebellum, hypothalamus, frontal cortex, vermex and brainstem, were weighed and homogenized with deionized water with a high-speed homogenizer at 23000 rpm. Homogenates with a 50-60% w/V (wet weigh) of brain tissue were

---

Ordered by the patient's gynaecologist.
keep frozen at -50 °C until analysis. Analysis specimens were brain slurry prepared in a concentration of 24% of brain tissue with nitric acid concentration of 5% V/V.

Cataractous human lenses were obtained by extracapsular extraction of patients of the oftalmology unit of the Hospital Militar “Carlos Arvelo” in Caracas, Venezuela. Samples were kept frozen at -5 °C until analysis. Cataract samples were added to a weighted glass vial and weighed again. Two ml of deionized water and 10 μl of 2000 ppm cobalt standard solution were added to the vial and the mixture homogenized. This was accomplished with a special device consisting of a glass vial and a cut glass stirrer which spins at 35 000 rpm. Time required for complete homogeneization varied from a few seconds to about 2 minutes depending on the sample. The homogenate was allowed to rest for about 15 seconds to allow bubbles to raise to the surface before the test specimen was taken.

Blood and urine samples were taken from cancer patients under treatment with Pt-containing drugs in the Pediatric Service at Instituto Oncológico Luis Razetti in Caracas, Venezuela. Blood samples, taken by venepuncture with disposable syringes, were centrifuged at 2400 rpm to separate the serum. Urine samples were taken in plastic vessels. Urine and blood samples were always taken 24 hours after administration of the drug in a dose of 100 mg/m². Samples were kept in a freezer at -10 °C until analysis. Vanadium was used as internal standard for blood serum and urine: 10 μl of 1000 ppm V standard solution was added to 1000 μl of serum or urine and homogenized by agitation in a closed vial.

To carry out the TXRF irradiation the same sample deposition procedure was used for all the materials discussed above. An amount of 10 μl of sample was deposited in the center of a clean, 30-mm diameter, specular surface pure quartz reflector. The sample drop was dried by vacuum, or when foam was produced, by the use of an infrared lamp.

Results and discussions

Human reproduction studies.

In ongoing studies of the role of trace element concentration and semen quality, samples have been analyzed by AAS and by TXRF. Several quantification procedures have been tried:

![Figure 1. Digested semen. 40 kV 20 mA Mo anode X-ray tube, TlAP monochromator.](image)

<table>
<thead>
<tr>
<th>Whole semen + Incoh. peak as internal standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Element</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>K</td>
</tr>
<tr>
<td>Ca</td>
</tr>
<tr>
<td>Fe</td>
</tr>
<tr>
<td>Cu</td>
</tr>
<tr>
<td>Zn</td>
</tr>
<tr>
<td>Br</td>
</tr>
<tr>
<td>Rb</td>
</tr>
</tbody>
</table>

Table 1. Trace elements in whole semen with TlAP monochromator.
i) digested + Cr internal standard, ii) digested + Compton peak as internal standard and iii) whole semen + Compton peak as internal standard. Fluorescent spectra of digested samples exhibit a large incoherent Compton peak indicating incomplete digestion as shown in Fig. 1. A semiempirical model of a standard was used for ii) and iii). Resulting trace element concentrations by the various methods did not differ significantly. Table 1 shows typical analysis results obtained with a TIAP crystal monochromator. Lead fluorescent signals were detected (see Fig 1) but were not quantified. With the limited reflectivity monochromator used, lead counts were too close to detection limits for attaining reliable results.

Neurologic studies.

The following results can be quoted.
1. A TXRF methodology for trace element determination in cerebrospinal fluid was established.
2. The TXRF analysis of cerebrospinal fluid and serum of patients with neurologic disorders allows establishing the possible existence of anomalous operation of the blood-brain barrier whose function, of preventing toxicity in the bloodstream from reaching the central nervous system, might be impaired.

Cancer studies.

Oncological patients undergoing certain cancer treatments receive platinum-containing drugs. Monitoring of platinum levels in body fluids and tissues is an effective means for optimal administration and evaluation of the effectiveness of the drug as well as to avoid the toxic effects of platinum. Additionally, platinum level determinations are required in studies of the mechanism of action of such drugs in the body and possible side effects. A TXRF method for measuring platinum in urine and blood serum was developed. Urine exhibits a variable matrix, which precludes analysis with the Compton peak. Hence cobalt was added as internal standard to affect platinum quantification. Serum, on the other hand, is a "good" stable matrix that may be reliably quantified using the Compton peak as internal standard. Typical spectra are shown in Fig. 2 and 3. Platinum detection limits that were obtained are 130 ppb in serum and in urine. These values are sufficiently low to reliably detect and quantify platinum blood levels and urine concentrations in samples taken 24 hours after the Pt-containing drug was administered. Table 2 shows the Pt concentrations measured. They are at least a factor of 10 higher than the measurement detection limit.
Ophthalmic studies.

A number of studies in the last decade have reported measurements of trace elements in the human eye lens. However, a relation between element concentrations or its importance in the cataract pathogenesis has not been clearly established. In an ongoing project and as a contribution to these studies a new technique for the analysis of human cataracts using TXRF has been established. Measured values of eight quantified elements show a very wide range of concentrations which corroborates previous literature values, see Fig. 4. Although certain relations between concentration and patient conditions have been suggested, further work is needed in a close collaboration with the ophthalmologist.

Figure 4. Measured trace element concentrations in cataracts of diabetic patients

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Pt in Serum (ppm)</th>
<th>Pt in Urine (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.9 +/- 0.1</td>
<td>4.8 +/- 0.4</td>
</tr>
<tr>
<td>3</td>
<td>1.1 +/- 0.1</td>
<td>2.7 +/- 0.3</td>
</tr>
<tr>
<td>4</td>
<td>1.0 +/- 0.1</td>
<td>2.9 +/- 0.2</td>
</tr>
<tr>
<td>5</td>
<td>1.1 +/- 0.1</td>
<td>4.6 +/- 0.2</td>
</tr>
</tbody>
</table>

Table 2. Concentrations of platinum in serum and urine that were obtained by TXRF with a TIAP monochromator. 40 kV, 20 mA Mo anode X-ray tube.

CONCLUSION

The use of the Compton peak as internal standard allows quantification of analytes without the need to add a reference element to each sample thus simplifying the procedure and reducing the possibility of contamination.

The procedures that have been described show that TXRF techniques are appropriate for simple accurate and precise analysis of heavy elements in fluids and tissue of human origin. They demonstrate instances where trace element determinations are of interest as an accessory in disease research or as a valuable tool in clinical diagnosis.

The direct analysis of platinum in minute amounts of serum is an instance where it is shown that TXRF is appropriate for routine monitoring of blood levels in cancer patients undergoing chemotherapy with platinum-bearing drugs.

Acknowledgments

The supply of the basic equipment for this work by the International Atomic Agency (VEN/2/007) is gratefully acknowledged. I want to thank the close collaboration of Laszlo Sajo-Bohus whose assistance has been invaluable in the success of our joint projects. The financial support of CONICIT of the government of Venezuela is gratefully acknowledged.
REFERENCES

23 E.D. Greaves, L.M. Marcó Parra, A. Rojas and L.Sajo-Bohus. Determination of platinum levels in serum and urine samples from oncological pediatric patients by TXRF. Submitted to X-ray Spectrometry, 1999
24 Dose units used by the physician.