Method development for the analysis of complex samples with inductively coupled plasma mass spectrometry

MARCUS KORVELA
Abstract


In this thesis the development of methods for handling the problems associated with analyzing trace elements in complex matrixes using inductively coupled plasma mass spectrometry is presented. Trace elements such as Cu, Fe, Se, and Zn, to name a few, do play important roles in different organisms. Therefore it can be of importance to study trace elements in different samples of biological origin. As trace elements are low in abundance, sensitive instrumental techniques such as inductively coupled plasma mass spectrometry (ICP-MS) are required for accurate determination. Due to the complexity of samples with biological origin, careful method development, both regarding the sample preparation and instrumental analysis has to be performed to minimize negative effects on the instrument signal and introduction of interferences.

For example the metal contents of mink livers were analyzed, after bomb digestion to investigate if the metal concentration could be linked to changes in the organ morphology as well as the minks’ environment. Morphological changes and capture locations could be linked to the metals investigated. The investigation of the elemental composition of cerebrospinal fluid from chronic pain patients using spinal cord stimulation electrode treatment on the other hand required less harsh sample treatment. No correlation between the spinal cord stimulation and element concentration could be found, but differences between patients and the control group were presented hinting that chronic pain intrinsically could affect the cerebrospinal fluid metal concentration. Another bodily fluid of interest is saliva and the use of paperpoint sticks as a sampling technique for Ti in saliva was investigated. As Ti is interfered by several components expected to be found in saliva, the use of reaction or collision gas was also investigated to reduce the effects of interferences. Simple leaching of the paperpoint sticks together with complexing the Ti with NH3 as reaction gas was shown to be optimal. Finally, how the selection of internal standard would be affected by the use of reaction and collision gases was also investigated. With collision gas most internal standards worked fine, while for reaction gas internal standard selection was harder. For elements with high ionization energy such as As, Se and Zn the choice of internal standard was very dependent on matrix. While ICP-MS suffers from problems when analyzing samples with complex matrixes many of them can be minimized by proper method development as shown in this thesis.

Keywords: ICP-MS, trace elements, complex matrix.

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To people who worry that they won’t finish in time
“I hate these nerds. Just because I'm stupider than them they think they're smarter than me!”
-Professor Farnsworth, Futurama
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


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Papers not included in thesis


## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>CSF</td>
<td>Cerebrospinal Fluid</td>
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<td>DRC</td>
<td>Dynamic Reaction Cell</td>
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<td>FAAS</td>
<td>Flame Atomic Absorption Spectroscopy</td>
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<td>GFAAS</td>
<td>Graphite Furnace Atomic Absorption Spectroscopy</td>
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<tr>
<td>HR-ICP-MS</td>
<td>High Resolution Inductively Coupled Plasma Mass Spectrometry</td>
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<tr>
<td>ICP-AES</td>
<td>Inductively Coupled Plasma Atomic Emission Spectroscopy</td>
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<td>ICP-MS</td>
<td>Inductively Coupled Plasma Mass Spectrometry</td>
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<tr>
<td>KED</td>
<td>Kinetic Energy Discrimination</td>
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<tr>
<td>LOD</td>
<td>Limit of Detection</td>
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<tr>
<td>PBS</td>
<td>Phosphate-Buffered Saline</td>
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<tr>
<td>PTFE</td>
<td>Polytetrafluoroethylene</td>
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<td>PFA</td>
<td>Perfluoroalkoxy alkane</td>
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<tr>
<td>QID</td>
<td>Quadrupole Ion Deflector</td>
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<tr>
<td>RF</td>
<td>Radio Frequency</td>
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<tr>
<td>RSD</td>
<td>Relative Standard Deviation</td>
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<tr>
<td>SCS</td>
<td>Spinal Cord Stimulation</td>
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<td>US</td>
<td>Ultrasonic</td>
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Introduction

Living organisms like humans are mainly comprised of O, C, H, and N, the main components of proteins and other biomolecules. While they are the most abundant, other important elements including Ca, P, K, S, Na, Cl, and Mg can also be found in relatively large quantities in the body, either as free ions or incorporated into biomolecules. And while the building blocks of life is mainly constituted of these elements, there are several other elements which exist in smaller quantities in the body either with known biological functions such as e.g. Cu [1], Fe [2], Mn [3], Mo [4], Se [5] and Zn [6], or with so far no known biological function such as e.g. Rb, Sr, Ti. For as long as humanity has been aware of all the different elements present in organisms, we have been trying to elucidate their role in life. Starting with early measurements of total element concentration in organs, leading up to more modern experiments where the distribution of certain elements and their chemical form in organ tissue are studied [7-9]. In a similar manner the interest in trace elements has gone beyond just the basic biological role to realizing that trace elements can both be indicators of, aggravate symptoms, or even in some cases be the cause of certain diseases [10-13]. Therefore, further understanding of interactions of trace elements in the organism may help in finding more ways of diagnosing, preventing and/or even curing diseases. While some elements are necessary there are of course the opposite with other elements being harmful or even deadly and sometimes essential ones being toxic in certain concentrations or when present as different molecular species. So, in addition to understanding the biological roles of element it is equally important to be able to detect them in the environment before they become a threat to flora and fauna and understanding what makes them toxic and how they are distributed in the environment. The traditional way to determine the spread of elements in nature is to analyze water, air or soil samples, but another option is to investigate the element concentration in animals that live in the area of interest. Species used for this purpose are termed sentinel species and the benefits of using them includes fewer samples needed compared to conventional samples and possible information on the toxicity of the elements that are investigated.

Unsurprisingly the increased understanding of trace elements and their role in organism has been closely linked with the development of the analytical
Instruments used [14] to detect trace elements mainly in regards to lower detection limits and higher sensitivity and selectivity. This is evident in the development of Flame Atomic Absorption Spectroscopy (FAAS) and Graphite Furnace Atomic Absorption Spectroscopy (GFAAS) leading to plasma-based technologies such as Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Developed in the early 80’s, the ICP-MS was a continuation of the work done by Gray et al. [15] and tried to combine the atomization ability of an ICP with the sensitivity of an MS. The idea was successful, and the use of ICP-MS took off and while it has now become a mature technique it is still an improving field with instruments becoming even more sensitive, faster, and more robust with each new model. The main idea of the ICP techniques was to replace FAAS and GFAAS by using higher temperature conditions to hopefully reduce interferences that plagued the older techniques, but it did not quite work out as expected [16]. Even though the ICP-AES instruments did overcome some of the problems associated with the older technologies, it did come with its own unique problems and interferences instead. While ICP-AES have a multi element capability and higher sensitivity it is more expensive to operate and not as robust as the FAAS, with risk of more physical interferences. To increase the sensitivity the MS was introduced as a detector, which worked out splendidly, but again this created new problems with different spectral interferences [17, 18] in addition to some of the physical ones already affecting the ICP-AES technique. Since then new methods have continuously been developed to overcome these issues and the increasing demands associated with modern scientific inquiry, which demands even lower detection limits in more challenging matrixes.

In this thesis methods for analyzing complex samples for specific elements in a variety of matrixes with ICP-MS are presented in papers I-III, as well as general technical aspects of getting better results in complex samples as presented in paper IV. In paper I the metal contents in digested wild mink liver samples are analyzed, both as environmental indicators and also to investigate links between morphological effects on organs and metal concentration. Some studies have been performed on mink livers but none from the specific area and the exact same set of elements using ICP-MS [19, 20]. In paper II the elemental composition of cerebrospinal fluid (CSF) of chronic pain patients using spinal cord stimulation (SCS) electrode treatment are investigated. As metals have been linked to other medical conditions it was of interest to investigate if they could be also be involved in the effects from using SCS. While CSF has been a popular sample to measure with ICP-MS the element composition in regards to chronic pain and the treatment of it has never been investigated. Paper III explores the possibility of using paper-point sticks as a sampling technique to determine Ti concentration in saliva.
as well as different techniques to remove Ti interferences. As Ti concentrations have been linked to inflammation in dental implants it is of interest to investigate [21]. Usually that has involved the use of biopsies which are much more invasive to the patient compared to using paperpoint sticks. No previous studies existed on the use of paperpoints sticks to sample saliva for the measurement of element composition. While an article describing the use of NH$_3$-gas to complex Ti using a Nexion 300D has previously been published [22], it served as proof of concept and did not compare the method to that of using collision gas or the delve in to the complexities of internal standard choice or method optimization. Paper IV investigates the selection of internal standards with the use of reaction or collision cells in conjunction with harsh matrixes for ICP-MS. While studies on internal standard choice for ICP-MS exists [23, 24] none of them have investigated how the introduction of collision or reaction gases will affect the choice of internal standards, and as such was therefore of interest to investigate.
Inductively coupled mass spectrometry

The ICP-MS instrument (Figure 1) can roughly be divided into the sample introduction part consisting of the pump, nebulizer, spray chamber, injector tube and torch; the ion optics part such as sampler cone, skimmer cones and ion lenses; and the analyzer part which contains a mass analyzer, such as a quadrupole, time of flight, electric-, or magnetic-sector field followed by a detector and a computer.

![Diagram of ICP-MS](image)

Figure 1. Schematic overview of an ICP-MS, based on the Nexion 300D configuration and not to scale. A) sample, B) peristaltic pump, C) nebulizer, D) cyclonic spray chamber, E) injector tube, torch and RF-coil, F) interface cones, G) quadrupole ion deflector, H) analyzer quadrupole, I) detector, J) computer.

The central unique feature of the ICP is of course the plasma, which is created by introducing an electrical spark to an Ar gas flow resulting in the ionization of some of the Ar atoms creating Ar⁺ ions and free electrons. A radio frequency (RF) field, either at 27 MHz or 40 MHz [25] is simultaneously
applied which promotes collisions between atoms and electrons further
knocking out more electrons leading to an equilibrium between neutral Ar
atoms, Ar$^+$ ions and electrons being formed. This creates an overall neutral
gas, but which still contains charged particles i.e. a plasma. The plasma will
be formed into a sort of plume in the torch due to the flow of argon gas, both
to cool the plasma and supply it with fresh Ar to maintain the plasma. The
plume can reach temperatures close to 10 000 K depending on which section
of the plasma is observed, with the cooler channel in the middle were the
sample is introduced usually ranging between 6000 K and 7500 K [26]. The
high temperature will atomize most compounds that enter the plasma and the
Ar$^+$ ions will ionize atoms and molecules that have lower ionization energy
compared to Ar (15.2 eV) [27]. The ionized atoms will then, by a pressure
difference, be drawn into the sampler cone and skimmer cones and then fur-
ther into the ion optics which directs the beam into the mass analyzer, such
as a quadrupole used in all papers of this thesis. Ions with a selected mass to
charge ratio (m/z) will then be transported into the detector, which will am-
plify the signal and send the information to the software.

The first step of analysis is of course the introduction of sample and while
solid, slurry and gaseous samples can be analyzed by ICP-MS acidified
aqueous samples are by far the most common. Either pumped or self-
aspirated the sample is lead to the nebulizer, which transforms it into a fine
mist in the spray chamber. Several different varieties of nebulizers exist e.g.
the concentric- (most common in ICP-MS) [28], crossflow- and Babington-
nebulizer (Figure 2) with each being designed with different parameters in
mind such as; sensitivity, precision, matrix tolerance, and sample consum-
ption [29]. The concentric type nebulizers consist of a capillary inserted into a
glass tube with a nozzle at the end. The capillary is positioned so that the end
meets up with the opening of the nozzle of the outer glass tube. When sam-
ple is pumped through the capillary and argon gas is flowing in the glass
tube, then the sample will be pulled out, by the gas through the annulus of
the capillary and then the outer nozzle resulting in spray formation. The con-
centric type generally provides high sensitivity and precision, but is sensitive
to matrix effects, especially salt build up on the tip, which may impede spray
formation leading to drift or even completely clog the nebulizer. In compari-
son the crossflow nebulizer has two capillaries, one with sample flowing up
towards the other capillary which has a perpendicular argon gas flow. The
argon gas blowing over the sample liquid shatters the liquid and creates the
spray, this makes the crossflow nebulizer more tolerant to salts and minor
particles, but it is slightly less sensitive and not as precise as the concentric
nebulizer. The Babington nebulizer works in its simplest form by leading a
sample liquid over an orifice that has argon flowing through it and thereby
creating a spray. This makes the Babington the most salt and particle tolera-
ble of all nebulizers, but on the other hand it also has the lowest sensitivity of them all.

Figure 2. Schematic overview of some different nebulizer types: A) Concentric, B) Cross flow, and C) Babington. Not to scale.

The purpose of the spray chamber is to create a more uniform droplet distribution, by removing mostly larger droplets (>10 µm) [30] before transport into the injector tube and finally the plasma itself. The need to remove larger droplets is to prevent too much cooling of the plasma, leading to instability, which affects the signal, or even in the worst case, extinguishing the plasma. The rationale behind the choice of spray chamber is like that of nebulizers i.e. depending on which sample to be analyzed, the sensitivity range and matrix tolerance, as well as sample flow rate and which nebulizers that are compatible. The two main types of spray chambers that are used are the cyclonic- and Scott double pass type (Figure 3), with the former being used for more manageable matrixes and the latter for tougher ones as well as accommodating higher sample introduction rates [31].
Variations in injector tubes, torches and cones are less common with difference mainly in the material of the components to withstand aggressive measurement conditions such as high HF-concentrations, organic solvents, and/or element specific problems e.g. changing to zirconium injector tube from a borosilicate one to avoid contamination when measuring low concentrations of Si.

Three types of mass analyzers are commonly found in ICP-MS instrumentation: time of flight, sector field instruments and quadrupoles with the last one being the most common [32]. Time of flight instrumentation separates different m/z based on their flight time through a tube. Basically, a specific kinetic energy is applied to the sample ions sending them towards a reflectron, which bends the ions into a path leading to the detector. Depending on the m/z ratio of the ions they will have different velocities in the flight tube, with smaller m/z traveling faster compared to those with higher m/z leading to different flight times, which can be related back to the mass of the ion striking the detector. This enables fast, precise, pseudo-simultaneous data acquisition, which is excellent for multi element analysis and transient signals. Thus the time of flight analyzer is especially useful when coupling the ICP-MS to online separation systems e.g. high-performance liquid chromatograph or size exclusion chromatography. Another common mass analyzer system is high resolution ICP-MS (HR-ICP-MS) using sector field instrumentation either electronic, magnetic or a combination of both. For a magnetic analyzer the basic principle behind the ion separation is that the ions are sent into an electro magnet with a bent channel in between its poles. A magnetic field is then applied perpendicular to the ions flight path which will bend the ion trajectory depending on the ion mass, velocity, and mag-
netic field strength. This will let only ions of a certain mass and ion velocity through a slit to the detector. The electrostatic analyzer consists of two bent plates which have a potential applied between them. When an ion beam passes through the plates the ions will be bent out of their trajectories depending on their m/z and the voltage applied and is led through a slit to the detector. To reduce the problems associated with ions having different starting velocities, two sector analyzers are usually placed in succession, with the most common combination being a magnetic followed by an electrostatic. The HR-ICP-MS has the highest resolution of all the mass analyzers and can therefore resolve a lot of molecular interferences and isobaric overlaps which would present problems with overlaps for the other mass analyzers. The drawbacks are the slower scanning speed and loss of sensitivity when operating at the highest resolution, as well as being more expensive compared to the other analyzers and the large physical size of the mass analyzer resulting in larger instrumentation. The quadrupole is the most popular mass selector delivering fast analysis with fairly good resolution, with a capability to resolve m/z between 0.5-1 amu, while not being as expensive as the other mass analyzers available. The quadrupole consists of four parallel metal rods (Figure 4) divided into pairs with different direct current voltages applied to each pair with an RF-voltage superimposed, which will cause any ions entering the central axis between the rods to oscillate. The oscillation will be dependent on the m/z ratio of the ions and the voltages of the rods will be set to only let ions of a certain m/z pass through the quadrupole to the detector, while other ions crash into the rods or the quadrupole container.

Figure 4. Schematic overview of a quadrupole. “U” is the applied direct current-voltage and “V*\cos(\omega t)” is the applied radio frequency alternating current.
The most common detector type are electron multipliers either the channel electron multiplier or the discrete dynode detector [27]. The basic principle of both types is that when a positive ion hits the surface of the detector it generates, depending on the kinetic energy of the ion, a number of electrons. These will in turn strike further into the detector releasing more electrons leading to a cascade of electrons resulting in an amplified signal, which is then translated into a readable output by the ICP-MS software.

In this thesis a PerkinElmer Nexion 300D ICP-MS was used in all papers. It was equipped with a peristaltic pump, concentric type nebulizer and a baffled cyclonic spray chamber. The injector tube and torch used were of Perkin Elmer factory standard, made of quartz, and it used Ni sample and skimmer cones with an Al hyper skimmer cone. The instrument also was outfitted with the option to either employ a collision (He) or reaction (NH3) gas in a cell prior to the main quadrupole analysis cell. Two different nebulizers were used, either a Meinhard type c concentric nebulizer or a concentric per-fluoroalkoxy alkane (PFA) nebulizer. The Meinhard type nebulizer was used in paper I and II, but due to problems with the inner capillary breaking when subjected to HF and/or high salt containing solutions it was changed to the concentric PFA in paper III and IV. While the change of nebulizer did increase the relative standard deviation (RSD) of the signal intensity almost 2-fold the change was only from 1 % to 2 % and the PFA nebulizer offered better stability for high salt solutions due to the slightly higher bore width.
Analytical challenges of complex samples using inductively coupled plasma mass spectrometry

After the sample collection one of the most important steps of an analysis is the sample preparation and the original matrix of the sample and the desired matrix for analysis will influence the sample preparation procedure. While this consideration is not unique to the ICP-MS, it is of great importance since almost all elements are possible analytes and therefore problems could come from anywhere. The demand for lower detection limits in complex matrixes therefore puts more pressure on the analyst to avoid possible pitfalls that could be introduced by the sample preparation or the matrix.

Sample preparation

Some preparation before analysis is normally required for most samples, except perhaps in the case of measurement of dilute acidified aqueous solutions or when using laser ablation ICP-MS to analyze mineral samples. The sample preparation in ICP-MS is necessary to make sure that certain conditions are met to enable a good analysis. Firstly, it is to ensure that the analytes in the sample can be properly introduced into the plasma either as free ions in an aqueous solution, suspended in a liquid, or in a gas phase mixture. Secondly sample preparation is done to reduce or remove matrix effects of the sample and make sure that the sample will completely decompose in the plasma. The last reason for sample preparation is to make sure that the analyte concentration in the sample will be within the proper concentration range.

Several ways exist to prepare a sample with the goal of creating an aqueous solution of which the simplest of course is to dissolve the liquid or solid in acid and dilute it with ultra-pure water. While this seems like a fairly straightforward procedure care still has to be taken not to inadvertently precipitate analyte by using non-compatible acids or bases, contaminate the sample with analyte through non-pure solutions or from the laboratory equipment, environment and/or chemist. Unless rarer elements at fairly high
concentrations, above 100 ppb, are being analyzed there is high risk of contamination from lab environment and equipment especially glassware and obviously metal equipment such as scissors, tweezers and airborne metal particles from outside the fume hood, but sometimes even from within the fume hood itself. Aside from contaminating a sample with the analyte, issues can arise from other elements or molecular species, which can cause interferences in the analysis. This is the reason why the use of HNO$_3$ for dissolving sample is most prevalent in ICP-MS analysis methods, as other acids such as HCl, HF, and H$_2$SO$_4$ tend to introduce severe interferences for several elements that can be of interest to the analyst, this will be discussed more in detail in the “spectral interference” section. Sometimes though, some element requires the use of other acids to stabilize them and to prevent precipitation e.g. Ti which need small amounts of HF to be stable in a HNO$_3$ solution [33].

Some samples though, especially biological and inorganic environmental samples may need more than a simple acid addition to get the analyte into solution and therefore more extreme approaches are required. Typically, the methods employed are open vessel digestion, bomb digestion either heated in a conventional or a microwave oven [34], or in cases of extremely resistant samples: fusion.

Open vessel digestion is the most simple system to use, since it requires only a heating source and vessels for heating the sample and reagents in. Depending on the harshness conditions required by the method, the material of the vessels used can be as simple as a polypropylene falcon tubes, glass test tubes, to more expensive quarts vessels. The open design allows for real time observation of the digestion progress and for the modification of the method by addition of more and/or different reagents to dissolve possible particles left untouched by the originally proposed method. The downside of the open system is that there is a risk of losing sample due to vigorous reaction of sample with the reagents, which also presents a risk to the chemist. Volatilization of some elements such as Cd, Hg, and Pb is also problematic which can lead to erroneous result if not precautions are taken to keep them stable. Another issue is of course the risk of contamination from the environment since the digestion procedure usually takes at least an hour, but usually several and in worst case scenarios even days, depending on the complexity of the sample, and even though fume hoods are used the longer a sample is open the higher the risk for contamination. Sometimes manual addition of reagent to the digestion vessel is needed during the digestion, which demands additional man hours of supervision from the analyst and makes it harder to process larger sample sets.
Bomb digestion is similar in principle but instead of an open system it usually utilizes a polytetrafluoroethylene (PTFE), pyrex or quartz glass tube sealed with a PTFE cap set in a steel cylinder referred to as a “bomb”, which can withstand high pressure. Placed in an oven the bomb will conduct heat to the tightly sealed tube and thus also the sample and added digestion reagents. Due to the bomb being tightly closed and able to withstand high pressure, the temperature range available is not restricted by the boiling point of the acid, which allows for higher temperatures, and in turn, increases the efficiency of the reagents. Therefore, lower amounts of reagents can be used for a bomb digestion compared to an open system, as well as reducing the digestion time. The risk of contamination from the surroundings is considerably decreased compared to an open system, but also introduces a slight risk of contamination from the bombs themselves. Losses from volatilized analytes are also lower, but some may still be in gaseous form if the bomb is opened before being allowed to cool enough, which could cause losses. Bomb digestion is also more suited to handle a larger number of samples simultaneously as the analyst does not need to be present or observe the digestion process to the same extent as would be required with an open vessel digestion.

A more modern development of the standard bomb digestion is the microwave assisted where the standard oven is exchanged for a microwave oven and the bomb material from metal to different PTFE-polymers. The change to microwaves as heating source and bomb material allows for a more targeted heating, with the microwaves absorbing more into the reagent solution, rather than the sample container walls. This makes it more efficient than traditional oven heating and therefore allows for faster digestion times compared to a bomb in a conventional oven as well as open digestion systems. The change of bomb material from metal to polymer reduces the risk of metal contamination, which also makes the microwave digestion a more attractive alternative to traditional oven-based bomb digestion. The one downside of microwave induced digestion is that the polymer bombs are less tolerant to temperatures above 200°C [35] and high pressures compared to their metallic counterparts of the same volume. So, if larger amounts of sample are to be digested, which requires higher temperature and pressure, metallic bombs with an oven must be used instead. While there is a theoretical risk of the bombs rupturing due to generation of too much pressure, e.g. caused by overloading the bomb, most modern bomb designs come with safety measures such as rupture seals and/or intentional structural weak spots in the bomb. Such designs prevent unwanted sudden explosive pressure releases or at the very least minimize the danger by directing the release downwards. Most modern digestion ovens also include online monitoring of the pressure and/or temperature of the bombs during the digestion, which further decreases the risk of explosion.
In paper I, the sheer number of mink liver samples with the general expectation of high elemental concentration in an organic matrix that needed to be removed, made the use of bomb digestion in a conventional oven followed by dilution, the most suitable approach for sample preparation. In paper III, the use of microwave digestion was considered to be a more appropriate approach since the goal was to be able to quantify close to low ppm Ti levels in patient saliva absorbed on a paperpoint stick, causing the use of metallic bombs not to be advisable, due to the risk of contamination. In addition to digestion extraction procedures were also carried out using ultrasonic (US)-extraction as it was plausible that most of the Ti in the saliva on the stick would be absorbed onto the stick an therefore it should easily desorb in dilute acid with ultrasound applied. The ultrasonic extraction also benefits from less sample dilution compared to a normal digestion as well as a lower risk of high background levels since the sticks themselves were not completely consumed. Aside from the possibility of higher background levels, digestion of the stick also carries the risk of introducing errors if the sticks are not homogenous regarding their elemental content both within and between batches. While of course this could still give some error in the US-extraction as well, but the magnitude should be much lower compared to the complete digestion of the stick. In addition, leaching of the paperpoint sticks was also performed, as using only dilute acid solutions was an even simpler alternative to ultrasonic extraction and microwave assisted bomb digestion. In contrast though, for paper II where CSF samples were prepared, no such measures were needed due to the small sample volume that was available which had to be diluted to a large extent in order to meet the sample volume requirements of the analysis. This meant that in preparing the CSF samples simple dilution with dilute HNO₃ was the only option and made sure that the matrix would not become too much of an issue. In paper IV, there were no samples in the traditional sense as everything measured on was calibration standards and blanks. The solutions still had to be prepared with utmost precision though, in regard to their true composition, as to minimize errors that could confound the results and obscure trends in the data. To achieve this all additions to solutions were weighed to ensure the best possible accuracy.

Matrix effects

One of the main sources of trouble for most analysis methods is the matrix of the sample and for ICP-MS it is not any different [17]. With varied applications ranging from environmental [36], medical, geological [37], food and industrials samples the possible matrixes can be anything from purely organic or inorganic in origin, but can also consist of complex combinations from both sources. The problems associated with matrixes are varied and in ICP-MS ranges from sample introduction problems and plasma effects, which are
called physical interferences, to molecular species and isobaric overlaps, which are termed spectral interferences [17, 38].

Physical interferences

Matrix interferences in the introduction system arise from effects on the nebulizer spray formation to spray chamber transportation issues. Changes in primary aerosol formation can be due to several factors such as changes in sample density [39], surface tension, and viscosity which would change the rate of Coulomb fission [38], volatility, aerosol ionic redistribution [40], and/or deposition of material on the nebulizer which will affect droplets size distribution compared to that of a water and acid based standard solution. The spray chamber also suffers from similar problems as the nebulizer, but mainly introduces changes to the aerosol due to droplet collisions and the products thereof and evaporation [41]. The ionization capabilities of the plasma will also be affected both by temperature changes due to the energy required to decompose the sample and ionization effects from sample components [42-44] e.g. effects from different carbon species as seen in paper IV. Plasma characteristics can also be affected by deposition on the sample injection tube, which will affect the characteristic of the sample flow introduced into the plasma and deposition on the torch, which can disturb the flow of argon into the plasma. Effects have also been observed in the ion optics such as deposition on interface cones [44], enlargement of cone orifices due to corrosion, as well as space charge effects, especially when low mass elements are present in much lower abundance compared to high mass elements. In the mass analyzer matrix interferences are mainly seen in the form of overlaps from molecular ions and/or isobaric isotopes, so called spectral interferences, which will be discussed later.

Unless there is the possibility to remove the physical interferences by dilution [45] or digestion methods as mentioned earlier, other methods have to be used such as: matrix matched standards, internal standardization, isotope dilution [46, 47], aerosol dilution [18], and/or a change to more robust instrument operating conditions [48]. No approach is appropriate for all sample types and analytes and all matrix problems though. Matrix matched standards is the norm and that should take care of most problems except deposition of matrix material in the sample introduction system. It is also sometimes difficult to match the matrix [49], either because it is hard to characterize properly or otherwise tricky to replicate. Internal standardization is also a commonly used method to alleviate matrix effects in the introduction system, as well as drift and temporary effects to the signal. The main limitation of internal standardization is the selection of a proper internal standard, i.e. one that is not present in sample from the beginning, is expected to behave like the analytes that it should compensate for, and not
interfere with them. Isotope dilution is one of the most precise methods available to ICP-MS, but it requires that somewhat stable isotopes for the analytes are available and it also has the potential to be very expensive as well as there must be enough sample available. One of the most powerful methods to compensate for matrix effects is standard addition [49], but it requires substantially more sample, time and reagent compared to other conventional methods. It can also only compensate for multiplicatively effects such as signal enhancement or suppression and not additive effects e.g. contamination.

As noted earlier the use of different sample preparation methods can help in minimizing the matrix problems. For example, in paper I, both a lot of carbon, salts, and particles that could clog the nebulizer were removed from the samples by the digestion sample preparation step. As an extra precaution internal standardization was also used. In paper II, one could have expected matrix problems from salt deposition to enhancement effects from carbon as CSF is a biological sample, which contains both proteins and salts in abundance, but due to the large dilution of the samples it was not necessary to take any further actions. Internal standards were not used in paper II with the ICP-MS, due to concerns of sample contamination, the re-measurement of standards after a certain number of samples were done to make sure that matrix effects that could induce drift in signal over time was not present. While this would not compensate for direct effect of the sample matrix on e.g. spray formation, the dilution together with matrix matched standards was probably enough to eliminate most problems. If there would have been any suspicion of matrix effects, standard addition should have been used on a subset of the samples as control or at the very least spiking some of the samples to determine the recovery. As seen in paper III and IV, heavy matrix interference problems can be reduced to a degree by internal standardization. In paper III the internal standard was introduced not only to compensate for possible variation from sample and standard matrix, but also drift in the system due to the harsh nature of the HF containing matrix, which was necessary to keep the Ti⁺ ions stable in the sample solution. Paper IV was mainly focused on elucidating the suitability of ⁸Be, ⁸⁹Y, ⁶⁹Ga, ¹⁰³Rh, ¹¹⁵In, ¹⁹³Ir, and ²⁰⁵Tl as internal standards for ²⁴Mg, ²⁷Al, ⁴⁷Ti, ⁴⁹Ti, ⁵¹V, ⁵²Cr, ⁵³Cr, ⁵⁵Mn, ⁵⁷Fe, ⁵⁹Co, ⁶⁰Ni, ⁶¹Ni, ⁶²Ni, ⁶³Cu, ⁶⁵Cu, ⁶⁶Zn, ⁶⁷Zn, ⁷⁵As, ⁷⁸Se, ⁸²Se, ¹¹¹Cd, and ²⁰⁸Pb under conditions of high matrix concentrations of either HNO₃, PBS-buffer, or Triton X-100 in reaction-, collision- and standard-cell modes. This was done to investigate if and which of the internal standards chosen would compensate matrix effects in the different cell modes (used to compensate for spectral interferences, which will be discussed in a later section). The analytes chosen were based on elements where the use of DRC and/or KED could be beneficial, with exception of Pb which was chosen as a control element due to its large mass and medium ionization energy as well
as few known spectral interferences. The internal standards were selected to cover a range of different properties such as mass and ionization energy, and how frequently they are used in the literature. The matrixes HNO₃, PBS-buffer and Triton X-100 were selected to represent some of the commonly encountered types of matrixes such as acid, inorganic salts and organic content respectively. The concentration range of the matrixes was chosen to range from 1 % to 10 % (v/w) except for Triton X-100 which had a range from 0.1 % to 1.0 % (v/w) due to the increasing risk of plasma extinguishing at higher concentrations. All solutions contained at least 0.14 M HNO₃, with other matrix components added to give desired concentrations. This was done to investigate whether internal standardization can be used to compensate for matrix effects when dilution is not an option e.g. due to low concentration of analyte in the sample. The effects of the different matrix types interestingly enough showed that while each matrix has a general trend of how it affects different elements, it is hard to assess exactly how each of the elements will interact with a matrix. For example, most elements increased in sensitivity when the matrix concentration of HNO₃ increased in KED-mode while others like Zn, As and Se, which have ionization energies above 9.0 eV actually lost sensitivity (Figure 5). Looking at the sensitivity changes in the PBS-matrix the same pattern was not observed, as all elements lost sensitivity with increasing matrix concentration. While the most commonly cited selection criterion has been mass similarity between analyte and internal standard, Figure 5 and 6 clearly shows that what is the most important criterion can sometime depend on the matrix. In figure 5 the elements with ionization energies above 9.0 eV clearly behave differently compared to those with lower ionization energy, while in figure 6 all elements behave in a similar fashion regardless of their ionization energy.
Figure 5. The relative sensitivity for a selection of analytes (Mg, Co, Pb, Zn, As, Se) and internal standards (Ga, Rh, Tl) for each concentration of HNO$_3$-matrix in KED-mode.

Figure 6. The relative sensitivity for a selection of analytes (Mg, Co, Pb, Zn, As, Se) and internal standards (Ga, Rh, Tl) for each concentration of PBS-matrix in KED-mode.
The choice of matrixes in paper IV was similar to that of Vanhaecke et al. [23] who used either H$_2$SO$_4$, HCl, H$_3$PO$_4$, and CH$_3$COOH as acid matrix; glycine or CH$_3$COOH as a carbon matrix; or either K, Rh or Lu solutions containing solids. While there were more matrixes investigated in comparison with paper IV, they were only tested at one concentration either 0.5 M for the acids or 0.5 g/l for carbon and suspended solids. This makes it impossible to know if the effects were linearly dependent on the matrix concentration or not, though the paper claims that enhancement or suppression effects seemed dependent on daily factors with day to day variation in the sign of the matrix effects. Their findings were not in agreement with some of the conclusions drawn in paper IV, especially in relation to the importance of the ionization energy of the elements for different matrixes. This discrepancy could be explained by a) that the instrumentation Vanhaecke et al. used was much older and b) that only one element with an ionization energy higher than 9 eV was measured, which appears to be the threshold at which the effect of the ionization energy becomes important, as seen in paper IV. Option b) seems more likely as Thompson and Houk [50] came to a similar conclusion as in paper IV i.e. that ionization energy is important for the choice of internal standards, while using instrumentation of the same era as Vanhaecke, albeit a different instrument model. The work of Finley-Jones et al. [51], which is a more recent approach, and should therefore be more comparable to paper IV, investigated the effects of NaCl and CH$_3$COOH as well as difference in pump speed and sampling depth on the choice of internal standard. They followed a similar protocol by using 1 % HNO$_3$ matrix standards as references to evaluate the effects of the different matrixes. The results are not in total agreement with paper IV, as they were not able to find the trend that mostly elements with ionization energies over 8.9 eV are affected more by ionization effects of the matrix compared to other elements. But nonetheless they noted problems with finding internal standards for elements with high ionization energies, such as Be for some matrixes. In a later paper Vanhaecke et al. [52] noted similar problems as seen in paper IV with elements with high ionization energies, but were hesitant to attribute it to the ionization energy, most likely due to not all of the elements being affected similarly. The different results regarding the importance of ionization energy could also be due to specific carbon species affecting hard to ionize elements differently as noted by Grindley et al. [53].

Spectral interferences

One main limitation of ICP-MS is the presence of spectral interferences such as overlap from element isotopes that have similar m/z ratio to that of the analyte. In addition to that there are interferences from polyatomic species [36] that are not decomposed by the plasma or formed in the plasma or directly afterwards in the transfer through the ion optics. There is a plethora of
studies detailing the different interferences and their possible mechanisms of creation. While some problems can be overcome by choosing different analyte isotopes, some elements have overlaps for all isotopes or are monoisotopic as in the case of $^{76}$As, which is interfered by $^{39}$Ar$^{37}$Cl. Sadly the spectral overlaps are more frequent in the lower mass range, which also contains most of the elements that are relevant to life sciences, such as e.g. Na, K, P, S, Mg, and Fe, requiring creative solutions to get around such problems. Some of these issues could be overcome by the complementary use of HR-ICP-MS, ICP-AES and/or even other techniques such as FAAS and/or GFAAS. However, that requires that there is enough time, resources, sample available, and that the concentrations are high enough and of course that the analyst has the extra instrumentation at hand, which in turn, as mentioned earlier, can suffer from their own limitations.

A simple method to overcome isobaric overlap from other elements is to use mathematical corrections [36]. The central idea is that you measure another isotope of the interfering element and then uses the theoretical or empirically derived isotopic abundance to calculate how much the interfering isotope will add to the analyte signal and subtract the contribution. While seemingly practical because it does not require extra sample preparation, extra measurement time might be required if all the other isotopes of the interfering element also suffer from interferences from other elements. In a worst-case scenario there might even be a need to add several extra isotopes of interfering elements into the analysis method to resolve only one of the original interferences, which will increase the analysis time as well as increasing the uncertainty of the final result. Mathematical correction is also not really suited to handle interferences from polyatomic species as their formation can be very dependent on the matrix composition of the measured sample and the instrumental settings.

Another ingenious way of solving overlaps from polyatomic species is to add gas [54] to the ion beam in an octapole, hexapole, or quadrupole cell [55], as used in the Nexion 300D, before the mass analyzer. Two different main ways of using gas exists: kinetic energy discrimination mode (KED) or ion-molecule reactions, for simplicity in this thesis referred to as dynamic reaction cell mode (DRC). With KED an inert collision gas, e.g. He, is flooded into the gas-cell with the ion beam leading to collisions with polyatomic interferences as well as analytes resulting in a reduction of the kinetic energy of all ions present. To exit the gas-cell an ion must have kinetic energy above a certain threshold, set by the instrument. Since polyatomic ions usually have larger cross-sectional areas compared to atomic ions with the same m/z, they tend to lose more kinetic energy in comparison and therefore being unable to leave the gas-cell. This can reduce or completely remove some of the interferences depending on the cross-section area difference.
compared to the analyte and the concentration of the interference. A simple example of this is the removal of CaO⁺ interferences on all Ti isotopes, using He as collision gas.

DRC uses a reactive gas e.g. NH₃ to fill the cell, with the hope of either neutralize interferences by charge transfers or forming new molecular ions by ion/atom transfer to shift the m/z ratio of the interferences away from the m/z ratio of the analytes. To be used efficiently the gas should be chosen to react readily with the interferents that should be removed and not with the analyte or at least with a much slower reaction rate compared to that of the interferents. A classic example of this is the removal of ⁴⁰Ar²⁰O⁺, which interferes with the most abundant Fe-isotope, ⁵⁶Fe, and makes analysis of low concentrations very tricky. By addition of NH₃ the ArO⁺ will lose its charge while the Fe⁺ ions will remain largely unaffected by the reactive gas. If the interferants don’t react efficiently enough or if they are present in such large quantities compared to the analyte that substantial loss of analyte signal will happen in addition to interference reduction, measuring on reaction products of the analyte and reaction gas might be a viable strategy. Sometimes relatively stable polyatomic adducts will form with the analyte and reaction gas which possibly shifts the m/z ratio away from that of the common interferences and hopefully does not introduce any new or at the very least less interferences [56]. An example of this is trying to analyze Ti in the presence of a large abundance of Ca, again creating overlap in the form of CaO⁺. Due to larger amount of Ca compared to Ti, KED will not be able to remove the interference, without reducing the Ti⁺ signal below the limit of detection (LOD), and the reaction rate with NH₃ is higher for Ti compared to Ca, so traditional DRC is not viable either. Instead one can monitor the complex TiNH(NH₃)₄⁺ at m/z 131.067, which is free from other interfering species [22].

In paper I, the risk of spectral interferences on ¹⁰⁷Ag, ¹¹¹Cd, ²⁰²Hg, and ²⁰⁸Pb, was not that high because of the lack of severe interferences due to the high m/z ratio of the analytes. While there could probably have been some ZrO, which interferes with Ag, and MoO, which interferes with Cd, the likelihood that either Zr or Mo would be present in such abundance to make a major impact on the outcome of the analysis was doubtful since the oxide formation ratio was around 3 %. In paper II, ⁴⁷Ti, ⁵¹V, ⁵⁵Mn, ⁶¹Ni, ⁶⁶Zn, ⁷⁵As, ⁸⁵Rb, ⁸⁸Sr, ¹⁰⁷Ag, ¹¹⁸Sn, ¹³⁸Ba, and ²⁰⁸Pb in CSF were analyzed with the ICP-MS, which offered a more challenging task with regards to spectral interferences. In addition to the ICP-MS, an ICP-AES was used to measure Ca, Cu, Fe, Mg, P, S, Si, Sr, Zn, K, and Na. Some of the spectral interferences encountered in the ICP-MS could have been solved with the use of collision gas for Ti, V, and Zn while other such as Ni and As would have required the use of reaction gas. But when looking at the results, the V and Zn signal...
intensity were below the LOD. While the Ti concentrations were lower in the CSF control samples compared to the patient samples, the relative difference of Ti was much lower compared to that of the Ca levels, which suggests that the difference was not a result of interference from CaO. For Ni the signal was below LOD and as discussed in paper II, the As signal was most likely an interference from ArCl. While this could have been removed, the As concentration would probably have been too low to detect due to the loss of signal with cell usage, and as such the ArCl served better as a measure of Cl concentration. The reduction in signal intensity was also the main reason why cell-technology was not employed in paper II, due to the high dilution factor of the samples. In paper III the risk of interference from CaO [21] was high, therefore using He as collision gas in KED-mode was first investigated to compare the different sample preparation strategies. But due to the paper-point sticks containing fairly high and varying amounts of Ca, analyzing $^{48}$Ti was not advisable even when using KED-mode as there is a risk of not removing all polyatomic interferences. This also meant that measuring in standard-mode was also not an option. And while $^{49}$Ti was not interfered by the Ca levels, it is a rather low abundance isotope. This makes the loss of Ti signal due to the He gas more pronounced, which contributes to the LOD of 240 ppb in undiluted samples. Therefore, to improve the LOD an approach using NH$_3$ as a reaction gas to create a TiNH(NH$_3$)$_4$-complex at m/z 131.067 was investigated. The formation of the Ti-complex should be free or at least suffer from less isotopic or polyatomic overlaps compared to non-complexed Ti due to less natural occurring isotopes with an m/z around 131. The LOD for the DRC-mode was lower (100 ppb) and also the sensitivity was higher which was an effect of reduced loss of signal from gas collisions, as well as no kinetic energy threshold, which is used in KED-mode. While the gain in LOD and sensitivity from using DRC-mode to create a Ti-complex makes it an attractive choice, one of its disadvantages is that it requires more optimization procedures compared to KED-mode. While optimization of the KED mode mainly consist of adjusting the gas flow to maximize interference removal while keeping signal loss low, DRC-mode and especially using DRC-mode forming complexes requires more fine tuning of parameters. The quadrupole ion deflector (QID) optimization has to be done specifically for the complexes and analytes to be measured as the software algorithm cannot extrapolate between masses, as it would for a standard use of the ICP-MS. For example the optimal settings for the complex at 131.067 will be the same as that of the Ti-isotopes and therefore using standard QID optimization would adversely affect the Ti-complex signal. And if the QID optimization would only be performed on the Ti-complex then other potential non-complexed isotopes of interest, such as internal standards, would in a similar way be adversely affected by erroneous instrument extrapolation of the QID. The reactant gas flow and axial field voltage has to be optimized with each parameter in mind as it will affect the scattering losses, the com-
plex order formation [57] as well as signal to background ratios. If the parameters were not set correctly the Ti-complex calibration curves could exhibit non-linear responses at concentration levels above 5 ppb, sometimes increasing sensitivity with concentration and sometimes the reverse. The non-linearity also seemed to be worsened the longer the instrument was running leading to nonlinear response for all standard solution concentrations if re-measured after sample measurement. This problem was not observed when the gas flow and axial field flow were optimized with each other in mind. Another issue with the using DRC-mode with settings that favored complex formation was the issue of finding an adequate internal standard. Internal standards candidates such as Ga, Y, Rh, and In were either reacting with the gas themselves or where interfered by complexes. Due to Tl being relatively unaffected by the NH$_3$-gas as well as having no overlaps it could be used as an internal standard even though it would only compensate for sample introduction effects. There could possibly be some elements that would behave similarly enough to Ti so that they could be used as internal standards and compensate for effects that change the complex formation. But due to the low band pass setting needed for efficient complex formation the risk of overlap from other complexes formed by matrix components would be even higher than that of normal DRC-mode usage. In paper IV, the main goal was to investigate the behavior of $^9$Be, $^{89}$Y, $^{69}$Ga, $^{103}$Rh, $^{115}$In, $^{193}$Ir, and $^{205}$Tl for potential use as internal standards in solutions with high concentrations of either: HNO$_3$, PBS-buffer, or Triton X-100 as the matrix, when used in conjunction with reaction or collision gas. While the main goal was more to investigate the effects of the conditions that are used for spectral interferences removal, it still highlighted some of the problems encountered when trying to remove interferences. Examples of that are the removal of the Ti, Y, and Ir signals in DRC-mode as well as the problematic creation of new interferences as seen in the increase in V signal in DRC-mode with increasing PBS-concentration. V was the only element, which behaved in this manner and the corresponding matrix blanks did not show this trend in signal increase, nor did it appear in any other cell-mode. This only leaves the option that it was an interference created together with another analyte under the present conditions, i.e. DRC-mode and PBS-matrix. While probably not a common phenomenon, as it was only encountered for V with a specific matrix in DRC-mode, it highlights a problem that cannot be solved by using normal matrix blanks and has the potential to completely be missed by an unwary analyst. Therefore, it might be wise to make additional single analyte standards if an analyte concentration seems unexpectedly high or low (which could happen if the standard series contains elements that the sample doesn’t).
Biological samples

In this thesis there has been a focus on biological samples ranging from semi-solid tissue samples in paper I to bodily fluids such as CSF in paper II and saliva in paper III. The common properties of biological samples are a tendency to contain organic material such as proteins, peptides and lipids. This can result in problems from C but also from S and P, which can be prevalent in biomolecules [58]. But S and P can also be found as different inorganic species in biological samples as well as together with other high abundance element such as Na, Cl, Ca, and K. While these elements sometimes are of interest to determine they are close to each other in mass as well as Ar, which makes their determination especially tricky. Therefore, using the ICP-MS together with other analytical instruments is usually preferred when analyzing biological samples, with the ICP-MS focusing on higher mass elements in low abundance. These problems have not stopped scientist from trying to analyze biological samples with the ICP-MS though, and some of the common sample types include: blood, plasma, serum, urine, saliva, and CSF, as they are relatively non-invasive to sample, and already in liquid form.

CSF has been popular to investigate because it is known to reflect processes in the brain and spinal cord tissues, which would be hard to sample without risk of causing harm to the subject. Several diseases such as Parkinson's disease [11, 59], amyotrophic lateral sclerosis [10, 60], dementia [12, 13, 61], Skogholt's disease [62], and cerebral vasospasm [63] have connections with altered element concentrations in the CSF of patients. Therefore, CSF was also deemed to be a good sample for analyzing the effects of spinal cord stimulation (SCS) in patients with chronic neuropathic pain, as presented in paper II. As there already were known interactions between chronic pain and metals including Fe [64], Zn [65-67] and Mg [68-71], it was warranted to investigate whether trace elements were involved in the pain relief from SCS treatment. Originally paper II followed a similar protocol compared to Nischwitz et al. [72], in which proteins are separated from small biomolecules using ultracentrifugation spin filters. The point of this approach was to get a more nuanced picture of the distribution of trace elements in the CSF i.e. if there was both an effect from the treatment on the total elemental concentration and/or on the distribution of free element versus bound to larger biomolecules. Except the centrifugation little other sample preparation had to be done due to the large sample dilution. In most other papers, large dilutions are not usually performed, rather other ways such as direct injection into the ICP-MS [73] or different chromatographic techniques coupled to the ICP-MS are used [74]. More destructive sample preparation methods are described in literature though, but mostly for method validation [73]. The choice of elements in paper II was made on basis of a pseudo-quantitative analysis with the ICP-MS of the control pool as well as measurements per-
formed with the ICP-AES. This narrowed the choice down to 22 possible elements: Ti, V, Mn, Ni, Zn, As, Rb, Sr, Ag, Sn, Ba, and Pb measured with ICP-MS and Ca, Cu, Fe, Mg, P, S, Si, Sr, Zn, K, and Na measured on the ICP-AES. The elements chosen to be analyzed and on which instrument was dependent on the expected element concentrations and possible interferences. Repeatability problems with the spin filters unfortunately meant that no comparison between distribution changes in free and biomolecule bound elements could be made, and focus had to be shifted to total concentration changes. While there was no significant difference for patient with and without treatment found with a t-test, a significant difference between patients and control were found (Table 1) for Ca, Sr, Na, K, P, Mg and Ti, which were present in higher concentrations in patient samples. This could have been attributed to environmental factors as has been reported by other authors as a source of potential error. But as noted in paper II this is probably not the case for the patients in paper II as most elements that were significantly different between patient and control are elements obtained through the food, and the Swedish nutritional intake is not different [75] in the regions from where the patients and control pool originates.

Table 1. Obtained p-values from the two tailed t-test for comparison of the CSF-control with “On” and “Off” samples.

<table>
<thead>
<tr>
<th>Element</th>
<th>Patient &quot;On&quot;</th>
<th>Patient &quot;Off&quot;</th>
<th>Difference compared to CSF-control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sr</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>Higher</td>
</tr>
<tr>
<td>As</td>
<td>0.163</td>
<td>0.167</td>
<td>None</td>
</tr>
<tr>
<td>Ba</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>Lower</td>
</tr>
<tr>
<td>Ti</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>Higher</td>
</tr>
<tr>
<td>Rb</td>
<td>0.645</td>
<td>0.774</td>
<td>None</td>
</tr>
<tr>
<td>Ca</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>Higher</td>
</tr>
<tr>
<td>Mg</td>
<td>0.049</td>
<td>0.047</td>
<td>Higher</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>Higher</td>
</tr>
<tr>
<td>K</td>
<td>0.010</td>
<td>0.025</td>
<td>Higher</td>
</tr>
<tr>
<td>Na</td>
<td>0.012</td>
<td>0.019</td>
<td>Higher</td>
</tr>
</tbody>
</table>

*a Measured with ICP-MS  
*b measured with ICP-AES
An even easier and less invasive biological sample to use, compared to CSF, is saliva and one might think that the measurement of trace elements in saliva would mostly be of interest to the dental related problems such as in paper III. There are however other areas where the trace elements in saliva are of interest such as, but not limited to, effects from smoking [76], stress [77], and oral carcinogenesis [78]. Most saliva sampling is either done by sampling whole saliva by letting the subject spit or drool into test tubes over a period of time, but other options are available as well e.g. suction, using a rinse solution, or an absorbent [79], such as the paperpoint stick used in paper III. Sampling of saliva from specific glands located at different positions in the oral cavity can also be performed, but as it is more intrusive [79] and more complex, it is a less attractive choice for most applications. In contrast to spit sampling, the paperpoint sticks in paper III yielded considerably lower sample volumes, microliters compared to milliliters, which could be considered a drawback. However, due to the paperpoint sticks being used to sample close to the Ti-dental implant Ti-concentration in the saliva should be higher compared to saliva from the whole mouth, offsetting the lesser volume sampled. The lower volume also means that the dilution of sample is higher compared to e.g. spit sampling, making the chemical purity as well as external contamination during sample preparation the main limitations, when using the method described in paper III. The low absorption volume of the paperpoint sticks does make it unlikely that they could be efficient for saliva sampling in general, due to saliva generally not containing trace elements at higher concentration levels. For more general sampling of saliva when investigating trace elements, larger absorption volume alternatives such as e.g. cotton swabs inserted into the mouth could be used, unless the elements of interest are expected to be present in high abundance. When it comes to sample preparation the collected saliva is often centrifuged to remove solid particles and blood contamination and the supernatant is then subjected to digestion before analysis. Most of the papers which digested the saliva used FAAS for analysis, while simple dilution with acid seemed to be best when using ICP-MS as described by Kim et al. [76], which was also one of the conclusions of paper III. The centrifugation and digestion steps were aimed at removing solids as well as contamination from blood, which should not be as much of an issue when absorbing saliva into paperpoint sticks. The high variability of Ti in the blanks when microwave digestion was used also highlighted another limitation when using paperpoint sticks, i.e. the natural trace element concentration in the sticks. This issue could be alleviated by using more sticks for each sample thereby lowering the impact of the variability. Another strategy might be to investigate alternative absorbing materials such as plastic polymers, which potentially could contain lower amounts of trace elements and/or be more resistant to acids allowing for leaching out elements from the sampling stick before use. While paperpoint stick sampling is limited to sample trace elements at rather high concentrations it might still be
useful for, in addition to the use described in paper III, biomonitoring human exposure from environmental sources of rarer trace elements not naturally present in the sticks.

While traditional environmental samples are mostly inorganic in nature such as water, air, soil or other geological samples, there is the option to use biological samples to monitor different kinds of environmental pollutions. Different kinds of plants, fungi and/or animals can be used as model systems for pollutant presence, concentration and give insight into possible threats that a pollutant poses. Species used as model systems are termed sentinel species and to be a good sentinel species, they must fulfill certain criteria. Examples of such criteria are: to be present in fairly large numbers, be high up in the food chain, be able to bioaccumulate pollutants and be sensitive to them, and finally be restricted to a certain area. Animals proposed as candidates as sentinel species include, but are not limited to, several species of birds [80], dolphins [81], mussels [82], oysters [82], crabs [83], toads [84] and minks [85], with the last one being used in paper I. One of the benefits of using sentinel species is the need for fewer samples to characterize the pollutants in a certain area, since the animals will roam the area and accumulate pollutant if present. Another benefit is that there is the possibility of the animals concentrating pollutants in their organs, increasing the chances that the pollutants will be detected if they are present in the environment in amount close to the detection limit of the analytical method. Sentinel species also functions as a warning system of which pollutants and at what levels they might be harmful to humans, i.e. if a sentinel species is showing abnormal physical characteristics that can be related to a pollutant the risk that it could be harmful to humans is also high.

Mink was chosen as a sentinel species in paper I, as it is legally hunted all year due to it being an invasive species, which means that seasonal samples were available. Aside from availability, the mink also feeds on several different animals, mainly fish and crayfish, but also mammals, birds, and frogs. This places the mink high up in the food chain and therefore making it good at bioaccumulating elemental pollutants in the environment. The territories of the mink tend to be small which also is beneficial since it will give a narrower geographical area to ascribe pollutants to. The effects of certain pollutants on the biology of the mink have also previously been studied and links between organ properties and pollutants have been established [86]. Hg, Cd and Pb were chosen as they are known environmental pollutants that tend to accumulate and as such, they were of interest to monitor. Traditionally Ag has not been considered a major pollutant and has as such not been widely studied in the environment. Therefore, the distribution and effects of Ag in the environment are still largely unknown. Though in recent years Ag has been proposed as possible threat to the environment in the form of nano-Ag-
particles, which are included in a lot of consumer goods from which they are transported into the environment. As such investigating the concentration of Ag in wildlife was warranted. Most studies on sentinel species measuring elemental concentration with ICP-MS use destructive sample preparation techniques e.g. digestion [83, 84], in order to gain the complete picture of the organisms exposure to elements of interest. The main objectives of paper I was to analyze the Ag, Cd, Hg and Pb concentration in the livers of wild mink to investigate the element distribution in the environment as well as what possible effects the elements could have on the organ morphology of wild minks. Not completely unexpected the elemental concentration in mink liver could be related to where the mink was captured. The Pb concentrations were lower in areas of lower human population density, which could be expected, as there would be less contribution of Pb from pollution. Hg on the other hand did not show this behavior, which contradicts studies made on pikes in Sweden which showed a clear positive correlation between Hg levels and human population [87]. This contradiction could be due to different mechanism of accumulation between mink and pike, but does point to the possible need for several sentinel species to get a representative picture of elemental spread in nature. The connection between Pb and Cd concentration levels and the season of capture was unexpected and lacked any logical explanation due to the metals rather long biological half-lives. More expected were the liver concentration levels of Pb, Cd, and Hg concerning correlation with age as the concentration increased with increasing age. For Ag this was not the case, most likely due to excretion of element being the same or close to the intake. For some of the organs the relative weight was related to the concentration of some of the metals in the mink liver. For the liver, the relative weight correlated positively with the Hg concentration and negatively with the concentrations of Pb and Cd. The relative kidney weight though showed only a negative correlation with the Pb concentration in liver.
Conclusion and future aspects

In this thesis four papers have been presented on utilizing ICP-MS to analyze trace elements in complex samples. They highlight the problems of analyzing low concentrations of elements in harsh matrices, but also the viability of using the ICP-MS to accomplish this task. In paper I the goal was to analyze Ag, Cd, Hg, and Pb concentrations in wild mink liver to ascertain the spread of the elements as well as possible morphological changes induced by them. The concentrations of Ag, Hg, and Pb varied between the sample collection areas. The concentrations of Pb and Cd varied with season of capture and the Pb, Cd, and Hg concentrations correlated positively with increasing age. The relative weight of some organs was associated with the concentration of some elements in the liver. The relative liver weight correlated positively with Hg and negatively with Pb and Cd. Relative kidney weight on the other hand only correlated negatively with the Pb concentration in the liver. For some of the metals, concentrations also varied between age and season, which showed that this should be taken into account when assessing levels of heavy metals in wild mink. While the Ag concentration was fairly low and did not vary with age or season there was a difference in sample location and as such there could still be worth investigating further. For example, it could be of interest to elucidate not only the concentration of Ag but any possible difference in the Ag species dependent on age, area or season.

In paper II the effects of spinal cord stimulation on element concentration in CSF from chronic pain patients was studied. No correlation of treatment on the element concentrations were found, but significant differences were found comparing the control pool and patients for Sr, Ba, Ti, Mg, P, Ca, Na, and K, regardless of patient treatment. This indicate that some of these elements could be involved in the condition of chronic pain, but in order to deduce that, the element concentration in CSF of patients with chronic pain that have not undergone spinal cord stimulation treatment should be analyzed. The elements analyzed could also be expanded if larger volumes of sample were made available enabling less dilution of the sample and then reaction and collision cells could be used. Any matrix effects introduced by the higher matrix concentration could be alleviated by the use of internal standards. Also, the question of the distribution of biomolecule bound ele-
ments and free ions could still be further investigated by using spin filters or other separation techniques.

Paper III focused on investigating the use of paperpoint stick sampling to assess Ti leakage from Ti-dental implants in lieu of using biopsies as well as whether measuring using KED-mode or DRC-mode to create Ti-complexes would be more sensitive. When it came to the extraction of the paperpoint sticks, leaching proved to be superior to both ultrasonic extraction and microwave bomb digestion in terms of reliability, time consumption and simplicity. The reason why leaching was better was due to that the paperpoint sticks contained varying amounts of Ti, which was released with the harsher sample preparation methods leading to higher and more varied blanks. The complexing of Ti with NH\textsubscript{3} resulted in a lower limit of detection as well as higher sensitivity compared to that of measuring on either \textsuperscript{48}Ti or \textsuperscript{49}Ti in KED-mode. The main drawbacks of using the DRC-mode were the more complex optimization procedures as well as the problem of finding an optimal internal standard. To further improve the method pooling of several paperpoint stick could be utilized as well as minimizing background Ti-levels from solutions and equipment.

As noted in paper IV, both internal standardization and the use of reaction and collision cells have been studied separately, their effects on each other have not been investigated earlier which was the point of this study. To test the internal standards, high concentrations of either HNO\textsubscript{3}, PBS-buffer, or Triton X-100 were employed as model matrixes to simulate harsh conditions. The results showed that all internal standards, except Be, provided a relative sensitivity with an RSD below 10 % regardless of matrix and cell mode used, for most elements. Zn, As and Se could not be properly compensated for by any internal standard element most likely due to their higher ionization energy compared the other elements. This questions the assumption that similarity in mass is the most important parameter when choosing an internal standard, which has been the norm. Investigating other matrixes to give a more thorough overview and elucidating more clearly the effects of ionization suppression and enhancement by matrixes could further verify and expand the general applicability of the results in paper IV. It could also be of interest to compare different instrument models from different companies to determine if the generalizations of the results in paper IV are possible.

The general direction for ICP-MS in the future will most likely mirror that of the past, i.e. being governed by the technical advances made in the instrumentation. For example, the fairly recent development of triple quadrupole instruments with reaction cells, introduced by different manufacturers [88, 89] have made it possible to use new types of reaction strategies analogous to what previously only been utilized in organic MS analyzers. Steps have
also been taken to increase the matrix tolerance of instruments e.g. by introduction of different ways to dilute the sample mist online with argon as used by some companies [18] or even completely change the RF-coil and generator design [90]. New analyzers coupled to the ICP might also push the boundaries of what was once possible e.g. such as the distance of flight mass spectrometer [91] or optical detectors to get some of the combined power of an ICP-AES [92] with ICP-MS extending the elements that can be analyzed as well as the dynamic range of the instrument. So as long as engineers of the instrument manufacturers will continue to push the instrumental capabilities of the ICP-MS, scientists will develop new methods in order to push the boundaries even further, and to publish papers.
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I denna avhandling presenteras fyra artiklar som belyser olika metoder för hur ICP-MS kan användas för att förbättra analysen av spårelement i komplexa biologiska prover. I artikel I var målet att bestämma koncentrationen av silver, kadmium, kvicksilver och bly i vilda minkars levrar för att utröna om de bidrog till möjliga fysiologiska förändringar hos minkarna. Det var även av intresse att undersöka om det går att koppla metallkoncentrationerna till spridningen av elementen i minkarnas omgivning. Koncentrationerna av silver, kvicksilver, och bly i minklevrarna varierade mellan de olika minkjaktområdena. Koncentrationerna av bly och kadmium varierade med fångstsäsong samt även med ålder. Den relativa organvikten hos minkarna visade sig variera beroende på koncentrationen av metallerna i levern. Fastän silverkoncentrationen var generellt ganska låg så fanns det variationer mellan minkar från olika platser och då silver är en populär tillsats i fler konsumtionsvaror kan det fortfarande vara värt att undersöka silverhaltarna ytterligare i framtida undersökningar. Speciellt kan det vara av intresse att undersöka i vilken form silvret föreligger och inte bara koncentrationen då det möjligtvis skulle kunna visa på fler skillnader än bara provtagningsplats.

I artikel II studerades effekterna av elektrisk ryggmärgsstimulering på elementskoncentration i ryggmärgsvätska från patienter med kronisk smärta. Detta gjordes för att se om det fanns någon koppling mellan elementkoncentrationen och den smärtlindring som ges av ryggmärgsstimulansen. Tyvärr kunde inget samband mellan behandling och elementkoncentrationerna påvisas. Däremot fanns det skillnader för ett flertal element mellan patienter som led av kronisk smärta och en kontrollgrupp som inte hade kronisk smärta. Så trots att spårelementen som undersöktes inte kunde kopplas till smärtlindring
så indikerade resultaten på att dessa element kan vara involverade i sjukdomstillståndet kronisk smärta. För att fastställa detta samband borde elementkoncentrationen i ryggmärgsvätska hos patienter med kronisk smärta som inte har genomgått ryggmärgsstimeringsbehandling analyseras.


Artikel IV undersökte hur olika element fungerar som interna standarder (element som tillsätts för att kompensera för instrumentella förändringar under analysens gång) då man använder sig av reaktions- och kollisionsgaser (såsom det gjordes i artikel III). Flera artiklar hade tidigare undersökt vilka element som skulle vara lämpliga som interna standarder, men inga artiklar hade systematiskt undersökt hur valet av interna standarder påverkas när antingen reaktions- eller kollisionsgas används. Tillsatsen av kollisionsgas visade sig inte nämnvärt inverka på valet av intern standard. Introduktionen av reaktionsgas kräver dock mera eftertanke då internstandardelement kan reagera olika jämfört med de element man är intresserade av, samt att det kan bildas jonkomplex som stör mätningen av internstandarden eller analyten. Intressant nog så noterades också att likheten i massa mellan element inte var av alltför stor betydelse för hur bra de skulle fungera som interna standarder. Den mest avgörande egenskapen visade sig vara om elementet var svårt att jonisera eller inte, såsom arsenik, selen och zink. Detta gick stick i stäv med flera tidigare vetenskapliga artiklar, men liknande resultats har publicerats av andra i nyare artiklar. Möjligtvis så är denna diskrepans främst en effekt av att modernare instrumentering, såsom den som använts i artikel IV, mera effektivt kan kompensera för effekterna av olika elementmassor så att joniseringsskillnader blir mera framträdande.
References


A doctoral dissertation from the Faculty of Science and Technology, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology. (Prior to January, 2005, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology”.)