Inductively Coupled Plasma Spectrometry for Speciation Analysis

Development and Applications

NIKLAS FORSGARD
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Abstract

In analytical chemistry the main goal is normally to determine the identity and/or concentration of one or more species in a sample. The samples analyzed are often natural samples, containing numerous different species in a complex matrix and the choice of technique for multi-elemental detection is in general inductively coupled plasma spectrometry. The chemical forms of an element can affect many of its characteristics e.g. toxicity, which makes speciation analysis important. Therefore, determination of the identity and quantity of an element is still important, but for many applications measurements of total element concentration provides insufficient information. To be able to perform speciation analysis, separation, identification and/or characterization of the various forms of elements in the sample has to be accomplished. Speciation analysis has been employed in a wide range of disciplines, including for example environmental science, biology and clinical chemistry.

This thesis describes work to improve and understand the elemental speciation analysis with liquid chromatography coupled to plasma spectrometry and also highlights the importance and potential of the synergy between atomic spectrometry and molecular mass spectrometry. The combination of the matrix tolerant, robust and very sensitive plasma spectrometry used together with molecular mass spectrometry, which provides structural information and the possibility to identify unknown species, is demonstrated to be a very powerful tool for speciation analysis. In this thesis methods are developed for on-line sample clean-up and pre-concentration coupled to liquid chromatography and plasma spectrometry, which makes handling of small sample volumes easier and also decreases the risk of contamination. The problems associated with organic modifiers in plasma spectrometry are also addressed. Applications of speciation analysis are exemplified by analysis of aluminium-chelated siderophores in field-soil solutions and organic phosphorus species in aquatic sediments. The possibility to analyze un-dissolved samples as slurries with minimal sample preparation is also discussed.

Keywords: Speciation analysis, Atomic emission spectrometry (AES), Mass spectrometry (MS), Inductively coupled plasma (ICP), Liquid Chromatography (LC), on-line sample pre-treatment, Organic phosphour, Siderophores, Slurry nebulization, matrix effects

Niklas Forsgard, Department of Chemistry, Analytical Chemistry, Box 599, Uppsala University, SE-75124 Uppsala, Sweden

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


IV  Screening for organic phosphorus compounds in aquatic sediments by liquid chromatography coupled to ICP-AES and ESI-MS/MS. Heidi De Brabandere, Niklas Forsgard, Per Sjöberg, Emil Rydin, Jean Pettersson, Monica Waldebäck. *Manuscript*

V  Analysis of non-dissolved organic materials by ICP-AES and ICP-TOF-MS. Barbro Kollander, Niklas Forsgard, Marit Andersson, Jean Pettersson. *Manuscript*

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Author’s contribution

Paper I: Planned the study together with the co-authors and was the main responsible for writing the article. Performed all the experiments in the study.

Paper II: Participated in the planning of the study and the experiments. Main responsible for the coupling of the flow system to ICP-MS. Assisted in the writing of the article.

Paper III: Was main responsible for planning the study and for writing the article. Performed all the LC and ICP experiments in the study. Performed the LC-ESI-MS/MS experiments together with Per Sjöberg.

Paper IV: Participated in the planning of the study. Performed all the experiments together with Heidi De Brabandere. Assisted in the writing of the manuscript.

Paper V: Planned the study together with the co-authors and performed the experiments together with Barbro Kollander. Assisted in the writing of the manuscript.
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Abbreviations

AES  Atomic emission spectrometry
CCD  Charge-coupled device
CE   Capillary electrophoresis
CID  Charge injection device
EC   Electro chemical
ESI  Electrospray ionization
FFF  Field flow fractionation
GC   Gas chromatography
HPLC High performance liquid chromatography
IC   Ion chromatography
ICP  Inductively coupled plasma
IDA  Information dependent acquisition
LC   Liquid chromatography
MALDI Matrix assisted laser desorption ionization
MS   Mass spectrometry
MS/MS Tandem mass spectrometry
NMR  Nuclear magnetic resonance
NP   Normal phase
PGC  Porous graphitic carbon
PREG Polar retention effect on graphite
Q    Quadrupole
r.f. Radio frequency
RP   Reversed phase
SEC  Size exclusion chromatography
SF   Sector field
SPE  Solid phase extraction
TOF  Time of flight
UV   Ultra violet
1. Introduction

The definition of speciation analysis set by The International Union of Pure and Applied Chemistry (IUPAC) is “Analytical activities of identifying and/or measuring the quantities of one or more individual chemical species in a sample”. A chemical species is a “specific form of an element defined as to isotopic composition, electronic or oxidation state and/or complex or molecular structure”.

In analytical chemistry the main goal is normally to determine the identity and/or concentration of one or more species in a sample. The samples analyzed are often natural samples which consist of a mixture of numerous different species. To be able to analyze a specific molecule in such a complex mixture great care has to be taken to the method of analysis used. To be able to perform a speciation analysis, separation, identification and characterization of various forms of elements in different kinds of samples have to be accomplished. The most commonly used strategy for speciation is to use some kind of separation step before a generic detector. The technique that is traditionally used in the chemical analysis of mixtures containing none-volatile organic substances is liquid chromatography (LC) with ultraviolet (UV) absorption detection. When higher sensitivity and/or analyte mass information is needed LC in combination with electrospray ionization mass spectrometry (ESI-MS) is often the choice. However, in some aspects neither of these techniques is ideal. The UV-detection requires the analytes to absorb light in the ultraviolet region and the electrospray may be incapable of ionizing all species or do not ionize all species with the same efficiency. Electrospray can also be very matrix sensitive which limits its use for direct analysis of natural samples. Other reasons for using alternative detection techniques can be if very accurate quantifications at trace levels are needed or if additional information of the analyte is wanted. Atomic spectrometry can provide information about the elemental composition of the analytes, which can be very interesting when looking at molecules that contain other elements than carbon, hydrogen, nitrogen and oxygen. The choice of technique for the elemental detection is in general inductively coupled plasma (ICP) spectrometry. The term ICP spectrometry is here used for both ICP atomic emission spectrometry (AES) and ICP mass spectrometry. ICP-MS is a relatively new technique that combines two well established analytical methods (ICP and MS) to produce an instrument with a great potential in the field of multi-element trace analysis.
The importance of speciation analysis originates from the fact that the chemical forms of an element affect many of its characteristics. Therefore, determination of the identity and quantity of an element is still important, but for many applications measurements of total element concentration provides insufficient information. One example is arsenic, where some organic species are not toxic, while the inorganic forms can be highly toxic [1-3]. The surrounding of an element in a molecule can thus have direct influence on its toxicity, bioavailability, transport, uptake, bio-geological distribution and environmental impact. Speciation analysis have been employed in a wide range of disciplines, including for example environmental science [4-6], biology [6-9] and clinical chemistry [10-14].

The development of elemental speciation techniques are now changing from those focusing on screening for the presence of known heteroatom-containing species in the direction of state-of-the-art speciation of much more complex multielemental systems. Among the most growing and most challenging areas is the analysis of elements in biological systems, broadly called metallomics [15, 16]. The role of various elements in cellular functions, disease states, and in establishing protein structures and functions are areas of increasing interest in the life science area. Another challenging area for speciation analysis in biological systems is drug metabolism studies [17]. Drug metabolism generally refers to the biotransformation that occurs when a drug enters the body. Metabolism studies usually include metabolite profiling, which is separation of the drug and its metabolite followed by quantification of each metabolite and finally identification of the major metabolites. In this area plasma spectrometry has been demonstrated to be a valuable alternative to radiochemical detection because the need of synthesized radio-labeled drugs is omitted and plasma spectrometry provides higher sensitivity. These investigations require the analysis of a large number of unidentified species present at various, often trace, concentrations in complex mixtures.

For these reasons, many investigators are pursuing strategies for improving plasma-source techniques for elemental speciation. This thesis is a contribution to the description and improvement of plasma spectrometry in combination with organic mass spectrometry as detectors for speciation analysis following separation, primary with liquid chromatography. In this field some issues are especially considered and described in more detail: the effect of organic solvents in LC eluents (paper I, III), sample clean-up and pre-concentration (paper II, III), identification of species (paper III-IV) and quantitative analysis with plasma spectrometry (paper II-V). The parallel hyphenation of LC to ICP spectrometry and ESI-MS/MS is exemplified by selected applications (paper III-IV) and the possibility to analyze undissolved samples and perform speciation analysis by immobilizing analytes on non-dissolved particles is discussed (paper V).
2. Plasma Spectrometry

An ICP spectrometer consists of a sample introduction device, a plasma region and a detector. The gas generally used to create the plasma is argon. The ionization properties of argon allow it to ionize most of the elements in the periodic table simultaneously, which makes multi element analysis possible. The sample can be introduced to the plasma as a gas, liquid or as a solid. In the case with a liquid the sample is pumped or self aspirated to a nebulizer where an aerosol is generated. The nebulizer sprays the aerosol into a spray chamber which main task is to remove aerosol droplets that are too large for the plasma. The nebulizer gas transports the aerosol through the spray chamber to the plasma. The plasma is a mixture of electrons, argon ions and argon atoms and has a gas temperature of 5000-9000K. It is created and sustained by the argon gas flow and a magnetic field generated by a radio frequency (r.f.) current in the induction coil (see figure 1a) In the plasma the compounds in the sample aerosol are vaporized, atomized and ionized. A part of the created atoms and ions are also excited. In an ICP-AES, the intensity of the light at a specific wavelength, emitted from the plasma by de-excitation of the sample components is measured. In ICP-MS, the intensity of the ions extracted from the plasma is measured at a specific mass-to-charge ratio (m/z).

2.1 Sample introduction

The introduction of the sample is a critical step in atomic spectrometric analysis and liquid sample introduction is the most common means for presenting samples to plasmas. The sample introduction system for introducing liquid samples into an ICP spectrometer normally consists of a nebulizer, a spray chamber and an injector tube. The predominantly used nebulizers for analytical spectrometers are pneumatic nebulizers and ultrasonic nebulizers. The most popular designs of pneumatic nebulizers include concentric (see figure 1), microconcentric, microflow and crossflow. A pneumatic nebulizer consists of a nozzle to accelerate the propellant gas (nebulizer gas) and a means to introduce the liquid sample into the flowing gas stream. When the liquid meets the high-speed gas at the nozzle an aerosol of nebulizer gas, liquid droplets and solvent vapour is created. The nebulizer is often mounted with its tip inside a spray chamber. The main purpose of the spray chamber is to remove the aerosol droplets which are too large for the
plasma.(>8 \textmu m in diameter)[18]. In the aerosol droplets as large as 100 \textmu m are created [19]. The large droplets do not undergo a sufficient fast desolvation, evaporation and atomization in the plasma. If these droplets are allowed to enter the plasma they will cool the plasma and cause a reduction in precision and an enhancement of interferences [20]. There are three main types of spray chambers commonly used; Scott double pass, cyclonic and single pass. The single pass spray chamber is used with systems that do not require a strong filtering of the aerosol and with this design a large part of the sample will reach the plasma. The most commonly used spray chambers are the double pass and the cyclonic spray chamber where generally less than 3% and 8% respectively of the sample enters the plasma [21]. The purpose of the injector tube is simply to transport the aerosol from the spray chamber into the centre of the plasma. A schematic of a plasma system equipped with a concentric nebulizer and a cyclonic spray chamber is shown in figure 1.

![Figure 1](image)

Figure 1. (a) Schematics of a sample introduction system with a concentric nebulizer and a cyclonic spray chamber. (b) Magnification of the nebulizer tip viewed end-on.

The main limitation of all pneumatic nebulizers is that they produce aerosols with a wide drop size range. This means that high transport efficiency can be achieved only at the expense of allowing large droplets to reach the plasma [22]. The answer to the problem is predominantly the ultrasonic nebulizer. In this nebulizer the aerosol is generated by a piezoelectric transducer. Together with a desolvation system it is one of the most efficient conventional nebulizers with an increase in sensitivity of typical one order of magnitude or more compared to pneumatic nebulizers, since it produces a larger fraction of small droplets. The more easily transportable droplet size will result in the transport and injection of a larger fraction (10-20%) of the initial aerosol into the plasma [23].
2.2 Ionization and excitation

Energy is transferred into the Ar ICP by the interaction of ionized argon with the electromagnetic field generated by the induction coil. Both the positive argon ions and the negative electrons are accelerated by the high-frequency field of the coil. When a spark is passed through argon in the presence of a r.f. field some electrons of the spark gain enough energy to ionize the argon and thereby releasing another electron. This electron can in turn ionize another argon atom and the plasma is initiated. A steady-state plasma is produced when the electron release rate is equal to the electron consumption by recombination to neutral argon. In ICP-AES the high temperature plasma is used to generate excited atoms and ions which produce element specific emission and in ICP-MS the plasma is used to generate positively charged ions. The aerosol is transported, by the sample injector tube, to the base of the plasma. As it travels through the different heating zones of the plasma it is desolvated, vaporized, atomized and ionized. During this time the sample is transformed from a liquid aerosol to solid particles and then into a gas. When it arrives at the analytical zone of the plasma, at approximately 6000-7000 K [24], it exists as atoms and ions. The average energy for an argon plasma is dominated by the first ionization potential of Ar (15.8 eV) and most elements have a first ionization potential below 16 eV and very few have a second ionization potential below 16. This means that most elements produces an abundant population of singly charged ions and that formation of doubly charged ions is rare [25, 26]. Most of the elements in the periodic table are highly ionized by an inductively coupled argon plasma. From calculation, presented by R. S. Houk, 54 elements are expected to be ionized with an efficiency of 90% or more at 7500 K [27]. Both the ionization and excitation process in the plasma is very complex and there are several mechanisms possible for an analyte atom. The three dominant ionization processes are penning ionization [28-30], electron impact ionization [28, 30] and charge transfer ionization [28, 31], which are described in table 1.

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penning ionization</td>
<td>$M + Ar^m \rightarrow M^+ + Ar + e^-$</td>
</tr>
<tr>
<td>Electron impact ionization</td>
<td>$M + e^- \rightarrow M^+ + 2e^-$</td>
</tr>
<tr>
<td>Charge transfer ionization</td>
<td>$M + Ar^+ \rightarrow M^+ + Ar$</td>
</tr>
</tbody>
</table>

2.3 Plasma atomic emission spectrometry

Emission spectrometry has great potential as a qualitative and quantitative tool since all elements can be made to emit characteristic spectra. In ICP-AES, the light emitted by the excited atoms and ions in the plasma is meas-
ured to obtain information about the elemental composition of the sample. Because the excited species in the plasma emit light at several different wavelengths, the emission from the plasma is polychromatic. This polychromatic emission must be separated into individual wavelengths so the emission from each excited species can be identified and the intensity can be measured without interferences from emission at other wavelengths. The separation of light according to wavelength is generally done using a monochromator, which is used to measure light at one wavelength at a time, or a polychromator, which can be used to measure light at several wavelengths at once. The detection of the emitted light is done using a photosensitive detector such as a photo-multiplier tube or more advanced detector techniques such as a charge injection device (CID) or a charge-coupled device (CCD).

2.4 Plasma mass spectrometry

A mass spectrometer is a device that after careful calibration precisely separates gaseous ions in high vacuum according to the ratio between mass and charge. The identity of the single charged atomic ions produced by an argon plasma can thereby easily be determined. The detector also collects information about the number of ions produced, which make quantification possible. Despite the popularity of ICP as an ion source for elemental analysis it suffers from some limitations. Most ICPs are created from argon and, because the first ionization potential of argon is 15.8 eV, elements with ionization energies near or above this value are not efficiently ionized in the plasma. This can cause bad sensitivity for elements such as selenium (9.8 eV), sulphur (10.4 eV) and chlorine (13.0 eV).

The ions created in the atmospheric pressure plasma are transported through an interface into the high vacuum area of the mass analyzer. The role of the interface is to transport the ions efficiently, consistently and with electrical integrity from the plasma to the mass analyzer region. The interface normally consists of two water cooled metallic cones, with very small orifices, that are maintained at vacuum of about 2 torr with a mechanical roughing pump. The first cone is called the sampling cone and the second is known as the scimmer cone. The ions emerge from the scimmer cone into the mass analyzer area, which is maintained at a vacuum of 10^{-6} torr with turbo pumps, where they are directed through the ion optics to the mass separation device. The vacuum interface is shown schematically in figure 2.

The role of the ion focusing system is to transport the maximum number of ions from the interface region to the mass separation device. The ion optics consists of one or more electrostatic controlled lens components, which can focus and steer the ion beam to the mass analyzer. Another important role of the ion optics is to stop particles, neutral species and photons from reaching the mass analyzer and detector.
The mass analyzers commonly used with ICP-MS are the quadrupole (Q), the time of flight (TOF) and the sector field (SF) (often used in double-focusing magnetic-sector instruments). Details of the different mass filters and the operation of them can be found elsewhere [32-34]. The quadrupole, which was developed in the early 1980s is the most used mass filter for ICP-MS and represent approximately 90% of all ICP mass spectrometers today [32]. The technique was the first to be commercialized and is today considered to be a very mature, routine, high-throughput, trace element technique. The popularity of ICP-QMS is due to its low cost and reasonably high speed and sensitivity. The main drawback with ICP-QMS is its limited resolution (1 Da), which can lead to interferences from polyatomic and double-charged ions [35]. The detection limit for elements such as Fe ($^{56}$Fe interfered by $^{40}$Ar$^{16}$O) and Cr ($^{52}$Cr interfered by $^{40}$Ar$^{12}$C) is thereby poor compared to heavier elements.

The solution to interference problem is the use of a sector field mass analyzer. High resolution ICP-MS with a sector-field mass spectrometer became commercially available in the early 1990s and today they can work with resolving power (m/Δm) of 5000 in practice, which is sufficient for avoiding most of the spectral interferences [35]. This should be compared with a quadrupole instrument which have a resolving power of approximately 400 [32]. Another good feature with SF instruments is their very high sensitivity combined with extremely low background levels. The drawback with SF mass spectrometers is the low speed, which is insufficient for high-throughput applications or multielement determinations on rapid transient
signals. Typical speed for a full mass scan (0-250 amu) of a SF instrument is in the order of 150-200 ms, compared to 100 ms for a quadrupole [32, 34].

The most recent mass spectrometer with ICP as ion source to be commercialized is the time of flight technology, which was first combined with ICP by Mayers and Hieftje in 1993 [36]. The TOF-MS offers extremely high data acquisition, high ion transmission and quasi-simultaneously detection of all masses for each ion packet extracted from the ion source. The rate at which the ions packets can be analyzed depends on the flight time of the heaviest ions through the field-free region which, when using flight tubes of 1 m in length, is less than 50 μs [34]. The system allows accumulation of thousands of mass spectra per second and the speed of TOF-MS analyzers exceeds that of quadrupole analyzers by at least two orders of magnitude.

Table 2. Comparison between three different mass analyzers. Q and SF data taken from reference [32] and TOF data from reference [34]

<table>
<thead>
<tr>
<th></th>
<th>Q</th>
<th>TOF</th>
<th>SF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Speed (full mass scan)</td>
<td>100 ms</td>
<td>50 μs</td>
<td>150-200 ms</td>
</tr>
<tr>
<td>Mass resolving power</td>
<td>~400</td>
<td>500-2000</td>
<td>300-400,</td>
</tr>
<tr>
<td>(m/Δm)</td>
<td></td>
<td></td>
<td>3000-4000,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8000-100001</td>
</tr>
<tr>
<td>Mass analyzer sensitivity</td>
<td>10-50</td>
<td>1-5</td>
<td>100-1000</td>
</tr>
<tr>
<td>(million cps/ppb)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Background level (cps)</td>
<td>1-10</td>
<td>20-50</td>
<td>0.1-0.2</td>
</tr>
<tr>
<td>Isotope ratio precision</td>
<td>0.2 %</td>
<td>&lt;0.1 %</td>
<td>0.02-0.2 %</td>
</tr>
<tr>
<td>(R.S.D.)</td>
<td></td>
<td></td>
<td>[37]</td>
</tr>
</tbody>
</table>

1 Depending on entrance and exit slit settings

The quasi-simultaneously detection of all masses originates from the extremely low difference in flight-time between adjacent masses. The ability of the ICP-TOFMS system to capture a full mass spectrum significantly faster than the other mass analyzers gives the possibility of multielement determinations on rapid transient signals, improved precision and shorter analysis time [34]. The improved precision originates from the ability to measure internal standard signals simultaneously as the analyte isotope, which also makes the TOF system well suited for high precision isotope ratio analysis. Quadrupole instruments can achieve typical limits of precision of 0.2% R.S.D. for single pairs of isotopes while TOFMS can achieve less than 0.1% R.S.D [38]. The largest drawback with the TOFMS is the sensitivity and detection limit, which is one order of magnitude worse compared to a quadrupole instrument, because a part of the sampled ions are lost during the ion-beam modulation before the mass analyzer [39]. A summary of the characteristics of the different mass analyzers is presented in table 2.
One of the key advantages with ICP-MS is its wide dynamic range, covering about eight orders of magnitude from low ppt up to hundreds of ppm [40]. This is achieved using dual stage, discrete dynode detectors which enables relatively small ion signals to be measured in pulse counting mode while higher ion signals are quantified using an analogue detection mode. The details and function of the dual-stage discrete dynode detector is described in references [41, 42]. Initial designs of ICP-MS instruments used ion multipliers to produce a pulsed signal which was direct proportional to the number of ions of a particular mass to charge ratio. This technology enabled low ion intensities to be monitored. However, in pulse mode ion multipliers experiences seriousdead time effects when high ion signals is measured [43], due to the finite time required to allow the detector surface to recover following an initial ion impact. Because of the dead time effect both the dynamic range and the isotope ratio precision of this type of detector is limited [44]. The alternative in early ICP designs was to measure the current produced by the amplification of a given ion input flux, the so-called analogue mode of data acquisition. The problem with analogue data acquisition is that the concentration levels needed to get a useful analogue signal was too high to be of practical interest. The dual strategy of pulse counting and analogue signal measurement used today offers the benefits of both techniques simultaneously.
3. Aspects on speciation analysis

3.1 Hyphenated techniques

In all atomic spectroscopic methods the analyte must be converted into the appropriate form before it can be detected. In both ICP-MS and ICP-AES the analyte and all other species in the sample is first converted into free atoms and then, to some extent, ionized and excited. The atomization efficiency should be very close to one hundred per cent to give optimal analytical signal and to minimize interferences from molecular species. In the atomization step all bonds in the analytes are ideally broken, which makes identification of the species and consequently speciation analysis more complicated. The strategy most commonly used for speciation analysis with plasma spectrometry is the use of an on-line separation system before the spectrometer to give time resolved signals for the different species in the sample. The use of hyphenated techniques gives the possibility of species identification and confirmation by matching of the retention or migration time with available standards.

For small volatile and thermo stable molecules gas chromatography (GC) has been used. For larger, unstable and none-volatile molecules liquid chromatography (LC) has been the separation technique of choice. Other separation techniques such as capillary electrophoreses (CE), supercritical fluid chromatography, gel electrophoresis and field flow fractionation (FFF) have also been used to perform speciation analysis with plasma spectrometry. LC is a powerful technique, which allows separation of complex mixtures into individual components. Sufficient resolution, reproducibility and simplicity of the interface between the LC and the ICP make it the most important technique for speciation studies. The most commonly used LC methods in combination with atomic spectrometry are ion chromatography (IC) for inorganic ions such as Cr(III) and Cr(IV) [45] and arsenic and selenium species and size exclusion chromatography (SEC) for macromolecules [46, 47]. Reversed phase (RP) LC is used for relatively small non-polar to medium polar compounds such as selenium species [48] and different kinds of biomolecules [III, IV]. RPLC is also used for larger compounds including peptides and proteins. The normal phase (NP) is less employed together with ICP spectrometry, but has been used in, for example, phospholipids analysis [49]. The coupling of a LC system to the ICP is easily achieved by connect-
ing the outlet of the column to the liquid sample inlet (nebulizer) using a suitable tubing.

The most commonly used RP stationary phases are the non-polar octadecylsilane and octylsilane stationary phases [50] which gives good retention for non-polar analytes. A RP stationary phase that also display some special retention mechanisms for polar compounds are porous graphitic carbon (PGC), marketed as Hypercarb™. The PGC material was developed by Knox and co-workers [51] and numerous reviews describe its properties [52]. PGC is composed of flat sheets of carbon atoms bound in a hexagonal arrangement. Its special retention behavior for polar analytes has been ascribed to the polar retention effect on graphite (PREG) [53, 54] and provides an alternative and attractive complement to IC [55]. The use of PGC material for speciation analysis with plasma spectrometry is scarce but it has been used for speciation analysis of arsenic [56], selenium [57], boron containing drugs and metabolites from BNCT patients [58] and gallium-chelated siderophores [59]. In paper III PGC in combination with plasma spectrometry has been used for speciation analysis of aluminium-chelated siderophores in field-soil solutions and in paper IV for organic phosphorous compounds in aquatic sediments.

Trends in speciation analysis is more and more towards biological samples, which often are very complex, salt rich and contain low concentration of the analyte. The sample volumes are also normally very small. Multidimensional approaches for complex samples are becoming increasingly popular, especially when peak purity is critical for characterizing species. SEC fractionation, which often gives broad peaks, can for example be followed by other kinds of HPLC techniques to improve the resolution. The small sample volumes and low concentrations of analyte often demand miniaturization of the separation system which in turn puts high demands on the sample introduction system. Capillary LC and CE are two low-flow techniques often used for these kinds of samples [15, 60, 61].

3.2 Investigations on the effects of organic modifiers (paper I, III)

Interferences are any effect that changes the signal while analyte concentration remains unchanged. Despite the early claims that plasma spectrometry was relatively free from interferences, it still suffers from several more or less serious interferences. Most of the interferences are caused by the sample matrix and the plasma gases. Both the mobile phase and a complex sample matrix may cause interferences when interfacing separation methods to
plasma spectrometry and the matrix effects can be classified into spectroscopic interferences and non-spectroscopic interferences.

In ICP-MS, spectroscopic interferences are caused by atomic or molecular ions having the same nominal mass as the analyte of interest. The ions interfere with the analytical signal by causing an erroneously large signal at the m/z of interest, i.e. isobaric overlaps. This effect can be caused by overlapping isotopes of other elements, but also by polyatomic ions formed from the matrix and from the plasma and the atmospheric gases. When only introducing pure water into the ICP, a number of polyatomic ions can be seen [62] which originate from argon, water (O, H) and air (O, N). The major polyatomic species includes O⁺, N₂⁺, NO⁺, NOH⁺, O₂⁺, Ar⁺, ArO⁺ and Ar₂⁺. Some of these species can cause serious spectral overlaps including N₂⁺ on ²⁸Si⁺, NOH⁺ on ³¹P⁺, O₂⁺ on ³²S⁺, Ar⁺ on ⁴⁰Ca⁺, ArO⁺ on ⁵⁶Fe⁺ and Ar₂⁺ on ⁸⁰Se⁺. If all isotopes of the interfering elements are taken into consideration, even the interferences from such a simple matrix as water can cause large problems. If a more complex matrix is used the matrix species may add considerable complexity to the background spectrum and the problem with atomic and polyatomic ions can get severe when using separation methods coupled online to the ICP-MS. The mobile phase of the LC system might contain high concentrations of buffer salt, organic modifiers and ion-pairing agents. These species creates a complex matrix which can give raise to many polyatomic species and a very high background on the m/z of interest.

Non-spectroscopic matrix effects are effects that can enhance or suppress the analytical signal. In contrast to the spectroscopic effects they are not caused by other elements or polyatomic ions with the same nominal mass as the analyte. Non-spectroscopic matrix effects are either phenomena from the sample introduction system or effects from the plasma or the ion sampling system. Sample introduction effects that can change the analytical signal are: (a) changes in the physical properties of the nebulized solution (e.g. viscosity, surface tension, density, evaporation rate and vapour pressure.) which can change the nebulization properties of the system; (b) analyte fractionation which can change the amount of analyte transported to the plasma. Plasma related effects which can suppress the analytical signal can be divided into: (c) ionization suppression by an excess of electrons from the matrix or by a lowering of the plasma temperature with increased loading of solvent; (d) mass discrimination of lighter elements; (e) a change in ion density in the zone where ions are sampled into the mass spectrometer. These interferences are caused either by the sample matrix or by the analyte itself, e.g. in the isotope dilution technique, where mass discrimination of the lighter isotope might occur due to the heavier isotope.

In paper I and III the matrix effects related to the use of organic solvents as modifiers for RPLC coupled to ICP-MS were investigated. The amount of organic modifiers can range from a few per cents up to 100% in gradient elution. The plasma is, as mentioned before, generally not compatible with
organic solvents. Because of these drawbacks the coupling of RPLC with ICP-MS has gained less attention than ion chromatography and size-exclusion chromatography [63]. When the matrix of a sample contains an organic solvent the properties of the solution is changed. The added organic solvent will have different surface tension, density, evaporation rate and vapour pressure compared to the water solutions that are normally used with ICP. The lower surface tension and higher evaporation rate of organic solvents normally used in RPLC creates a larger distribution of small droplets in the aerosol [63], and thereby increases the spray efficiency, i.e. a larger part of the sample will reach the plasma. The higher evaporation rate of organic solvents will also cause a larger part of the organic matrix being transported to the plasma in the form of vapour [64]. These effects combined will result in an increased loading of the plasma and more energy will be required to vaporize, atomize and ionize all sample molecules in the plasma. If too much of the sample reach the plasma, the energy loss might be too large, which can lead to a lowering of the plasma temperature and even extinguish the plasma [65]. To increase the stability of the plasma and counteract the temperature decrease a higher r.f. power can be used. The higher r.f. power will increase the energy transmitted to the plasma and at least partially reduce the negative effects of the increased solvent load. Typically a r.f. power 500W higher compared to aqueous sample can be needed in order to sustain a stable plasma if high concentrations of organic solvents are used [66]. An addition of a few percent of oxygen to the sample aerosol or to the nebulizer gas is also often used to increase the stability of the plasma and to prevent carbon deposition on the torch and the cones. The oxygen converts the carbon into carbon monoxide and dioxide [67].

In paper III the determination of aluminium in biomolecules was hampered by a spectroscopic interference. The use of organic modifiers favoured the formation of $^{13}$C$^{14}$N$^+$, $^{12}$C$^{15}$N$^+$ and $^{12}$C$^{14}$N$^+$H$^+$ which gave an isobaric overlap at the same m/z as the only aluminium isotope, $^{27}$Al. The isobaric overlap produces a large background signal which will cause increased limits of detection and varying signals if gradient elution is used. The effect of acetonitrile on the aluminium background signal is illustrated in figure 3.

The use of organic solvents generally causes a suppression of analytical signal but in some cases an increase in signal has been seen, especially for elements with a high first ionization potential. This enhancement is believed to originate from an increased ionization of the analyte caused by charge transfer reactions between carbon ions and the analytes [68-70]. This effect may enhance the sensitivity for analytes with a first ionization potential between 9-11 eV. The carbon non-spectroscopic interference on more easily ionized elements (Li, Na, Al, Mn, Fe etc.) works in the other direction. With these elements the carbon charge-transfer mechanism has little effect, because the analytes is already ionized with 95-100% efficiency. Therefore, the overall matrix effect of carbon matrixes on these elements is signal suppres-
sion by space charge effects, causing mass discrimination by the increased ion current in the interface to the mass spectrometer.

**Figure 3.** Comparison of the CN-interference on the Al signal at m/z 27. (a) shows the signal of blank solution of water and (b) the signal of a blank with 15% ACN.

In paper I, a study was performed with the aim to investigate the decrease in signal when measuring boron species in organic matrixes with ICP-MS. Different organic solvents were tested to see if the signal suppression was less critical for some of them. In figure 4 the response for boronophenylalanine in different mixes of methanol, propanol and acetonitrile are shown and the results ranks them in order of declining influence on signal as ACN > PrOH > MeOH.

**Figure 4.** Background corrected and normalized boron-11 signal intensity for boronophenylalanine with increasing amounts of added organic solvent.

The fact that acetonitrile is less well tolerated by the ICP than methanol has been described by several authors. The plasma is probably loaded with more acetonitrile than methanol at the same molar flow of solvent to the nebulizer, because both the viscosity and the surface tension of water-
acetonitrile mixtures are lower than water-methanol mixtures. In addition, the enthalpy of atomization is estimated to be about 30% higher per mole of gaseous acetonitrile than for methanol vapour at 298 K. If this relationship in enthalpy is assumed to be valid at the temperature of the plasma, the plasma temperature should be more reduced by acetonitrile than by methanol.

To study the possibility of a decrease in plasma temperature when organic solvent are present in the matrix an investigation was performed on an ICP-TOFMS and an ICP-AES instrument. Solutions containing boron, lithium and cadmium in different mixtures of water-acetonitrile were measured on both instruments. These elements were chosen because of their masses and first ionization potentials. Lithium has a mass very near the mass of boron but have a lower ionization potential than boron (5.29 eV compared to 8.30 eV). Cadmium has a higher mass but nearly the same ionization potential as boron (8.99 eV). The difference in first ionization potential between boron and lithium did not give any difference in the degree of signal decrease in the ICP-MS. In the ICP-AES the intensity of the ionic cadmium line increased with increased concentration of ACN. This is the opposite from what would have been expected if the plasma had been cooled by the organic solvent and the degree of ionization was decreased. The atomic lines for cadmium and lithium was almost constant or increased in the ICP-AES, while the boron signal was somewhat decreased. These results indicate that the ionization properties of the plasma are not lowered to a large extent by the organic solvent. Since cadmium has nearly the same ionization potential as boron the large reduction in boron signal in the ICP-TOFMS instrument can only to a minor extent be explained by a decrease in ionization efficiency due to cooling of the plasma.

As indicated above, matrixes containing organic solvents can cause many non-spectral interferences. The possibility of mass discrimination effects when using organic solvents was investigated by comparing measurements on both ICP-MS and ICP-AES equipped with the same sample introduction system. In the MS technique one can expect to see signal depression caused by mass discrimination effects when introducing a heavy matrix. Mass discrimination effects should not take place in an ICP-AES system [71] which lacks both the vacuum interface and the ion sampling system. In analogy with the theories of mass discrimination the cadmium signal had the smallest difference in signal between the two spectrometers while boron and lithium showed a larger decrease in the MS technique (see figure 5).

The signal of the light elements $^7$Li and $^{11}$B were decreased to less than 20% even when low concentration of ACN were present in the matrix while $^{111}$Cd were depressed to about 40%. The depression was not seen in the ICP-AES, where the signals for all elements except boron where enhanced by the matrix. The difference in signal decrease between the instruments can be explained by mass discrimination of light elements in the presence of an increased ion current from the carbon matrix. The enhancement effect by
charge-transfer is probably small compared to the suppression effect by mass discrimination when the concentration of carbon ions in the plasma is high. The space-charge theory was further confirmed by an experiment where addition of a heavy element was used as a simulated heavy matrix ion. Aqueous solutions containing 200 ppb Li, B and Cd were used. The concentration of Cd was then increased and the signal suppression was observed. A large decrease of the boron and lithium signals occurred in the ICP-TOFMS instrument while the ICP-AES signals remained almost unchanged, see figure 6.

The problem with organic solvents in plasma spectrometry has been addressed by several authors and numerous techniques have been developed to decrease the negative effect of the solvents. Some of the more commonly used techniques are listed in table 3.

Addition of oxygen to the aerosol and an increase of plasma power have been discussed above. A standard technique to reduce the solvent load in ICP-MS is aerosol cooling and desolvation. The aim of these techniques is to remove the solvent from the aerosol prior to the plasma. Aerosol cooling is usually performed with a chilled spray camber operating down to -5° C. Desolvation can be performed by heating and cooling the aerosol one or
several times [72] and/or by using a membrane that separates the solvent vapour from the particles of a heated aerosol [73]. Another desolvation technique commonly used is cryogenic desolvation were the temperature in the cooling device is set as low as -80°C, which permits the determination of elements in pure methanol or acetonitrile at ml min⁻¹ flows without extinction of the plasma [74].

Table 3. Techniques used to minimize the effect of organic solvents in combination with plasma spectrometry.

<table>
<thead>
<tr>
<th>Technique</th>
<th>Effect</th>
<th>Drawbacks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Addition of oxygen to the aerosol</td>
<td>Removal of carbon depositions by converting the carbon to carbon monoxide and dioxide [75]</td>
<td>Increase of oxide species in the plasma, erosion of the cones. (ICP-MS only)</td>
</tr>
<tr>
<td>Increased Plasma power</td>
<td>Gives increased plasma stability and counteracts lowering of plasma temperature [66]</td>
<td>Increase of some spectral interferences</td>
</tr>
<tr>
<td>Chilled spray chamber</td>
<td>Lowers the solvent loading of the plasma by decreasing the amount of solvent vapour transported to the plasma [76, 77]</td>
<td>Formation of larger droplets which can cause increased background noise levels and decrease transport efficiency [78]</td>
</tr>
<tr>
<td>Desolvation units</td>
<td>Lowers the solvent loading of the plasma by desolvating the aerosol [72-74]</td>
<td>Possibility of analyte losses and memory effects [79]</td>
</tr>
<tr>
<td>Collision/reaction cells (ICP-MS only)</td>
<td>Decreases spectral interferences by breaking up or altering polyatomic ions [80, 81]</td>
<td>Loss of analyte signal, creation of new interfering polyatomic species [80, 81]</td>
</tr>
<tr>
<td>Decreased flow rate</td>
<td>Decreases the solvent loading by lowering the sample flow [82]</td>
<td>Lower mass transport might lead to lower sensitivity, peak broadening (LC) or low analyte capacity in separation techniques (e.g. capillary LC)</td>
</tr>
<tr>
<td>Post-column make-up flow</td>
<td>Dilutes the organic matrix and increases the plasma tolerance [83]</td>
<td>Loss of sensitivity due to dilution of analyte.</td>
</tr>
<tr>
<td>Post-column eluent splitting</td>
<td>Decreases the solvent loading by lowering the sample flow [49]</td>
<td>Decreased analyte transport to the ICP</td>
</tr>
</tbody>
</table>

Trends in ICP spectrometry using organic solvents include post column eluent splitting or dilution and/or a decreased flow rate. In an application concerning phospholipid analysis solvent load to the plasma was reduced 5-fold by splitting the flow before the nebulizer, chilling the spray chamber to -5°C and optimizing the carrier gas flow for maximum condensation of organic vapours [49]. The use of low flow techniques such as capillary HPLC and nano HPLC is likely to alleviate the problems with organic solvents in the mobile phase. At such low flow rates introduction of up to 100% organic solvent becomes possible without either oxygen addition or cooling of the spray chamber. A considerable signal loss was however observed at acetonitrile concentrations exceeding 80% introduced into an ICP at 4 µl min⁻¹ [82].
As mentioned before, the formation of overlapping polyatomic species from the matrix can influence the detection limit of an isotope. These spectroscopic interferences are caused by molecular ions having the same nominal mass as the analyte of interest. In a complex matrix like an aqueous-organic HPLC mobile phase numerous interferences can occur. In such a carbon rich matrix carbon-based spectral interferences can be formed e.g. C$_2^+$, CO$^+$, CN$^+$, and ArC$^+$. There are many ways of removing these interferences but one of the most commonly used is the use of a reaction cell consisting of a quadropole or hexapole where the analyte ions collides with an inert or highly reactive gas [84, 85]. Through various mechanisms, the interfering ions are converted into species of different mass or to neutrals. Kishi et. al. have showed the reduction of both C$_2^+$ and ArC$^+$ interferences from isopropyl alcohol by four to five orders of magnitude by using a dynamic reaction cell operated at a flow rate of 0.6 mL min$^{-1}$ with NH$_3$ gas [86]. Neubauer and Vollkopf have showed the reduction of both carbon and chloride matrix species, which allowed detection of ng/L levels of Cr, Mn and As in carbon- and chloride-based matrixes [87].

3.3 On-line sample cleanup and preconcentration (paper II-III)

The major attraction of ICP techniques is its exceptional multi-elemental sensitivity combined with high speed of analysis. In addition, the possibility of performing isotope and isotope-ratio analysis with ICP-MS offers a unique field of applications. As shown in the previous sections ICP-MS suffers from many spectral and non-spectral interferences which can degrade the advantages of the technique. The problem with interferences can be especially severe when working with natural samples, which often contains low concentrations of the analyte and a complex matrix consisting of e.g. easily ionized elements, salts, mineral acids, and organic compounds. To be able to quantify and determine isotope-ratios of species in this kind of samples it might be necessary to remove the matrix before the detection or separation step and/or to perform a pre-concentration of the analyte. Liquid-liquid extraction, followed by solvent evaporation is a traditional method for sample preparation in many types of applications. However, liquid-liquid extraction usually involves several steps which are time consuming and often imprecise. The liquid-liquid extraction also consumes large quantities of organic solvent, which is in conflict with environmental concerns [88]. Another commonly used off-line method is the use of solid-phase extraction (SPE) on disposable cartridges, which have been reported to increase laboratory throughput and to replace many liquid-liquid extractions [88].
In recent years an increasing number of LC methods, using online pre-concentration and sample clean-up, have been developed. On-line techniques offer many advantages over off-line techniques because they require smaller sample amounts, are faster and minimizes the risks of losses and/or contamination. Pre-concentration before LC separation of aqueous samples can easily be performed by solid phase extraction by merely employing a guard column with a suitable stationary phase (e.g. ion-exchanger, C18) before the separation column. When the sample is injected the analyte of interest becomes stuck on the guard column while the matrix is washed away by the mobile phase. Once the pre-concentration is complete, the mobile phase is changed and the analyte is eluted. Cai et al. [89] found that selenium from 100 ml water effectively could be retained on an ion-exchange column and complete peak separation could be achieved in less than 4 minutes with this method.

A more sophisticated approach, which is demonstrated in paper III and references [58, 90] is to use a column-switching method, which utilizes a pre-column to trap the analyte and a reversed flow to transfer the analyte to a second column used for separation. By this approach the major part of the matrix can be transported to waste and do not have to enter the separation column or mass spectrometer and two different stationary phases can be used for the trapping and the separation. One disadvantage of many of the solid-phase methods used is the requirement of organic solvents to elute the analytes. This demand can severely complicate the analysis with ICP spectrometry.

In paper II an electro-chemically (EC) controlled solid-phase extraction method for sample clean-up and pre-concentration before analysis with ICP-MS was developed. The extraction step uses a conducting polymer as a stationary phase which offers several advantages compared to traditional solvent based SPE. The main advantage is that both the extraction and the desorption step can be controlled merely by changing the applied potential of the conducting polymer coated working electrode. There is thus no need to alter the composition of the solution in order to elute the analytes. Extraction of negatively charged species is performed by applying a positive potential (typical about +800mV) to the working electrode. At this potential the polymer is oxidized and becomes positively charged. This results in an electro-sorption (extraction) of anions. The polymer is subsequently reduced to its neutral state by the application of a negative potential (typically about -800 mV). During the reduction step the anions are expelled (desorbed) from the stationary phase. In the same way the polymer can be used to extract positive ions by first reduce the polymer and then oxidize it. Electrochemically controlled solid-phase extraction is a very interesting alternative to conventional SPE and offers a means to selectively pre-concentrate and remove the matrix from either anions or cations without changing the mobile phase for desorption.
As shown in figure 6, it is also possible to alter the structure of the polymer to increase the selectivity towards a specific anion e.g. increase the selectivity for bromide in a matrix containing other ions by doping the polymer with bromide during polymerization.

![Figure 6](image)

**Figure 6.** The effect of the anion used during the electropolymerisation on the bromide peak area in the presence of sulphate. The error bars represent the standard deviation (n = 4 for 0.125 mM bromide using PPy(ClO₄) and n = 2 for 0.125 mM bromide using PPy(Br), n = 2 for 0.125 mM bromide and 0.125 mM sulphate using PPy(ClO₄) and n = 3 for 0.125 mM bromide and 0.125 mM sulphate using PPy(Br)).

For a solution containing 0.125 mM bromide a pre-concentration factor of 84 was achieved, but a higher pre-concentration factor could probably be achieved in a solution with lower concentration. A method where the flow through the cell was stopped during desorption was also tested, which decreased the desorption volume and further increased the pre-concentration.

![Figure 7](image)

**Figure 7.** (A) Analyte signal as a function of time using the stopped-flow desorption technique. (B) peak profile comparison between (a) a regular desorption at constant flow rate and (b) the stopped-flow technique.
An example of a pre-concentration of a 0.125 mM bromide solutions with and without stop-flow desorption is shown in figure 7. The EC-SPE technique is a promising extraction method which can be used for matrix removal, desalting and pre-concentration before a separation technique coupled to ICP-MS or ESI-MS detection.

### 3.4 Identification of species (paper III-IV)

In recent years, most of the speciation studies have been aimed at understanding the role of various elements in biomolecules. The analytes are often unknown species from living organisms or other natural samples, which demands the involvement of techniques that can provide molecular and structural information for successful characterization. The loss of structural information during the element detection in ICP-AES and ICP-MS makes identification of unknown species impossible by these techniques alone. Molecular MS, such as electrospray ionization mass spectrometry and matrix assisted laser desorption ionization mass spectrometry (MALDI-MS), has been recognized as an essential tool for speciation analysis, usually applied for identification and confirmation of unknown peaks seen in LC-ICP-AES or LC-ICP-MS [60, 91]. Both ESI and MALDI are soft ionization techniques, which normally produces ions consisting of the entire molecule with essentially no fragmentation taking place during the ionization process. To obtain structural information tandem mass spectrometry (MS/MS) can be utilized, where collision-induced dissociation is used to reproducibly fragment a selected molecular ion. MS/MS has been used by a large number of researchers for positive identification of detected species [92-94]. Combining the information from plasma spectrometry, molecular MS and MS/MS can give a complete picture of the species distribution for a given element within the sample (see figure 8.).

As with any technique there are some disadvantages with molecular MS. Unlike the plasma techniques ESI is highly dependent on the matrix components and a significant amount of peak suppression can be possible due to the presence of high concentrations of salt in the mobile phase [95]. In addition, routinely achievable detection limits for ESI are generally two to three orders of magnitude poorer than for ICP-MS [96]. The sensitivity of the ionization process towards matrix components and instabilities in the spray makes quantification intricate. Usually internal standards are required to compensate for these effects. When analyzing biomolecules standards are often not available or the species are unknown. The discrepancy with detection limits can become a problem for identification of ultra-trace elements detected by ICP-MS. MALDI has been used less frequently than ESI be-
cause its coupling to separation techniques is not straightforward since the sample must be immobilized before analysis. In some cases however, ESI is replaced by MALDI because it is less vulnerable to matrix effects [97].

![Figure 8](image_url)

Figure 8. Schematic diagram illustrating the contribution of various analytical techniques to the accomplishment of speciation analysis.

In paper III ESI-MS and ESI-MS/MS were used to identify unknown aluminium-containing biomolecules from soil solution samples found by LC-ICP-MS. The data from ICP-MS showed two aluminium-containing peaks which eluted at the same time as Al-desferrichrome and Al-desferricrocin standards. To further confirm the identity of the species and check the purity of the peaks the chromatographic system was coupled to an ESI-MS/MS instrument and information dependent acquisition (IDA) experiments were performed. The obtained fragmentation spectra were compared with fragmentation spectra from standard solutions of the two aluminium-chelated siderophores. These comparisons clearly confirmed the identity of the aluminium species. Siderophore speciation is further discussed in section 4.1.

In paper IV organic phosphorus species in aquatic sediments found by LC-ICP-AES were characterized by ESI-MS and ESI-MS/MS. No standards were available for these totally unknown species. Speciation of organic phosphorus compounds is further discussed in section 4.2.
3.5 Quantitative analysis with plasma spectrometry (paper II-V)

Analytical determinations with an accuracy of better than 10% are considered quantitative and determinations with an accuracy of 30 to 50% are known as semiquantitative [98]. Semiquantitative analysis of unknown samples is normally performed by simply multiplying measured isotope intensities by predetermined relative sensitivity factors for the specific isotope of interest. Such concentrations usually are accurate to within a factor 2 of the amount present. Because relative sensitivity factors are dependent on specific operating conditions and the sample matrix, they have to be adjusted if large changes are done or samples with varying matrixes are analysed. If better accuracy is needed an internal standard approach can be used where the sensitivity factors are normalized against the intensity of an added internal standard. This can give an accuracy within ± 25% of the amount present [98]. If even higher accuracy is needed a quantitative analysis must be performed. Quantification of analytes to which identical standards of well defined concentrations are available is normally straightforward. In the external calibration mode the standard is analysed at different concentrations and the signal intensity is plotted versus the analyte concentration. The sample concentration of the analyte is evaluated from the curve or the equation of the curve. If the sample is subject to matrix effect the method of standard addition might improve the accuracy [99]. When using a separation method before the detection the analyte can be separated from the matrix, which can make the time consuming method of standard addition unnecessary.

In many cases reference materials or standards are lacking and in speciation analysis the analytes are sometimes unknown. This problem is often encountered e.g. in environmental and biological analysis. The fact that the signal is independent of the structure of the molecule gives the advantage of the possibility to use inorganic standards for quantification of unknown analytes or analytes with lacking standards. As long as the molecule concentration is sufficient low, the matrix interference from the surrounding molecule has been found to be almost negligible and inorganic standards could be used for quantification with an accuracy of 10% or better [100]. Caution has however to be taken when working with separation techniques coupled to ICP-MS. Because of the sensitivity to interferences caused by changes in the matrix the response of an isotope can change during a chromatographic run, especially if gradient elution is used and especially when isotopes of low mass are analyzed. The effect on sensitivity and background for isotopes has previously been discussed in section 3.2 and in paper I and III.

The accuracy and precision in LC-ICP techniques may also be fairly poor due to time dependent instabilities in the plasma and changes in the nebulization and ion sampling. The accuracy and precision can be improved by
using some kind of internal standard. The internal standard, which can be the analyte isotope or another element/isotope, can be added to the sample or be added post-column. The problem with these methods is to find a good internal standard which behaves similar as the analyte isotope.

The technique of coupling chromatography with ICP-MS detection may be extended by making use of the ICP-MS capability to detect several isotopes quasi-simultaneously. Isotope dilution is a technique whereby the natural isotopic abundance ratio of an analyte is altered by spiking with a standard that has a different isotopic abundance. This technique requires that the analyte has more than one stable isotope. Isotope dilution is regarded as a supreme technique. The precision and accuracy is unsurpassed by other analytical methods and it overcomes some problems associated with instrumental drift and matrix effects [101]. With LC-ICP-MS isotope dilution can be performed in two ways. The isotopically labeled analyte species can be added to the sample and is then supposed to co-elute with the analyte species [102]. The use of this technique (species-specific isotope dilution) is limited by the availability of isotope labelled analytes, which can be lacking or at least are very expensive. If isotopically labeled calibration standards are unavailable, the technique of continuous introduction of an isotopically labeled species-unspecific spike solution can be used after the separation step [102].

In paper II the bromide concentration in tap water was determined with external calibration with bromide standards by ICP-MS after on-line electrochemical controlled solid-phase extraction. In paper III aluminium-containing siderophores in filed-soil solutions were quantified on ICP-MS with external calibration after separation by RPLC. In paper IV unknown organic phosphorus compounds were quantified with ICP-AES by using standards containing the phosphorous compound 3’-5’-cyclic adenosine monophosphate (c-AMP) for external calibration. In paper V, different elements were quantified with ICP-AES and ICP-MS, in slurries of biological materials, with external calibration utilizing internal standards to compensate for matrix effects.
4. Selected applications on speciation analysis with hyphenated techniques

4.1 Siderophore analysis (paper III)

Siderophores are low molecular mass organic compounds with high iron(III) stability constants. They are produced by many microorganisms, including microbes, fungi and some plants, often in combination with low molecular mass organic acids. Siderophores are excreted during iron-limited conditions to form water soluble complexes with iron(III). The formation of these complexes facilitates the uptake of iron into the organism [103]. Although siderophores are iron-chelating agents, they are not really iron-specific, but they form the most stable complexes with iron. For example, the stability constant for desferrioxamine B with iron(III) is $10^{30.6}$ compared to $10^{24.1}$ with aluminium [104]. Nevertheless, several investigations have found stable complexes of siderophores with metals including Al, Ga, Cu and Mo [105-107]. Despite the large number of investigations dealing with siderophores, the role of other metals than iron is not clear. How, if at all, these metals influence siderophore production and thereby the sensitivity towards them is not yet known. The identification of specific siderophore complexes and determination of their concentrations are of special interest in the understanding of plant nutrition. Siderophores are also believed to contribute to the weathering of minerals.

Siderophores are not only useful in microorganisms to enhance iron acquisition. The siderophore desferrioxamine B is used as a drug, under the commercial name Desferal to treat transfusional iron overload. In addition other siderophores are also antibiotic, e.g. ferrimycins and ferrioxamine E [108].

Clearly, the interest for siderophores takes many perspectives and covers many fields of science, therefore analytical methods to study these compounds in soil solutions, cultural samples and biological fluids are of great importance [109-111]. As these compounds are often found in low concentrations in complex matrixes, the analytical methods employed for identification and quantification have to provide excellent selectivity and sensitivity. Due to the complexity of the natural samples, unknown siderophores are not easily detected by, for example, LC-ESI-MS [112]. The reason is that other compounds, also present in the sample will give rise to a number of addi-
tional peaks in the mass spectra. The same siderophore may also form complexes with several metals e.g. iron and aluminium, which may elute very close to each other from the LC and generate overlapping complex mass spectra. Also the low concentration of siderophores, typically in the low-nanomolar range, found in natural field soil solutions makes the identifications with LC-ESI-MS even more difficult. Instead of ESI-MS inductively coupled plasma can be used as the ionization source for selective monitoring of metals in known and unknown siderophores. ESI-MS/MS can then be used to further identify the detected siderophores. With the problems listed above, the advantages of ICP-MS as an element specific detector for the LC system are evident. In paper III a study was made to investigate the applicability of ICP-TOFMS following reversed-phase LC separation for screening of aluminium-chelated siderophores in field-soil solutions.

![Figure 9](image)

**Figure 9.** Background signal at m/z 27. (A) pure milliQ-water, (B) 10 mM ammonium formate buffer, (C) 30 % MeOH in 10 mM ammonium formate buffer, (D) 15 % ACN in 10 mM ammonium buffer

The separation of siderophores was performed on a PGC column and sample clean-up and pre-concentration was performed on-line with the column-switching methodology. The detection of Al in carbon rich matrixes with ICP-MS is normally hampered by isobaric overlaps from polyatomic ions formed from the matrix. When adding carbon rich solvent, such as methanol or acetonitrile, the $^{13}\text{C}^{14}\text{N}^+$, $^{12}\text{C}^{15}\text{N}^+$ and $^{12}\text{C}^{14}\text{N}^+\text{H}^+$ with the same mass as $^{27}\text{Al}^+$ will form in the plasma [113]. The nitrogen is either entrained from the surrounding atmosphere or, as in the case with acetonitrile and ammonium formate, from constituents in the mobile phase. Small amounts of carbon dioxide is also always entrained from the atmosphere or dissolved in all solutions.
To investigate the background signals from interfering ions, both acetonitrile and methanol were tested as organic modifier. With aqueous buffer alone, an increase in background signal compared to pure milliQ-water was seen. Addition of 30% methanol did not increase the background signal further. When 15% acetonitrile was added, the background increased significantly because of the nitrogen content (see figure 9). In previous investigations of siderophores on PGC-columns acetonitrile has been used as organic modifier, because it has good eluent strength on PGC-columns. In this work, methanol was chosen to reduce the background signal. The tolerance of the ICP towards methanol is also generally better than towards acetonitrile.

To further decrease the background signal, cold plasma conditions were used. With cold plasma the Al signal increased about a thousand times while the background was decreased by 50% compared to the normal plasma conditions. This is probably due to less ionization of carbon which has a high first ionization potential (11.26 eV) compared to aluminium (5.99 eV). This will cause less carbon-containing polyatomic ions to be formed and also reduce other polyatomic interferences in the plasma. When analyzing elements of low mass, such as Al, great care has to be taken to minimize the formation of heavier ions, such as $\text{Ar}^+$, $\text{Ar}_2^+$ and other ions formed from the matrix, which could give rise to space-charge effect and lower the ion transport efficiency of the analyte ion [25, 68, 114]. Al-Ammar et al. showed that even low concentrations of carbon in the samples will cause a signal suppression for light elements with an ionization potential below 9 eV [68]. Cool plasma conditions have been found to reduce the level of ionization in the source plasma by 2-3 orders of magnitude compared to normal conditions [115]. With the extracted ion current substantially reduced, it is to be expected that space charge effects in the ion optics are much less important [116].

To investigate the potential of the developed method to make fingerprints of the aluminium-chelated siderophore content in natural samples with different origins, a gradient separation was performed on a field soil solution sample and the resulting Al trace from this experiment clearly revealed two peaks. By external calibration with standard solutions containing aluminium-chelated desferrichrome the concentrations of aluminium in the sample was estimated to 1.1 and 0.7 nM (30 and 19 ng l$^{-1}$) respectively. The same sample was also analyzed using an ESI-MS/MS instrument to confirm the origin of the peaks. The obtained fragmentation spectra were compared with fragmentation spectra from standard solutions of aluminium-chelated siderophores, see figure 10. These comparisons clearly confirm that the first eluting peak corresponds to Al-desferrichrome and the second to Al-desferricrocin. The similar retention behaviour of these peaks with corresponding aluminium-chelated siderophore standards also validates these results. Further information that support the identity of the Al containing siderophores were the detection of the corresponding iron species, providing characteristic isotopic
pattern in the ESI-MS, due to $^{54}$Fe/$^{56}$Fe/$^{57}$Fe, and similar fragmentation pattern as the Al containing species.

The hyphenation of LC and ICP-MS provides high selectivity in the identification of both known and unknown metal-containing compounds. In this study aluminium-containing siderophores have been examined, but the used methodology could easily be adapted to other applications with other aluminium complexes. The strategy to use cool plasma conditions and nitrogen-free organic modifiers to minimize isobaric overlaps and other interferences gives good sensitivity and low detection limits for the $^{27}$Al$^+$ ion even when low-resolution ICP-MS is used without collision cell or desolvation of the aerosol.

**Figure 10.** ESI-MS and ESI MS/MS data of a podzolic soil solution sample from Heden, Sweden, using the column-switched system. (A) XIC of EMS, 712 amu, 742 amu and 771 amu. Elution order: (1) Al-desferrichrome (2) Al-desferricrocin (3) ferrichrome (4) ferricrocin. (B) MS/MS spectra of peak (1) (C) MS/MS spectra of peak (2). "orn" denotes the modified ornithine (N5-acyl-N5-hydroxy-L-ornithine) and "gly" is used for glycine.
4.2 Organic phosphorous in sediments (paper IV)

Eutrophication, i.e. the enrichment of nutrients in aquatic systems, has become a widespread problem in many coastal and inland waters during the last couple of decades. Eutrophication can be induced when nutrient enrichment exceeds the normal uptake capacity and the word is thus used when anthropogenic over enrichment occurs with unwanted ecosystem consequences. One of these consequences is cyanobacteria blooms and oxygen depletion in the water body. Phosphorus is a key element for primary production in aquatic systems and is thus in many cases a regulator of trophic status. In lakes, the primary source of P is the drainage area. However, P available for lake primary production is also heavily dependent on recycling of P, either within the water column or after sedimentation. Sediment P may either be buried permanently or recycled into the water column. The general principles of P fluxes on a catchment scale are shown in Figure 11.

![Figure 11. Phosphorus sources in the environment. Figure by Andreas Dahlin](image)

Although organic P most likely constitutes a major proportion of sediment P [117], most studies have focused on inorganic P forms [118-120], which may be due to the limitations of the currently used analytical techniques for extraction and identification of organic P forms in sediments. Studies that have focused on the organic P part in sediments, have in most cases only been able to identify groups of organic P compounds (i.e. phosphate mono-
esters, phosphate diesters, polyphosphates, pyrophosphate, orthophosphate), most often using the $^{31}$P-nuclear magnetic resonance ($^{31}$P-NMR) technique [121-125]. However, a few studies have focused entirely on individual organic P compounds. Suzumura and Kamatani [126] extracted inositol phosphates by hypobromite oxidation followed by isolation with anion exchange chromatography and analysis with $^1$H-NMR and gas chromatography. The phospholipid phosphatidylcholine, isolated from sediment pore waters and extracts, has been analyzed with chemiluminescence via an enzymatic reaction [127]. Additionally, several sediment phospholipids were identified via a flow-blending extraction combined with liquid chromatography electrospray ionization tandem mass spectrometry [128].

![Figure 12. Chromatogram of a sediment sample obtained by LC- ICP-AES by measuring phosphorus at 178.2 nm.](image)

To gain a deeper understanding of the turnover of sediment P, it would be of interest to characterize all the individual organic P compounds in a sediment sample. Since existing methods focus either on groups of organic P compounds or on one specific P compound, there is a need for analytical methods that determine and characterize several individual P containing compounds simultaneously.

In paper IV a LC-ICP-AES method was developed for screening of organic phosphorous compounds in aquatic sediments. The separation step was performed with gradient elution on a PGC column and eight P-containing peaks were observed. The obtained chromatogram is shown in figure 12.
The same separation system was then coupled to ESI-MS/MS to obtain structural information from the peaks. An information dependent experiment was set up with a survey scan consisting of a precursor ion scan monitoring parents of either m/z 79 or 97. The survey scan triggers the selection of parent ion and automatically switches to MS/MS mode during elution of the plausible P compounds. Some structural information from the peaks were obtained, e.g. a possible identity of the 346 ion could be adenosine monophosphate (AMP).

The LC-ICP-AES method was also used to detect variation in concentration of the phosphorus compounds at different depths of sediments. The different depths correspond to different age of the sediment and the variations may reflect degradation processes over time as well as loss of sediment P to the overlaying water column. The top layer (0-1 cm) and an older layer (29-30 cm) were investigated. To compensate for drift in sensitivity 3'-5'-cyclic adenosine monophosphate (c-AMP) was added in the same amount to both samples and the obtained peak area for each compound was normalized against the c-AMP area. The difference in normalized signal for six P-containing peaks is illustrated in figure 13.

![Figure 13](image)

*Figure 13.* Comparison of the P-signal from six different chromatographic peaks for two samples collected at different depths of the sediment. Peak 1-2 and 6-7 in figure 12 is integrated together because of the poor separation.

This study shows the potential of ICP-spectrometry to detect low concentrations of phosphorous compounds in the complicated matrix of the sediment. The found peaks can then further be characterized online by ESI-MS/MS or off-line by $^{31}$P-NMR by collecting fractions. This is an important step towards the ultimate goal to identify all organic P compounds in an aquatic sediment extract.
5. Analysis of non-dissolved materials (paper V)

Analysis of non-dissolved material, i.e. slurries, of both inorganic and organic materials by different plasma techniques is of great concern due to several reasons. First of all slurry analysis reduces the time spent on sample preparation in forms of digestion and complete dissolution of a sample before analysis. This also leads to less contamination of the sample from digestion reagents and to reduced consumption of aggressive chemicals, which is of environmental concern. Additionally, loss of volatile analytes is minimized by the softer sample treatment prior to slurry analysis. The limited dilution in slurry analysis is beneficial because it enables analysis of elements present in low concentrations in the sample, which after a digestion procedure would have ended up in concentrations below the detection limit of the instrument. It also is advantageous when the amount of sample is limited, which could be important in analysis of, for example, medical or forensic character.

Although it is well known that the conditions in the inductively coupled plasma enable atomisation of elements included in a particle, there are limiting factors affecting the result. Most relevant are, the size of the particles and the transport efficiency. Ebdon et. al. [129] concluded, for example, in their comprehensive review of slurry nebulization in plasmas in 1997, that particles larger than 5 μm do not reach the plasma but also that the particle size cut off depends on the density of the particle. For slurry particles with high density (>7 g ml⁻¹) the particle size cut off is smaller [130]. Since particle size is of importance in slurry analysis some requirements are set for the sample preparation procedure. Difficulties with homogenization, i.e. to reduce particle size to usable ranges, are discussed in several works [131-133] for different sample types. When the slurry sample finally reaches the plasma there are several matrix effects to consider from both inorganic [70, 134, 135] and organic origin [135-137].

The intention of paper V is to study how analysis of non dissolved organic material could be performed with ordinary ICP instrumental settings and with the equipment most commonly used without any special modifications for slurry analysis. The reference materials selected, bovine liver and whole blood, are thought to be model systems for other kinds of organic samples, for which no reference material is available, such as cell cultures.
for mentioning one. Sample preparation was performed by simply diluting the sample with a small volume of a suitable mixing solution followed by 4×15s homogenization with a polytrone homogenizator at 14000 rpm.

With ICP-AES both Meinhard and cross flow nebulizer could be used to analyze slurries with ordinary settings, with the exception that Meinhard gave better sensitivity (i.e. higher slope) for most of the analytes. Meinhard also could keep a linear response with higher slurry concentrations. In 40% (v/v) whole blood it was possible to quantify 8 of 14 elements, within 10% of the certified value, with Meinhard and 7 of 14 with cross flow nebulizer. By reducing the slurry concentration to 20 % (v/v), the number of elements possible to quantify, increased to 10 for both nebulizers.

ICP-MS was shown to suffer more from interferences from the slurry matrix than ICP-AES. A clear trend was seen were the analytes with low mass deviated more from the certified values than the heavier elements. These results indicate that an internal standard with a similar mass as the analyte is needed to get results according to the certified values. To avoid deviations originating from the level of ionization, the internal standard should also have a similar first ionization potential as the analyte. These requirements make it difficult to find a good internal standard for all elements and also make multi element detection more complicated. With the right choice of internal standard, several elements were quantified within 25% of the certified value for 5% blood and for some cases up to 15% blood. Some elements were also quantified within 10% of the certified value in low slurry concentrations.

It can thus be concluded that this simple homogenization method of organic material before direct measurement by ICP-MS and ICP-AES can be used for quantification of a number of elements in biological samples, when suitable internal standards are selected.

In the future the possibility to introduce and completely atomize undissolved particles into the plasma open up the possibility to extract analytes from samples by antibodies or other selective chelating agents immobilized on nano-particles. Size exclusion chromatography or field flow fractionation can then be used prior to plasma spectrometry to separate the analytes from the complex matrix of a biological sample. This kind of selective extraction, sample clean-up and separation could be used for extremely sensitive and selective determination of biologically important molecules, such as metalloproteins.
6. Concluding remarks

This thesis describes efforts to improve and understand the elemental speciation analysis with plasma spectrometry and also highlights the importance and potential of the synergy between atomic spectrometry and molecular mass spectrometry. The cooperation between people working with elemental analysis and the people working with molecular mass spectrometry has in the past been scarce. Delightfully, during the last decade more and more applications have been published were the combination of the matrix tolerant, robust and very sensitive plasma spectrometry has been used together with molecular mass spectrometry, which can provide structural information and the possibility to identify unknown species. The development of elemental speciation techniques are now changing from those focusing on screening for the presence of known heteroatom-containing species in the direction towards state-of-the-art speciation of much more complex multielement systems. Among the most growing and most challenging areas is the analysis of elements in biological systems, broadly called metallomics. In these types of speciation analysis researchers often have to face the problems with low concentrations of analyte, multielement detection, complicated matrixes and small sample volumes.

In this thesis methods are developed for on-line sample clean-up and pre-concentration coupled to liquid chromatography and plasma spectrometry, which makes handling of small sample volumes easier and also decreases the risk of contamination. The problems associated with organic modifiers in plasma spectrometry are also addressed. Nowadays some of these problems can be avoided by the use of advanced instrumentation, but better understanding of the problems is still needed to make further development possible and also to be able to do good and simple speciation analysis without expensive equipment such as high resolution mass spectrometers. The applications exemplified in this work show that in some cases good detection limits and identification of species in complex samples can be achieved with low resolution spectrometers even without desolvation and/or collision/reaction cell. This is accomplished by using optimal plasma conditions and ion optic settings in combination with on-line clean-up and pre-concentration coupled to a suitable separation system.

Analysis of non-dissolved materials, i.e. slurries, is also discussed. The possibility to introduce biological materials as slurries, without the often time consuming digestion and complete dissolution step, can be of great
benefit for analysis where small samples volumes and low analyte concentration is a problem. In the future the possibility to introduce and completely atomize non-dissolved particles into the plasma open up the possibility to extract analytes from samples by antibodies or other selective chelating agents immobilized on nano-particles. Size exclusion chromatography or field flow fractionation can then be used prior to plasma spectrometry to separate the analytes from the complex matrix of a biological sample. If different antibodies are bound to particles of different size, separation can also be achieved between different analytes. This kind of selective extraction, sample clean-up and separation could be used for extremely sensitive and selective determination of biologically important molecules, such as metalloproteins.

I hope that this thesis has provided a deeper understanding of both the benefits of elemental speciation analysis in a wide range of disciplines and the advantage of combining elemental spectrometry with molecular mass spectrometry to create a “dream team” for speciation analysis. It is my hope and belief that, although significant progress already has been made in developing instrumentation and methods with improved sensitivity and versatility, the development of these hyphenated techniques is an ongoing progress that ultimately will provide us with the complete picture of elemental speciation analysis.
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8. Summary in Swedish

Denna avhandling beskriver specieringsanalys med induktivt kopplat plasma (ICP) spektrometri som detektor för olika separationstekniker. Med speciering menas att alla olika föreningar som innehåller ett speciellt grundämnen i ett prov identifieras och/eller kvantifieras. Specieringsanalys är ofta av stor betydelse, eftersom ett grundämnes kemiska form kan påverka ämnets kemiska och fysikaliska egenskaper. Ett vanligt exempel är arsenik, där en del organiska arsenikföreningar inte är giftiga medan de oorganiska formerna kan vara dödligt giftiga. I sådana fall räcker det inte att bestämma totalhalten av grundämnet i proverna utan de olika föreningarna måste mätas var för sig.

Spektrometri med ICP är idag en av de vanligaste teknikerna för detektion av grundämnen. Plasmat är en blandning av elektroner, argonjoner och argonatomer och har en temperatur på 5000-9000 K. Plasmat skapas och upprätthålls av ett argongasflöde och ett radiofrekvensfält från en induktionsspole. För att kunna mäta grundämnen med ICP-AES eller ICP-MS måste föreningar omvandlas till lämplig form. I plasmat bryts molekyler först ner till atomer, varav en del joniseras och/eller exciteras. Signalerna från provet mätts antingen som ljusemission från exciterade grundämnen (atomär emissionsspektrometri, AES) eller genom att mäta intensiteten av de joner som bildas i plasmat med en massspektrometer (MS).

Eftersom alla bindningar i en molekyl bryts i atomiseringssteget är det omöjligt att identifiera vilken förening grundämnen finns i med enbart ICP. För att ändå kunna identifiera olika föreningar används vanligtvis någon form av separationsteknik i kombination med ICP. Separationstekniken används för att separera föreningarna från varandra så att de kan detekteras skilda åt i tid. På det sättet kan man identifiera föreningar genom att jämföra elueringsstider med kända standarder. Om föreningarna i provet är helt okända eller om separationstekniken inte fullständigt separerar de olika föreningarna från varandra kan molekylär masspektrometri eller tandemmasspektrometri (MS/MS) användas för att få struktuell information. Den mest använda tekniken för jonisering är elektrospray (ESI) eftersom den är lätt att koppla till separationssystem och är en mjuk joniseringsteknik som kan ge jonisering utan att molekylerna fragmenteras i nämnvärd utsträckning.

Utvecklingen inom specieringsanalyse går nu från mätningar som fokuserar på att hitta kända föreningar i olika prover mot speciering av mer komplexa multielements-system. Ett av de mest växande och mest utmanande områdena är mätningar av grundämnen i biologiska system, generellt kallat

I artikel I beskrivs de negativa effekterna av de organiska lösningsmedel som ofta används som modifierare i vätskekromatografi. Dessa lösningsmedel kan försämra stabiliteten hos plasmat, samt även minska teknikens känslighet genom att påverka provintroduktionen och ge upphov till andra spektrala och ickespektrala matriseffekter. Det lättta grundämnet bor har visat sig påverkas kraftigt vid MS-detektion redan när ett fåtal procent organisk modifierare används. Den minskade signalen orsakas troligtvis av massdiskriminering när jonerna som bildas i plasmat ska transporteras till masspektrometer. Det stora antalet koljoner som bildas i plasmat ger upphov till en vidgning av jonstrålen vilket missgynnar lätt element i en större utsträckning än tunga.


I Artikel III utvecklades en metod för att speciera aluminiuminnehållande biomolekyler med vätskekromatografi kopplat till ICP-MS. Hög känslighet erhölls vid mätningarna på aluminium, trots den negativa effekten från de organiska modifierare som är nödvändiga för att eluera föreningarna, genom att använda kallt plasma och välja en modifierare som ger minimala interferenser. Metoden applicerades på sideroforer, vilket är relativt små biomolekyler som produceras av mikroorganismer, svampar och vissa växter och vanligtvis fungerar som komplexbildare för järn i naturen. Två aluminiuminnehållande sideroforer hittades och kvantifierades i ett markvattenprov. Samma kromatografiska system kopplades sedan till ESI-MS/MS för att identifiera sideroforerna. Både desferrikrom och desferricrocin visade sig
komplexbilda aluminium och detekterades i låga nanomolarkoncentrationer i markvattenproverna.


Artikel V beskriver möjligheten att analysera icke upplöst material uppslämmat i vätska (slurry). Metoden kan användas för att förenkla provbearbetningen av biologiska material genom att ta bort behovet av att bryta ner provet innan det analyseras, t.ex med starka syror, vilket minskar tidsåtgången, risken för kontamination och förlust samt användandet av miljöfarliga kemikalie. Genom att provet endast homogeniseras i en lämplig lösning innan det introduceras till ett ICP blir det mindre komplicerat att utföra mätningar på små provvolymer. Dessutom minskar utspädningen av provet, vilket gör det enklare att mäta låga halter. I förlängningen kan möjligheten att mäta icke upplösta partiklar även användas till att selektivt extrahera analyt med hjälp av antikroppar bundna till nanopartiklar i olika material och storlekar. Nanopartiklarna kan sedan separeras med fält- och flödesfraktierung (FFF) eller exklusionskromatografi (SEC) innan de detekteras med ICP-AES eller ICP-MS.
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