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Analysis and Speciation of Lanthanoides by ICP-MS

Abstract:
Inductively coupled plasma mass spectrometry (ICP-MS) is based on formation of positively charged atomic ions in a high-frequency inductively coupled Argon plasma at atmospheric pressure. The ions are extracted and transferred from the plasma source into a mass analyzer operated at high vacuum via an interface equipped with a sampling and a skimmer cone. The ions are separated in the mass analyzer according to their charge to mass ratio. The ions are converted at a conversion dynode and are detected by use of a secondary electron multiplier or a Faraday cup.
From an analytical point of view, ICP-MS is a well-established method for multi-elemental analysis in particular for elements at trace- and ultra-trace levels. Furthermore, methods based on ICP-MS offer simple quantification concepts, for which usually (liquid) standards are applied, low matrix effects compared to other conventional analytical techniques, and relative limits of detection (LODs) in the low pg g^{-1} range and absolute LODs down to the attomol range. For these applications, ICP-MS excels by a high sensitivity which is independent of the molecular structure and a wide linear dynamic range. It has found acceptance in various application areas and during the last decade ICP-MS is also more and more applied for detection of rare earth elements particularly in the life sciences.
Due to the fact that all molecules introduced into the high temperature of the plasma in the ion source were completely dissociated and broken down into atoms, which are subsequently ionized, all elemental species information is completely lost. However, if the different species are separated before they enter the plasma by using adequate fractionation or separation techniques, then ICP-MS can be used as a very sensitive element-specific detector. We will discuss this feature of ICP-MS in this chapter in more detail at hand of the speciation of gadolinium-containing contrast agents.

Keywords: analysis of lanthanoides, ICP-MS, speciation of Gd-containing MRI contrast agents
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1 Introduction

Inductively coupled plasma mass spectrometry (ICP-MS) is one of the most successful analytical methods in atomic spectroscopy. It has found widespread acceptance in various application areas including environmental (e.g. drinking, river, sea and waste water) [1, 2], geological (e.g. trace element patterning), clinical (e.g. determination of trace metals in blood, serum and urine) [3] and industrial [4] analysis. ICP-MS is a multi-element detection method which offers simple calibration and quantification most often by liquid samples and excels by limits of detection (LODs) in the sub pg mL^{-1} range.

2 Fundamentals of ICP-MS

In MS, the fundamental properties of electric, magnetic and radio frequency (Rf) fields are used to separate ions with different mass to charge ratios. The ions to be separated are generated in an ion source which is the inductively coupled high-frequency plasma in case of ICP-MS. Therefore, most ICP-MS instruments consist of the following components which are schematically shown in Figure 1.

– A sample introduction system where the sample is converted to a physical state which is optimized for the function of the ion source. In case of ICP-MS, sample introduction is most often realized by pneumatic nebulization of a liquid sample.

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- A plasma ion source, where ions are generated from atoms by use of external energies from an inductively coupled high-frequency electromagnetic field. In the plasma source, the aerosol is dried, decomposed, dissociated and atomized, excited and finally positively ionized.
- An interface system, which consists of a sampling and a skimming cone and a vacuum fore pump, by which the ions are extracted from the plasma. This device is also needed to reduce the pressure from the ion source to the vacuum needed in the mass analyzer region.
- A lens system to focus the ions into the mass analyzer.
- The mass analyzer (quadrupole [ICP-QMS], magnetic sector field [ICP-SFMS], time-of-flight analyzer [ICP-TOFMS]) where the ions are separated due to their mass to charge ratio.
- A unit by which the ions are detected (secondary electron multiplier or Faraday cup).
- A computer which controls all functions of the mass spectrometer, acquires data and delivers the mass spectrum, where the mass to charge ratios of the ions are represented with their measured intensities.

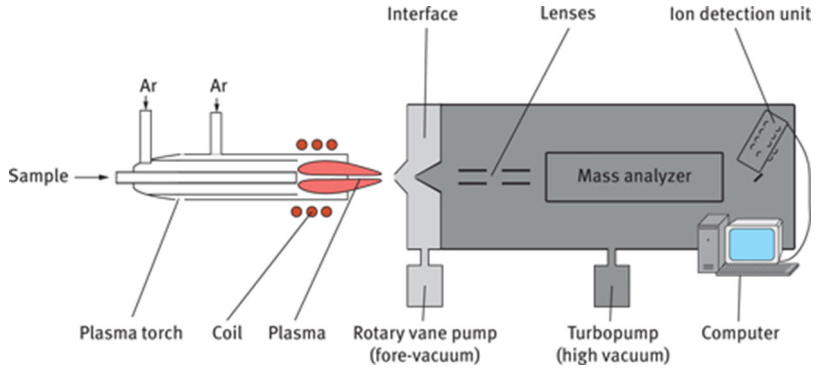

Figure 1: Schematic view of an ICP-MS instrument (with courtesy of Frank Bierkandt).

2.1 Sample preparation

ICP-MS is a method which conventionally is used for liquid analysis. For this purpose, the sample, in particular solid or particulate materials, has to be converted into a solution from which an aerosol is generated by a pneumatic nebulizer (see Section 2.2). The aerosol is transported continuously by a transport gas (most often Argon (Ar)) and a tubing to the plasma ion source, which is operated at atmospheric pressure.

Most often, digestion by use of mineral acids at high temperatures and high pressures is required to get solids and medical samples such as body fluids, tissues and bones into solutions. These mineral acids are often combined with oxidizing reagents in particular if carbon-rich materials have to be digested. Microwave-assisted digestions are generally sufficient to get many environmental and medical relevant materials into clear solution, which are compatible with the needs of ICP-MS (for more details see Ref. [5]).

Analyte quantification is commonly carried out by calibration with external single or multi-element standards and concerning sample handling usually an auto-sampler is applied to measure up to hundred samples a day and in some routine analytical labs the ICP-MS instruments are even working overnight. When instrumental drift or matrix effects are expected, an internal standard can be added in liquid form to compensate for intensity drift, as well as transport and ionization effects. The matrix load of an ICP-MS is usually limited to less than 1,000 $\mu g\ mL^{-1}$ and even at low $\mu g\ mL^{-1}$ levels matrix effects have to be compensated. Alternatively to internal standards, matrix addition is a laborious but sensible approach if accurate data are needed and higher salt loads can be tolerated, which is the case for REE. They have to be analyzed in geological or environmental samples at ultra-trace levels due to the very low natural abundances of all these elements and thus dilution most often should be avoided.

Special care is needed in case of a speciation analysis of gadolinium (Gd)-containing contrast agents. To preserve the original species during all sample preparation steps, the common addition of nitric or hydrochloric acids to the samples has to be avoided to prevent the degradation of the chelates.

2.2 Sample introduction

The robustness of the ion source and the fact that the ion source is operated at atmospheric pressure enables a large variety of sample introduction systems to be used. The most common samples are in a liquid form and

have to be converted into an aerosol. The aerosol is tolerated by the plasma if the droplets are small enough to be vaporized and the water load is not getting too high to effect the plasma properties. Commonly, pneumatic and, in particular, concentric nebulizers are used for aerosol generation (for a more detailed description and additional references see Refs. [6, 7]).

The most common pneumatic nebulizer consists of a nozzle in front of a concentrically centered capillary containing the analyte solution in which the liquid is accelerated by the Venturi effect using a pressurized nebulizer gas (Argon). By the turbulences generated in front of the nozzle, the liquid surface is disrupted and the liquid is dispersed into small droplets which are transported by the nebulizer gas via tubing to the plasma ion source.

Pneumatic nebulizers are typically combined with a spray chamber for removal of the larger droplets [6–8]. Additionally, cooling of the spray chamber significantly reduces the solvent load of the plasma and as a result water- and acid-based interferences. An alternative way to reduce the solvent load, which is particularly important if organic solvents are nebulized, is the use of a desolvation system. The desolvation system in most cases consists of a heated selective membrane, through which the gaseous solvent molecules are transported, whereas the dried aerosol is retained in the gas stream and transported to the plasma.

The main drawback of conventional nebulizer is their low efficiency (most often less than 5 %), which means that only a few percent of the sample liquid is injected into the plasma. This limitation can be overcome by application of high-efficiency nebulizers among which the ultrasonic nebulizer is one of the most successful ones. Micro-concentric pneumatic nebulizers working at extremely low sample introduction rates also achieve a higher efficiency. They are most often combined with separation systems requiring low mobile phase flow rates (capillary electrophoresis [CE], nano-high-performance liquid chromatography [HPLC]).

Nebulizers and spray chambers manufactured from inert polymers (such as Teflon or PFA) have to be used when aggressive chemicals, such as HF are applied. In this context, these nebulizers are most often combined with a torch injector tube manufactured from sapphire and cones made from Pt.

Many alternative sample introduction systems have been investigated with the aim of improving the sensitivity, reducing spectral interferences or handling small volumes of sample. Other sample introductions systems are used in the context of speciation (e.g. chromatographic systems) or for direct analysis of solids (e.g. laser ablation, electro-thermal vaporization).

2.3 The ion source

The ICP is one of the most successful analytical ion sources in atomic MS. This plasma is generated electrodeless in an atmospheric pressure discharge gas (Argon) by application of high-frequency voltages to an induction coil located at the top of a torch consisting of three concentric quartz tubes. Usually three different Argon flows are used. A relatively low gas flow rate (called inner gas, nebulizer gas, transport or carrier gas) of about 1 L min^{-1} is used for aerosol production of the liquid sample and to transport and inject the aerosol into the hot core of the plasma plume. A much higher flow rate (10–15 L min^{-1}) (called outer gas, plasma gas or coolant gas) is introduced tangentially. This flow of Argon thermally isolates the plasma from the outer quartz tube and thus prevents melting. The third flow (Argon support gas, intermediate gas, auxiliary gas) is optionally used for alignment of the plasma in the torch. The outer quartz tube is surrounded by a water-cooled induction coil connected to an Rf generator power supply with a frequency of 27 MHz. An oscillating magnetic field induces high-frequency oscillating electrons and an ion current in a closed circular path, by which the plasma gas is heated to high temperatures (gas-kinetic temperature of 6,000–8,000 K, ionization temperature ca. 7,500 K, excitation temperature of 6,500–7,000 K, electron temperature ca. 10,000 K). The plasma is ignited by a high-voltage spark.

The ICP is thus maintained by inductive heating in the high-frequency electro-magnetic field, but an electrical power of up to 1,500 W is required for continuous operation. The injected aerosol travels through a narrow axial channel, surrounded by the high-temperature plasma core forming a donut-shaped structure, where volatilization, atomization, excitation and ionization of the sample components take place. Predominantly positively singly charged ions are generated from most elements of the periodic table. The fraction of ionization of a given element is usually calculated by use of the so-called Saha–Eggert equation if the electron densities and electron temperatures are known. From this formula, it can be estimated that most elements with first ionization potentials below 8 eV are completely ionized and elements with potentials in a range between 8 and 12 eV are partly ionized. Thus, the mass spectrum acquired during a mass scan consists mainly of isotopes of singly charged elemental ions.

Due to the high temperatures and the high electron densities the ICP is a very robust electrical flame, which shows only little matrix effects. The fundamental plasma properties and analytical figures of merit are not significantly affected when the sample composition is altered. This is a tremendous advantage over many other

analytical methods. Therefore, these plasmas are operated in ICP-optical emission spectrometry (ICP-OES) with quite high concentrations in the percent range; however, due to space charge effects in ICP-MS the maximum concentration tolerated is limited (see Section 2.1).

2.4 Interface

As mentioned before, the plasma of the ion source is operated at atmospheric pressure, whereas the mass spectrometer has to be operated under high vacuum conditions. Thus, an interface is required to extract the ions from the plasma and to stepwise reduce the pressure on the way to the mass analyzer. Due to the fact that the interface is in direct contact with the hot plasma, it is usually water-cooled and made of materials with high thermal conductivity and sufficient chemical resistance. It generally consists of two cones, both often made of Nickel (Ni): the sampler, which is in direct contact with the plasma and the skimmer cone, which separates the first pumping stage from the second one. In the center of the metallic conus, an orifice (0.8–1.2 mm) is used for extraction of the ions from the plasma. The second cone (skimmer) is typically pointed and has a smaller orifice. The cones are placed coaxially in a distance of a few millimeters allowing a sequential pressure decrease. When analyzing corrosive solutions or measuring low Ni concentrations, platinum cones are preferred in order to avoid abrasion of the cone metal and Ni background, respectively. In order to reduce effects of electrostatic coupling between the load coil and the plasma discharge – resulting in an electrical discharge between plasma and sampler cone – the load coil is usually grounded at the side facing the sampler cone.

The ions generated in the plasma are sucked into the sampler orifice by the pressure drop between the atmospheric plasma and low pressure of a few mbar in the first vacuum stage. The ions are accelerated in this first pumping stage to ultrasonic velocities and are picked up by the skimmer by the flow dynamics of the hot expanding argon gas. Behind the skimmer cone a lens system is arranged for ion focusing.

2.5 Lens system

Behind the skimmer an additional pressure drop is generated by turbo-molecular pumps. Electrostatic fields can then be applied to an electrostatic lens system to accelerate the ions and focus them into the mass analyzer region. For reduction of the noise coming from neutrals and photons, different technical means (ion mirrors, photon stops, Omega-lenses, bended geometries in case of sector field and time-of-flight instruments) are applied to hinder them from reaching the detector.

2.6 Mass analyzers

Ions are separated in the mass analyzer due to their mass to charge ratio by using the fundamental properties of electric, magnetic or Rf fields. The most commonly used mass analyzer in ICP-MS is the ICP-QMS. Historically, it was first coupled to an ICP by Robert Samuel Houk in 1980 (for more details see the famous publication [5]) and was already commercially available a few years later in 1983. Since then, it had become one of the most successful multi-element techniques in atomic spectrometry.

2.6.1 Quadrupole analyzer

The quadrupole analyzer usually consists of four cylindrical metallic rods arranged symmetrically around a central axis. The pair of facing rods is supplied by a positive Rf and a direct current (DC) voltage, whereas the second pair is supplied by negative Rf and DC voltages. For a given combination of these voltages, only ions of a given single mass to charge value have a stable trajectory and are transmitted through the rod system whereas all others hit the wall or the rods and thus are removed from the ion beam. Ramping the ratio between the Rf and the DC voltage offset allows fast scanning across the whole analytical mass range sequentially. ICP-QMS provide a unit mass resolution all over the mass range, which means that one peak (isotope) is separated from the neighboring peak (isotope) only. They do not reach high-resolution mode, and thus spectral interferences can limit applications of ICP-QMS.

Among spectral interferences polyatomic species are most problematic, because they are less predictable and depend on the sample composition and the operational parameters of the ICP-MS system. The formation of cluster ions from the most dominant species in the plasma (Ar, H, O, C, N) and the matrix solution (water, mineral acids, salts, etc.) is a major source of polyatomic interferences in ICP-MS.

Other technical means are therefore required to overcome these limitations by spectral interferences. Reaction and collision cells as well as double focusing sector field devices are used to overcome this limitation.

2.6.2 ICP-QMS combined with reaction and collision cells

Collision and reaction cells can be used in quadrupole instruments exclusively. In this approach, a pressurized cell usually is located behind the skimmer cone. Two types of gases are used in the cell: inert buffer gases (collision cell) and chemically reactive (reaction cell) gases. In the first case, the buffer gas collides with the ions from the plasma. Big molecular ions, in particular polyatomic molecular species, collide more often with the buffer gas than atomic ions due to the higher impact cross section. This results in a higher energy loss. At the exit of the collision cell, a retarding field is applied where only the atomic ions have sufficient kinetic energy to overcome the barrier. In the second approach, a reaction gas is applied that either reacts with the analyte element to shift to an un-interfered mass range, or with the polyatomic species to a product molecular ion with a significant shift of the mass. Also, charge transfer reactions are often applied to neutralize the interfering species directly. In modern instrumentation both approaches are accessible. Recently, a new technology was launched to the market, where a quadrupole mass filter is arranged in front of the reaction cell so that only preselected ions can enter the cell for reactions. This approach significantly reduces the number of unwanted reactions in the cell. Due to its similarity to organic mass spectrometers, this new approach is also known as MS/MS approach, because two quadrupoles are used synchronized to each other.

Although very powerful and quite often used, collision and reaction cell devices have a significant disadvantage: the loss in multi-element capability.

2.6.3 ICP-sector field mass analyzer

Most of the hardware items of a sector field device are identical to quadrupole devices. This holds true for the sample introduction, the ICP, the interface, as well as the detector and computer system. However, they contain a more complex lens system, an entrance and an exit slit and a magnetic and an electric sector field. The lens system is needed for acceleration of all ions to high energies >8,000 electron volts (eV) and for beam shaping to adopt the circular geometry of the ion beam behind the skimmer to a more linear geometry which fits to the shape of the entrance and exit slit (for more details see Ref. [9]). The high energies are required for optimal function of the magnetic sector, which is used to separate the ions due to their mass to charge ratio. The electric sector is needed to compensate differences of the ion energy, which otherwise can compromise the resolving power of the magnetic field.

In general, sector field instruments (for more details see Ref. [9]) are equipped with up to two single detectors (Faraday cup, secondary electron multiplier or channeltron detectors) and are usually operated in scanning mode for multi-element analysis. In contrast to this type of sector field instruments, a multi-collector (MC)-ICP-MS is operated in static mode and is equipped with multiple detectors (Faraday cups, multiple ion counting systems or channel plates). The latter configuration has the advantage that all isotopes of an element are measured at the same time, which improves the precision of signal intensity measurements by orders of magnitude in comparison to all scanning devices. Due to the fact that MC-ICP-SFMS instruments are mainly applied for isotope ratio measurements, so far they are not yet applied for REE analysis and thus will be discussed elsewhere in more detail [9–11].

Compared to quadrupole devices, sector field instruments show a higher sensitivity (slope of the calibration graph) at a low resolving power and significantly reduced background due to their bended geometry (less than 1 count per second (cps) in the mass range above 100 Da) and thus lower detection limits are achieved. The main advantage of sector field instruments is the capability to apply a high mass resolving power that separates the spectroscopic interferences from the signal of the isotope of interest by variation of the slit width of the entrance and exit slit.

The resolution of the ICP-SFMS instrument is defined by

$R = (m/z)/\Delta(m/z)$

R: mass resolution [without units]. tem m: nominal mass of measured signal (peak) [should be given as a reference, because the mass resolution of an sector field instrument is constant over the mass range, and thus the peak width (Δm) of a signal increases proportional to mass. This is quite opposite to a quadrupole machine, where the peak width is constant over the whole mass range and thus the resolution increases with increasing mass].

Δm: mass difference between a peak at the nominal mass m and a peak at $m + \Delta m$.

z: absolute charge number of the isotope of interest.

Commercial instruments provide a resolution R of up to 10,000 by which most of the common spectral interferences originating from polyatomic species can be resolved from the isotope of interest. However, it should be mentioned that the sensitivity is indirectly proportional to the resolution: higher resolutions are paid by a loss in sensitivity.

In Table 1, typical spectral interferences and the respective theoretical resolution for separation from the analyte isotopes are compiled.

Table 1: Mass resolution (R) necessary to separate typical interferences.

Nominal mass (m/z)	Polyatomic species	Analyte isotope	Resolution R
	Solvent H_2O and HNO_3		
31	$^{14}N^{16}O^{1}H^{+}$	$^{31}P^{+}$	968
	$^{15}N^{16}O^{+}$	$^{31}P^{+}$	1,458
54	$^{40}Ar^{14}N^{+}$	$^{54}Cr^{+}$	2,031
	$^{40}Ar^{14}N^{+}$	$^{54}Fe^{+}$	2,088
	Solvent HCl		
51	$^{35}Cl^{16}O^{+}$	$^{51}V^{+}$	2,572
52	$^{35}Cl^{16}O^{1}H^{+}$	$^{52}Cr^{+}$	1,671
75	$^{40}Ar^{35}Cl^{+}$	$^{75}As^{+}$	7,775
	Solvent H_2SO_4		
48	$^{32}S^{16}O^{+}$	$^{48}Ti^{+}$	2,519
64	$^{32}S_2^{+}$	$^{64}Zn^{+}$	4,261
64	$^{32}S^{16}O_2^{+}$	$^{64}Zn^{+}$	1,952
65	$^{32}S^{33}S^{+}$	$^{65}Cu^{+}$	1,939

From Table 1, it can be seen that a resolution of about 4,000 is already sufficient to separate most isotopes from polyatomic species. However, the resolution of commercial instruments is often not sufficient to overcome isobaric interferences from isotopes of different elements located at the same nominal mass (e.g.^{54}Cr and^{54}Fe) for which a resolution of more than 100,000 (73,900 to separate^{54}Cr from^{54}Fe) is required.

2.6.4 Time-of-flight mass analyzer

In a "time-of-flight" mass analyzer (ICP-TOFMS), the ions are accelerated in an electric field by a voltage applied to a grounded plate and an ion transparent grid. All ions in the electric field receive the same energy. They are separated according to the difference in time that they need to reach the detector in a fixed distance. After passing the grid the ions travel in a field-free region with velocities dependent on their mass. Low-mass ions hit the detector first, followed by heavier ions. Thus, the detector registers ion signals with µs time resolution. Depending on manufacturer up to 30,000 full mass spectra can be recorded per second. These instruments are therefore very useful for measurement of very short transient signals.

A specialized ICP-TOFMS – well known under the name "mass cytometer" – is schematically shown in Figure 2. This instrument [12] was introduced to the market as a trademark "CyTOF^{TM}" by the company DVS Sciences Inc. (now Fluidigm, CA, USA). The instrument addresses the challenges of a special detector for biological and medical cell flow cytometry.

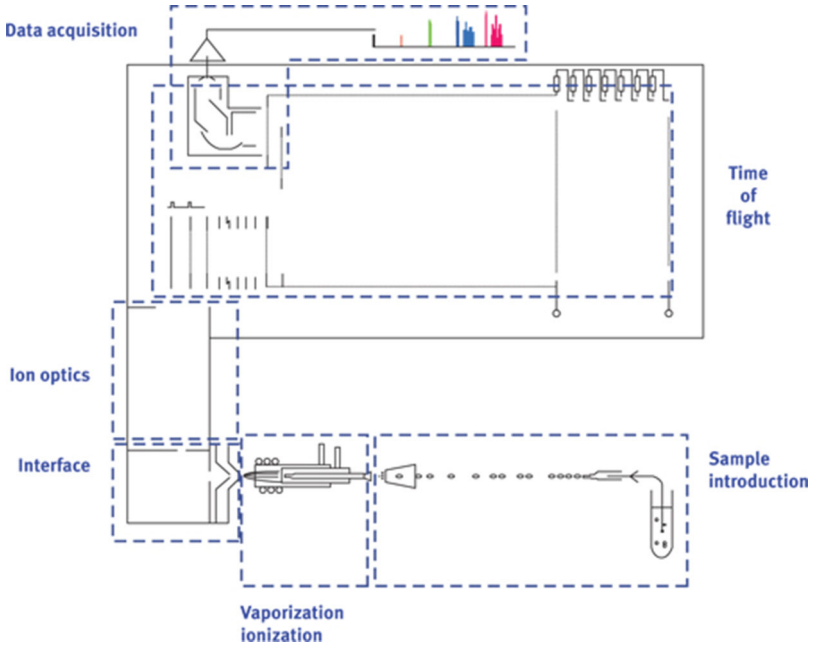

Figure 2: Schematic set-up of a mass cytometer (see Ref. [12]).

It employs a fast ICP-TOFMS with an integral electrostatic quadrupole deflector minimizing the exposure of ion optics and detector to unionized particles and an Rf-quadrupole ion guide is used as a low-mass cut-off filter ($m/z <$ approx.82) rejecting abundant interfering plasma species (for instance Ar^+, ArO^+, Ar_2^+). But this mass cut off also limits the range of detectable masses to >82 Da with a mass resolution $R = 600$. The sample introduction of the CyTOF^{TM} is based on a concentric nebulizer designed and used to nebulize a cell suspension with a sample uptake rate of <60 µL min^{-1}. The nebulizer is connected to a heated spray chamber, to which a make-up argon gas flow (typically at 0.7 L min^{-1} is supplied via a mass flow controller. In the spray chamber, the droplets with imbedded cells are dried (all water and droplets without cells are vaporized) and transported to the ICP in a laminar flow. The instrument allows multi-element analysis of a few thousand single cells just in the time frame of a second.

This instrument opens a new door for application of lanthanoide elements. A whole article (see: Novel Applications of Lanthanoides as Analytical or Diagnostic Tools in the Life Sciences by ICP-MS-based Techniques) is dedicated to this new technology, because lanthanoides are applied in this application for tagging of antibodies to detect biomarkers in highly multiplexed immune-assays, and is therefore of high interest in clinical diagnosis and cell biology.

2.7 Detector and computer

Most ICP-MS instruments are equipped with an ion-to-electron conversion dynode and an electron multiplier (discrete dynode detector or channeltron), which can be operated in analogue or counting mode. That allows to cope with a large variation in the number of ions that reach the detector per unit of time (cps) so that a linear dynamic range of up to 9 orders of magnitude can be achieved. For sector field instruments, an additional detector, a Faraday cup, is optional which allows extending the linear dynamic range even to 12 orders of magnitude.

Finally a computer, which controls all functions of the mass spectrometer, the generator and the vacuum system, acquires data and delivers the mass spectrum, where the mass to charge ratios of all measured isotopes are represented by their intensities. Most instruments provide all software needed for calculation of mean and integrated intensity values and standard deviations of the measured intensities. From these values, calibration, drift correction and quantification of specific elements can be performed. The operator selects all isotopes needed for analysis and arranges the samples in a given sequence defined in the software and finally evaluates the results of the whole sequence with the software.

Specialized software for peripherals, in particular special sample introduction systems, as well as for performing time resolved measurements, for instance in hyphenated techniques, is also usually provided by the instrument manufacturers.

3 Analytical figures of merit

All multi-element methods in atomic spectrometry are in principal suited for analysis of lanthanoides. However, analysis by ICP-OES of lanthanoides in particular REE can be problematic due to very line-rich emission spectra causing significant spectral interferences. In comparison, the mass spectrum in ICP-MS is quite simple and consists only of peaks of all isotopes of the element of interest. In the mass range of the REE, polyatomic molecules are not observed and thus ICP-MS is often applied in industry, geology and in the life sciences. Concerning spectral interferences, the main limitation of REE analysis is oxide formation which is rather a big problem compared to other elements. Careful tuning allows reducing this value below 1 %, but if all REE are present at similar concentrations, as it is the case for many geological applications, than this problem can become an important issue.

For most of the applications, in particular for industrial quality and purity control of starting products, the oxide problem can be neglected. The LODs are quite low for lanthanoides, because their natural background is rather low. However, the LODs strongly depend on the application and thus will be discussed in more details in the application section of this book.

The analysis of lanthanoides, and in particular of REE, is a general topic of this book, thus different applications will be presented where different analytical methods are applied for multi-element analysis in different matrices (environment, geological samples, new materials) and of course ICP-MS can be applied for all these applications – a critical review is presented elsewhere [13].

In the next sections, we want to focus on a quite new application of ICP-MS where this method is used for a speciation analysis mainly of Gd-containing contrast agents in environmental waters [14].

4 Speciation of Gd-based contrast agents

Speciation has been defined by the International Union for Pure and Applied Chemistry as "speciation analysis is the analytical activity of identifying and/or measuring the quantities of one or more individual chemical species in a sample; the chemical species are specific forms of an element defined as to isotopic composition, electronic or oxidation state, and/or complex or molecular structure; the speciation of an element is the distribution of an element amongst defined chemical species in a system." [15].

The most important tool for speciation studies of metals is the combination of a separation and/or a fractionation technique with ICP-MS, and most often the ICP-MS instrument is only used as a metal-specific detector, which provides a time-resolved intensity measurement for the isotope of interest. The compound of interest then is identified by standards having the same retention time and if standards are available they also can be used for calibration, just to get quantitative results.

Different separation and fractionation techniques are described in literature, and their selection depends strongly on the compounds of interest. So far, the most common separation techniques applied in ICP-MS couplings are HPLC, GC and CE. They allow speciation analysis aiming to solve analytical problems in the life sciences and in environmental research.

ICP-MS, by itself or hyphenated to separation techniques, is a powerful tool for the analysis of contrast agents for magnetic resonance imaging (MRI). The mechanism of these compounds is based on the magnetic characteristics of Gd. In ionic form, Gd enhances the MRI signals. Since the Gd ion is highly toxic for humans, is it complexed with polyaminocarboxylates for a safe application and fast excretion.

Several Gd chelates are on the market as contrast media. Chelating agents can be macrocyclic or open chains as shown in Figure 3. According to the number of carboxylic groups, ionic or non-ionic complexes are formed. Because of their similar extracellular distribution, administration of any of the contrast agents results in the same diagnostic information. The only exception is cartilage; here the distribution of anionic agents is lower than neutral agents because of repulsion from the negatively charged glycosaminoglycans.

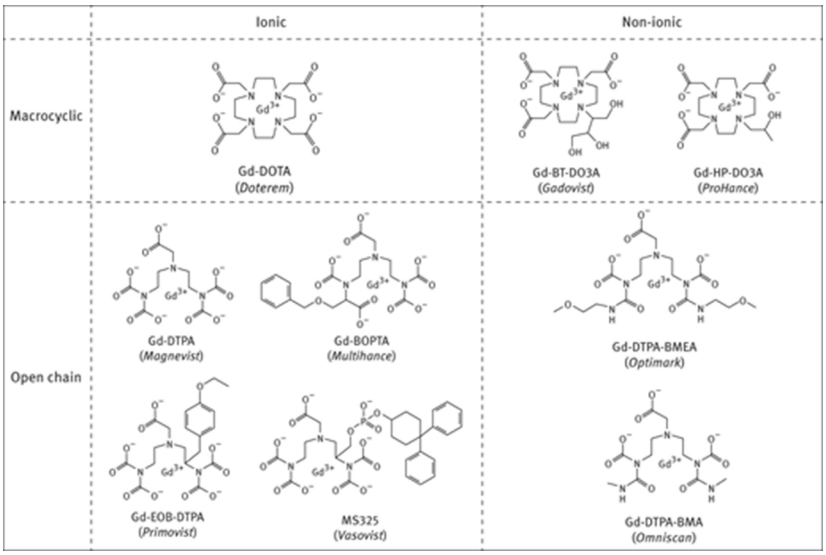

Figure 3: Structures of the Gd complexes employed as MRI contrast agents. The chelates can be separated in ionic and nonionic complexes and in complexes with linear or macrocyclic ligands.

Gd-based MRI contrast agents are administered intravenously eliminated rapidly by the kidneys and excreted unchanged in the urine. Due to the concentration level in which the contrast agents are administered (about 1 g/kg body weight) and the demand of detailed information even in low concentrations, an analytical method with low detection limits is required for the analysis of Gd in human body fluids. ICP-MS is often used for this kind of studies.

As described in Section 2.1 sample preparation is necessary for most ICP-MS applications, especially for samples with a high matrix content such as human body fluids. Many studies in the past included an acid-based digestion step in their procedure in order to free samples from unwanted organic compounds. Although Gd is not normally considered as a contaminant easily picked up from the environment, sample preparation has to be carried out with care. Analysis of blood and urine samples for Gd with ICP-MS has in the past been shown to be reproducible with a high precision. Many studies investigating the excretion and pharmacokinetics of the contrast agents have been successfully employed ICP-MS.

The Gd chelates have in clinical studies been shown to be excreted unchanged. However, the discovery of the cutaneous disease nephrogenic systemic fibrosis, a rare but fatal condition that has been linked to these compounds, demonstrated the need of further investigations. Especially potential species transformations within the patient's body came into focus. Because analytes are ionized completely in the plasma, ICP-MS only detects Gd signals without molecular information. The analysis strategy needed to be extended for the characterization of the respective Gd species.

Hyphenation to a separation system is commonly the method of choice. The coupling of HPLC to mass spectrometers has in particular been the focus of researchers for speciation analysis.

All Gd chelates that are being used as active ingredient in contrast agent formulations can be separated on a range of HPLC columns. As the complexes are highly polar or even ionic, finding a suitable column is crucial. The type of stationary phase determines its ability to separate analytes of different nature. Hydrophilic interaction chromatographic (HILIC) stationary phases have been applied for the separation of the complexes with great success.

HILIC columns can consist of any polar material and several surfaces, including for example amide- or silanol-bonded phases. The Gd chelates have been separated on zwitterionic bonded phases with great efficiency. The separation mechanism is described as a hydrophilic partitioning combined with ionic interactions. Eluents are similar to reversed-phase chromatography, often based on acetonitrile or methanol.

Other stationary phases that have been used in the past for contrast agent separation are porous graphitic carbon, C_{18}, as well as silica-based gel-filtration columns and size-exclusion columns. Many of these columns, however, were generally not suitable for the simultaneous separation of all available Gd complexes, but only for two or three of the complexes.

Hyphenation of HPLC systems to an ICP-MS requires an interface. In the simplest case fitting tubing for the transfer of the HPLC outflow directly into the nebulizer of the ICP-MS is sufficient. When the flow is very high, a splitting might be necessary. Often, the addition of oxygen is necessary after nebulization. Common ICP-MS instruments tolerate about 2% organic solvent. Higher percentages overburden the plasma and the plasma extinguishes or carbon depositions on skimmer and sampler cones can cause signal drift effects. The addition

of oxygen results in the reaction of the organic content to carbon dioxide. The use of common organic solvents like acetonitrile or methanol in HPLC characteristic percentages is possible.
Figure 4 shows the difference between a common ICP-QMS set-up for the analysis of Gd concentrations in directly introduced samples as described in Section 2.2 and an HPLC system hyphenated to an ICP-QMS. When the sample is introduced directly into the plasma, a spectrum is recorded and the intensity documented. The concentration of the analyte can be determined with the intensity of the selected isotope and respective calibration measurements as described in Section 3.

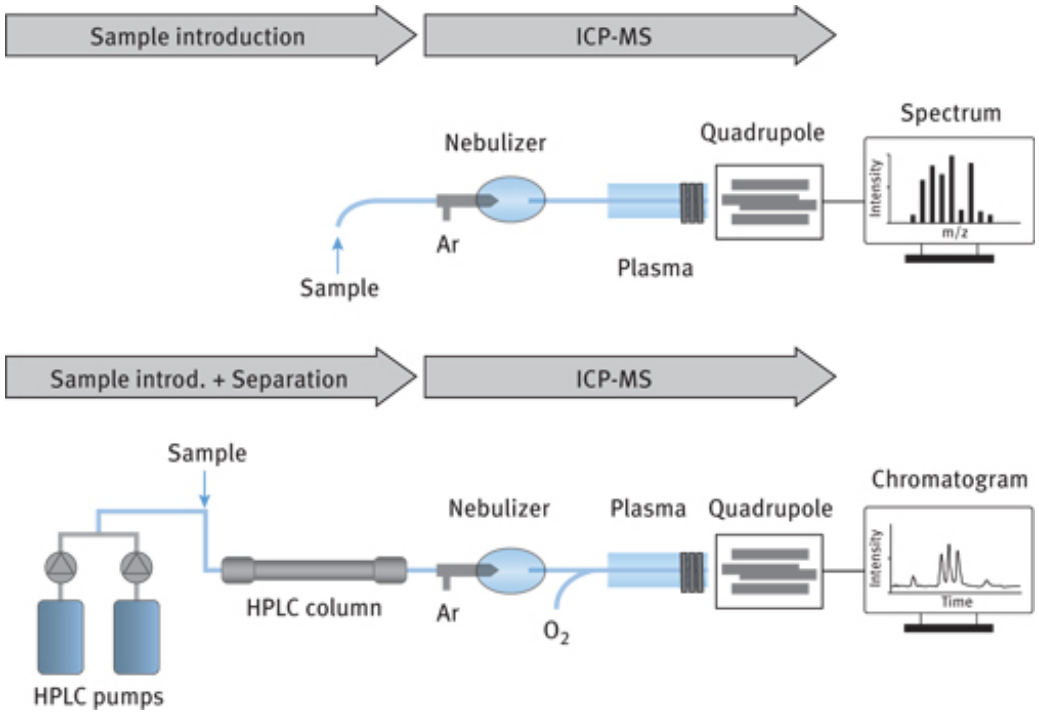

Figure 4: Schematic of direct sample introduction into an ICP-QMS (top) and hyphenation of an HPLC system to an ICP-QMS (bottom).

In the HPLC system, two pumps provide a mixture of two solvents for the chromatographic separation. The sample is introduced into the flow which then enters the column. On the column, the analytes are separated. After their respective retention time, the analytes exit the column and are being transferred with the HPLC flow into the nebulizer and subsequently into the plasma. The plasma ionizes all analytes, but due to the distinct retention time and transient signal recording, the chromatographic peaks can be assigned to the respective substance. This is done by comparing the chromatogram of the sample with chromatograms of standard solutions with known analyte composition and concentration that were run under the same conditions. Analytes in the samples can then be identified by comparing retention times which are specific for individual molecules.

Figure 5 gives an overview on different analytical methods for Gd quantification and speciation, respectively.

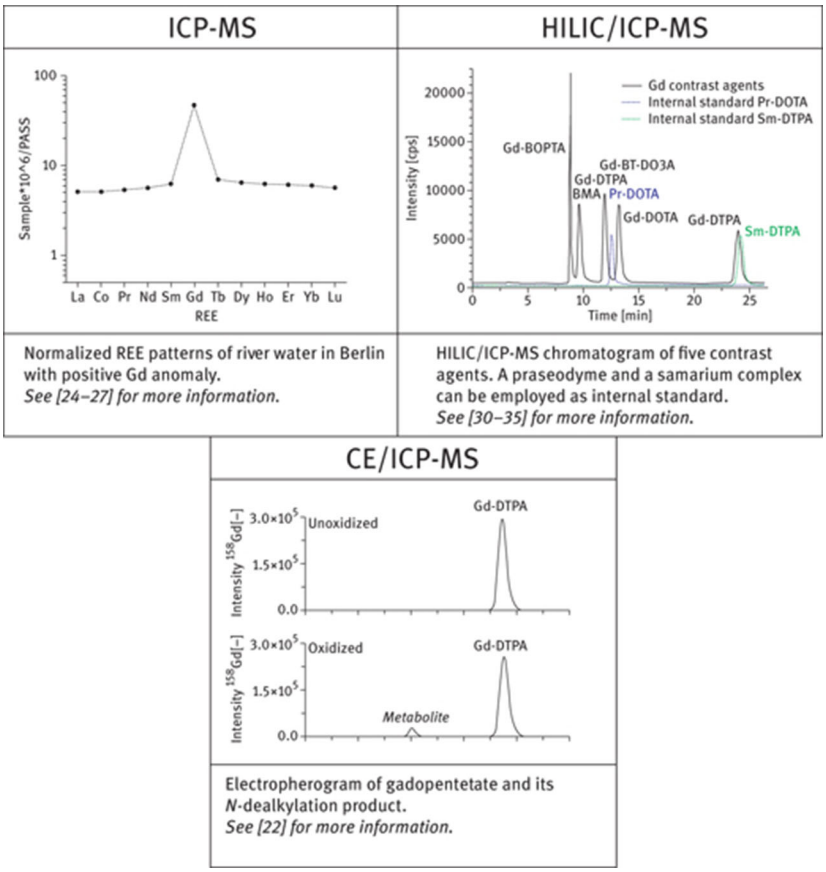

Figure 5: ICP-MS was employed for the determination of rare earth element concentration in river water. A chromatogram and an electropherogram show the power of separation techniques hyphenated to ICP-MS for Gd species analysis.

5 Analysis of Gd-based contrast agents in medical samples

The contrast agents are excreted rapidly and especially fast in the first few hours after application. Excretion monitoring and the assessment of pharmacokinetics were the first studies carried out with ICP-MS. The excretion can be monitored well with the procedure mentioned above. Elimination rates can be determined and total excretion from the patient's body could be confirmed. In comparison to other analytical methods such as ICP optical emission spectroscopy or total reflection X-ray spectroscopy, ICP-MS exhibits the necessary low detection limits for a full monitoring of Gd concentrations.

ICP-QMS has also been applied for the analysis of Gd in tissue, fingernail and hair. The high sensitivity of the method showed great potential for accurate pharmacokinetic studies down to very low concentration levels in a wide range of sample matrix [16–18].

With the ability for speciation analysis and therefore the distinction between different Gd species, several studies concerning the potential of contrast agent transformation were carried out. The first analysis of urine samples of an MRI patient by means of HPLC/ICP-QMS showed that the injected Gd-based contrast agent was excreted fast, undissociated and almost quantitatively (>99 %). The separation of free Gd ions and gadopentetate was carried out with a size-exclusion chromatographic column [19]. The assessment of complex stability identified a difference between linear and macrocyclic ligands. HPLC/ICP-QMS analysis detected Gd ions in human serum samples of chelates with linear ligands, indicating their release from the complex [20]. Similar results were found in a study concerning potential transmetalation of the complexes with iron ions. Transmetalation and the associated release of toxic Gd ions was shown to take place in samples with Gd complexes with linear ligands but not in samples with macrocyclic ligands. These results were shown by the complementary employment of HILIC/ICP-QMS and HILIC hyphenated to electrospray ionization (ESI)-MS [21]. The combination of the two analytical methods was also successfully employed for the investigation of metabolic reactions of the contrast agents. Electrochemical simulation of the oxidative metabolism and subsequent analysis of the respective samples by means of HILIC/ICP-MS and HILIC/ESI-MS indicated a ligand dealkylation and therefore destabilization of the complex. The same study employed another separation technique hyphenated to

ICP-QMS in order to achieve more information: ionic reaction products, especially Gd ions, were investigated by means of CE/ICP-QMS [22].

The analysis of a skin biopsy sample from a suspected nephrogenic systemic fibrosis patient was carried out complementary by laser ablation ICP-QMS and HILIC/ICP-QMS. It revealed the presence of the intact Gd complex that had been administered 8 years before the analysis [23].

As can be seen from the presented studies, ICP-MS is a powerful tool for the investigation of contrast agents and their behavior in the human body. It is very important, however, to choose the appropriate analytical strategy corresponding to the objective of the study. The hyphenation of separation techniques to ICP-MS is most often necessary for species identification.

HPLC/ICP-MS will without question continue to be one of the most important analytical techniques for the detailed investigation of contrast agents as well as a whole range of other (metallo-)drugs. The constant development of new stationary phases as well as the constant improvement of ICP-MS detection limits will encourage speciation analysis of pharmaceuticals in the future.

6 Analysis of Gd-based contrast agents in environmental samples

A second challenge concerning Gd-based MRI contrast agents was the discovery of high Gd concentrations in surface waters. In an extensive study, rivers, tap water, sea water as well as the effluent of a wastewater-treatment plant and a hospital were sampled. The concentrations of REEs, determined by ICP-QMS, were normalized to post-Archaean Australian Shale and showed a distinct Gd anomaly that indicated its anthropogenic nature [24]. Many studies were carried out since, all with ICP-QMS, all showing a similar pattern. Positive Gd anomalies ranging between 1.5 and 240 were exposed in many rivers that run through populated areas [25–27]. In sharp contrast, rivers in thinly populated areas did not show Gd anomalies [24]. The Gd source was soon to be traced back to the employment of MRI contrast agents [24]. Effluents of wastewater treatment plants were analyzed with ICP-SFMS and a significant output of Gd by the plants was proven. A balancing of Gd input and output with ICP-SFMS, employing isotope dilution analysis in order to avoid matrix effects, showed that only about 10 % Gd is removed during treatment [28, 29].

In most studies, sample preparation consisted of filtration and acidification before ICP-QMS analysis. For an even more efficient determination of trace levels of Gd in natural water samples, a pre-concentration method based on solid phase extraction was developed by Raju et al. A C18 phase coated with bis-(2-ethylhexyl)-phosphate yielded in hundredfold-Gd enrichment. Recovery for several Gd complexes was >95 % [30].

Due to the toxicity of the un-complexed Gd ion, studies soon focused on the investigation of the present Gd species. Wastewater treatment plant effluent was analyzed complementary with ICP-QMS and HILIC/ICP-QMS. The results showed that the complexes are mostly intact. However, total concentrations determined by ICP-QMS were higher than the concentration of complexed Gd determined with HILIC/ICP-QMS, indicating species transformation [31].

The dilution of Gd discharged by a wastewater-treatment plant into the adjacent channel was monitored by means of ICP-QMS. The highest concentration was found close to the plant outlet as expected. Already after 2 km, the Gd concentration was constant [32]. Samples from a nature reserve into which the effluent from a wastewater-treatment plant enters the environment were examined by HILIC/ICP-SFMS. Samples were introduced as dry aerosol generated by desolvation, a process that improved detection limits for all major Gd complexes to well below 0.1 nmol L^{-1}. Several contrast agents were determined and the mass balancing showed that the contrast agent concentration accounts for about 80 % of the total Gd concentration [33]. The analyte enrichment by means of surface evaporation enhanced the ability to analyze sample with very low Gd concentrations. Lake water samples were gently heated by infrared light by Raju et al. HILIC/ICP-QMS analysis of the samples detected Gd complex concentrations in the ng L^{-1} range [34].

Gd complexes cannot only be found in surface waters; the analysis of tap water samples with HILIC/ICP-QMS proved the presence of several of the chelates in river bank filtration water. The application of a praseodymium complex as internal standard successfully corrected intensity drifts as well as changes in sample volume and matrix effects [35].

HILIC/ICP-QMS was also an appropriate analytical technique for the investigation of the uptake of Gd-based contrast agents by plants. The complexes could be extracted from cress plants that were exposed to the contrast agents. The highest Gd concentration relative to the concentration in the growing solution was observed in the leaves. Lower concentration where determined in stem and roots, indicating a concentration gradient. Speciation analysis was important for these experiments to demonstrate that Gd is transported from

the roots to the leaves in complexed form [32]. An overview of studies employing different HPLC columns for separation of Gd complexes is presented in Table 2.

Table 2: Overview of different studies employing different HPLC columns for the separation of Gd complexes as well as different ICP-MS systems. The detection limit (LOD) is presented for five of the most used and most investigated Gd contrast agents [35].

		LOD in pmol L^{-1} (absolute mass in fmol)				
	Method	**Gd-BOPTA**	**Gd-DTPA**	**Gd-DOTA**	**Gd-DTPA-BMA**	**Gd-BT-DO3A**
Lindner et al. [35]	ZIC-*c*HILIC – ICP-QMS	14(0.14)	13(0.13)	14(0.14)	22(0.22)	19(0.19)
Lindner et al. [32]	ZIC-HILIC – ICP-QMS	400(2.00)	250(1.25)	310(1.55)	260(1.30)	390(1.95)
Birka et al. [23]	HILIC – ICP-SFMS (desolvation nebulizer)		90(0.18)	100(0.20)		80(0.16)
Telgmann et al. [21]	HILIC – ICP-SFMS	830(1.66)	830(1.66)	830(1.66)	830(1.66)	830(1.66)
Raju et al. [34]	ZIC-HILIC – ICP-QMS	130(0.65)	170(0.85)	110(0.55)	120(0.60)	150(0.75)
Künnemeyer et al. [31]	ZIC-HILIC –ICP-QMS	1,000(4.00)	1,000(4.00)	1,000(4.00)	1,000(4.00)	1,000(4.00)

7 Summary and outlook

ICP-MS is a powerful tool for analysis of lanthanoides in various matrices. This method will always be applied if multi-element analysis of as many as possible lanthanoides is required with lowest limit of detection.

Concerning speciation the determination of Gd concentrations in environmental samples will remain a focus for future research in analytical chemistry. In particular the question how far a bio-enrichment can occur in the biosphere of aquatic compartments has to be answered. The application of MRI contrast agents based on Gd will continue and no sewage treatment stage is in sight to prevent Gd from reaching surface waters. The improvement of ICP-MS detection limits, be it through instrument upgrades, advanced pre-concentration methods or enhanced sample introduction systems, will be of major interest for environmental studies because of the low Gd concentration in many samples. This will be especially important for speciation analysis in order to give a detailed overview of the contrast agent behavior during and after wastewater treatment.

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