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Inductively Coupled Plasma Mass Spectrometry for Stable Isotope Metabolic Tracer Studies of Living Systems

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ABSTRACT

Sub-nanogram per gram levels of molybdenum (Mo) from human blood plasma are isolated by the use of anion exchange alumina microcolumns. Million-fold more concentrated spectral and matrix interferences such as sodium, chloride, sulfate, phosphate, etc. in the blood constituents are removed from the analyte. The recovery of Mo from the alumina column is 82 ± 5% (n = 5). Isotope dilution inductively coupled plasma mass spectrometry (ID-ICP-MS) is utilized for the quantitative ultra-trace concentration determination of Mo in bovine and human blood samples. The average Mo concentration in reference bovine serum determined by our method is 10.2 ± 0.4 ng/g, while the certified value is 11.5 ± 1.1 ng/g (95% confidence interval). The Mo concentration of one pool of human blood plasma from two healthy male donors is 0.5 ± 0.1 ng/g.

The inductively coupled plasma twin quadrupole mass spectrometer (ICP-TQMS) is used to measure the carbon isotope ratio from non-volatile organic compounds and bio-organic molecules to assess the ability as an alternative analytical method to gas chromatography combustion isotope ratio mass spectrometry (GC-combustion-IRMS). Trytophan, myoglobin, and β-cyclodextrin are chosen for the study, initial observation of spectral interference of $^{13}C^+$ with $^{12}C^1H^+$ comes from the incomplete dissociation of myoglobin and / or β-cyclodextrin. The interference species is most sensitive to the aerosol gas flow rate. Carefully decreasing this parameter can eliminate $^{12}C^1H^+$ interference. $^{13}C/^{12}C$ ratios in myoglobin and β-cyclodextrin can be determined precisely and quantitatively. The

best relative standard deviation (RSD%) obtained in the $^{13}C/^{12}C$ study is 0.91%, which is close to the limiting precision predicted by counting statistics (1.16%).

Many improvements have been made to the current ICP-TQMS device. A new skimmer interface together with the new ion optics arrangement improves the ion collection efficiency 19-fold. A second-generation ion beam splitter has been designed to reduce the background count rate and improve the ion transmission. A modified ion beam splitter transmits 5 times more ions while maintaining the performance characteristics of the ICP-TQMS system. The precision is further improved by measuring each data point for a longer dwell time (9s instead of 1.5s) and keeping the total ion collection time low ($\leq$ 50s instead of 150s).

CHAPTER 1. GENERAL INTRODUCTION

Historical perspective of the inductively coupled plasma

Science and technology are advancing ceaselessly since the creation of mankind. From the discovery of a simple element to deciphering the origin of the universe, chemistry today is scarcely recognizable as the subject that chemists knew a century ago. From the ancient gravimetric analysis of grams of sample to the modern spectrometric determination of 10^{-9} grams of unknown, what once seemed beyond reason now defines the frontier of science. Advancement in analytical chemistry has led to significant progression in biotechnology and vice versa. With the aid of the computer engineering today, biochemists are able to pinpoint the location of a mutant base pair of a gene. Scientists in all fields are working together for the ultimate goal of unlocking the secret of life. Traditionally, the analytical chemist is the one that carries the burden of constantly developing new and improving techniques for qualitative and quantitative determinations of substances that are found on our planet Earth and in outer space. One of the milestones of modern science is the development of the inductively coupled plasma mass spectrometry, the prime candidate for trace and ultra-trace analyses.

This dissertation focuses on the development of methods for stable isotope metabolic tracer studies in living systems using inductively coupled plasma single and dual quadrupole mass spectrometers.

The origin of the inductively coupled plasma (ICP) can be dated back as far as nearly 60 years ago. The first atmospheric pressure ICP was invented by a Russian Physicist who resided in Leningrad (now Saint Petersburg) during the dark age of the human society [1]. During World War II, the ICP researches were hindered by the lack of electricity, which is the vital ingredient for generating the inductively coupled plasma. This infant ICP had stopped and remained undeveloped for nearly two decades. Not until the early 1960's, Reed continued the development of the atmospheric ICP [2, 3]. Reed also described many of the physical properties of this plasma and suggested its possible application as a spectral source for solids [4]. Later, a group of American and British scientists led by Fassel at Iowa State University in the United States, and by Greenfield at Albright and Wilson Ltd. in England studied the ICP as an analytical emission source [5-7]. It was Fassel and co-workers who were responsible for the complete development and maturing of this fiery plasma [8, 9].

Inductively coupled plasma spectrometry

At the time this new atomic emission ionization source was ready to be introduced to the world, ICP had to compete with the contemporary flame atomic absorption spectrometry (AAS) that was expanding exponentially and gaining worldwide acceptance as an analytical tool [10-15]. Meanwhile, the established arc source atomic emission spectrometry (AES) was declining in general usage. The combination of the growing AAS and the declining of AES had established the conditions for the ICP source being less accepted by the scientific community. Not until 1975 was the first commercial ICP spectrometer born. Since then, the ICP-AES has become the most common technique for routine multi-elemental analyses [9, 16, 17]. At the same time, the analytical capability of the ICP continued to be explored as an ionization source for mass spectrometry. Houk and co-workers obtained the first analytical mass spectra from an ICP in 1978 [18]. Five years later, Sciex launched the first commercial quadrupole based ICP-MS instrument, which stimulated the growth of interest in the technique. Since then, ICP-MS has gained world wide recognition as one of the most complete and versatile analytical technique for elemental determinations and isotopic analyses in geochemistry [19, 20], semiconductor industry [21, 22], environmental chemistry [23, 24], clinical chemistry [25, 26], and nuclear chemistry [27].

ICP-MS system

In general, the ICP-MS system consists of five parts: the sample introduction system, the ICP ionization source, the ICP-MS interface, the mass analyzer, and the electronics (including the detector, computer and data collection and processing). Gas samples can be directly introduced into the ICP, whereas solution and solid samples are introduced as fine aerosols and fine particles. There are a variety of sample introduction techniques for both cases.

Solution samples

Solution samples are usually introduced to the ICP as aerosols. Several types of nebulizers have been used to produce the fine mists. The standard nebulizer is the pneumatic type [28], which produces aerosol by shattering the liquid stream as it leaving the tiny tip of the quartz tube with a fast flowing gas stream on the outer concentric tube. The pneumatic type nebulizer produces primary aerosols with a wide droplet size distribution (diameter from 2 to 40μm) [29]. The larger aerosol droplets are removed as they pass through the spray chamber. A double pass Scott type spray chamber is normally used for this purpose [30].

The secondary aerosols are then sent to the ICP for desolvation, vaporization, dissociation, atomization, ionization, and excitation. An ultrasonic nebulizer (USN) in conjunction with a conical spray chamber has also been widely used for generating solution aerosol [31]. In more recent times, the USN system is usually equipped with a desolvating and condensing system to remove most of the solvent. The tertiary aerosol produced by the USN has a narrow aerosol size distribution with 90% of the droplets with only 5 μm diameter [32, 33]. A Babington type nebulizer is designed specially for a solution with high solid contents to avoid clogging [34]. There are other types of nebulizers specifically used for low flow nebulization of effluent that comes out of a HPLC or CE column. These include the microconcentric nebulizer (MCN) [35], direct injection nebulization (DIN) [36, 37], microflow ultrasonic nebulization (m-USN) [38], high efficiency nebulization (HEN) [39], and electrospray nebulization (ESP) [40-42]. Recently, Brown developed a low flow nebulizer known as the oscillating capillary nebulizer (OCN) with nebulizer efficiency of 100% [43]. Other less commonly used nebulizers and spray chambers are discussed in reference 44-46.

Recently, the monodisperse dried microparticulate injector (MDMI) developed by French is used to inject a single liquid droplet with diameter of ~ 40 μm to the ionization source [47]. This type of sample introduction technique is used mainly for the study of matrix effects [48-50] and will be discussed later. Finally, electrothermal vaporization, which was developed for atomic absorption spectrometry, can also be used as an alternative sample introduction technique for ICP-MS [51, 52].

Solid samples

Solid sample can be introduced to the ICP by several techniques: laser ablation [53-55], arc or spark nebulization [56, 57], and direct sample insertion [58, 59]. Slurry nebulization of fine particles has recently been used for geological samples [60]. Solid sample introduction has several advantages, including the reduction in analysis time because of no need for sample dissolution. Furthermore, spatial information can be achieved if laser ablation is used. However, the major drawback of direct solid sample analysis is the lack of matrix matched standard materials, making quantification difficult.

ICP operation

An ICP is a weakly ionized plasma. Physicists have described plasma as the fourth state of matter. The simplest definition of plasma is gases of ionized atoms and molecules. More precisely, plasmas are ensembles of charged particles interacting with one another through coulombic forces. Strong electric forces attracting opposite charges in the plasma provide its quasineutrality. Plasmas behave like fluids. They can flow, they follow physical boundaries, they carry waves, and they react with electric and magnetic fields in many ways.

The inductively coupled argon plasma is energized by a high frequency electromagnetic field. The allowable operating frequency is 27 or 40 MHz with power output from 700 to 1500 Watts. Figure 1 shows the cross section of the ICP torch and the load coil surrounding it. The ICP torch consists of three concentric quartz tubes. The outer gas (usually argon) flows tangentially and spirals between the outer and the middle tubes with flow rate of ~ 15 L / min. This outer flow is the main supporting gas for the plasma and also acts as the cooling gas for the outer quartz tube. A second argon flow is known as the makeup

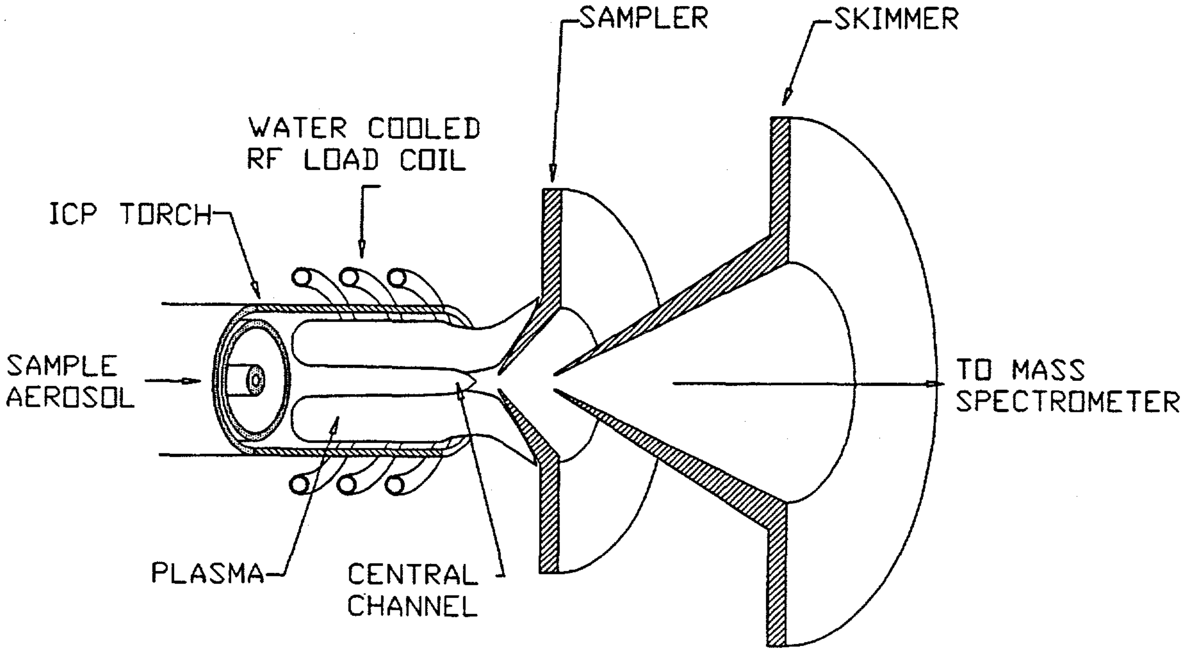

Figure 1. Schematic diagram of the ICP torch and ICP-MS interface [61].

or auxiliary flow with a flow rate of ~ 1 L / min. and flows between the outer and the middle tube. This makeup flow keeps the hot argon plasma away from the tip of the central tube known as the injection tube, and protects it from melting. A third argon flow called the nebulizer gas flow or aerosol gas flow, punches a hole through the hot plasma to form a relatively cool region known as the axial channel and carries aerosols from the sample introduction system to the ICP. The flow rate of this third flow is ~ 1 L / min. The relatively cool axial channel confines the sample aerosols in the central channel and improves the ion extraction efficiency at the ICP-MS interface [62-69]. Since the sample aerosols travel through the central channel well separated from the induction region, the basic electrical processes that sustain the plasma are not overly sensitive to the changes of sample composition.

The argon-ICP produces mostly singly charged species. The exact mechanism of ionization in the ICP is not known. However, there are at least three processes responsible for the ionization.

Electron impact

The most effective pathway for the production of charged particles is the result of collisions between electrons and atoms or molecules.

$A + e^{-}_{fast} \rightarrow A^{+} + 2e^{-}_{slow}$

Penning ionization

The Penning process involves the production of a free electron via the collision of excited atom (A^*) with atom or molecule (B) provided the excitation energy of A is greater than the ionization potential of B.

$A^* + B \rightarrow A + B^{+} + e^{-}$

Non-resonant charge transfer reaction

The ionized species A^+ in a higher energy state collides with the species B in a lower energy state to produce the ionized excited B^{+*} and the neutral lower energy species A. Energy is thus transferred from A^+ to B. The process is highly selective, the two energy levels of the A^+ and the B^{+*} have to be closely matched (± 0.5 eV), and the energy difference between B^{+*} and B should not be greater than the energy difference between A^+ and A [70]. The whole process can be summarized as follow.

$A^{+} + B \rightarrow A + B^{+*}$

There are other reactions between atoms and ions occurring at the same time in the plasma such as resonance charge transfer, excitation of atomic level by electron impact, second ionization reactions.

ICP-MS interface

The ICP-MS interface provides the means of extracting the positive ions from the ICP into the mass analyzer. Differential pumping is used to reduce the pressure from 760 Torr to ~ 10^{-5} Torr. The sampler and skimmer serve as the first and second differential pumping orifices as well as the ion extraction media [71] [Figure 1]. The pressure behind the sampler is ~ 1 to 2 Torr. A supersonic free jet expansion is formed behind the sampler and terminated at the Mach disk as the extracted beam collides with the background gases. The skimmer is usually positioned at ~ 1/2 to 2/3 the distance downstream from the sampler orifice to the onset of the Mach disk for optimum ion extraction [72]. Somewhere behind the skimmer, the quasineutrality of the beam breaks down and electrons are lost. Now the beam is composed mainly of positive ions (detailed description of the ion extraction process is given in reference 73). The skimmed beam is then focused and guided by a series of ion optics to the third pressure stage (~ 10^{-5} Torr) that houses the mass analyzer which differentiates ions by their different m/z values.

Mass spectrometer

The quadrupole is the most widely used mass spectrometer in conjunction with the ICP owing to its strong focusing properties, robustness, simplicity, ease in operation, high performance to cost ratio, and tolerance to moderate pressure environments (~ 10^{-5}) [74-77].

A quadrupole mass analyzer is composed of four metal rods. Opposite pairs are connected together, and dc and rf potentials are applied to each pair in opposite sign and phase. For simplicity, the trajectory and stability of the ion motion inside the quadrupole can best be described with the stability diagram [Figure 2] where

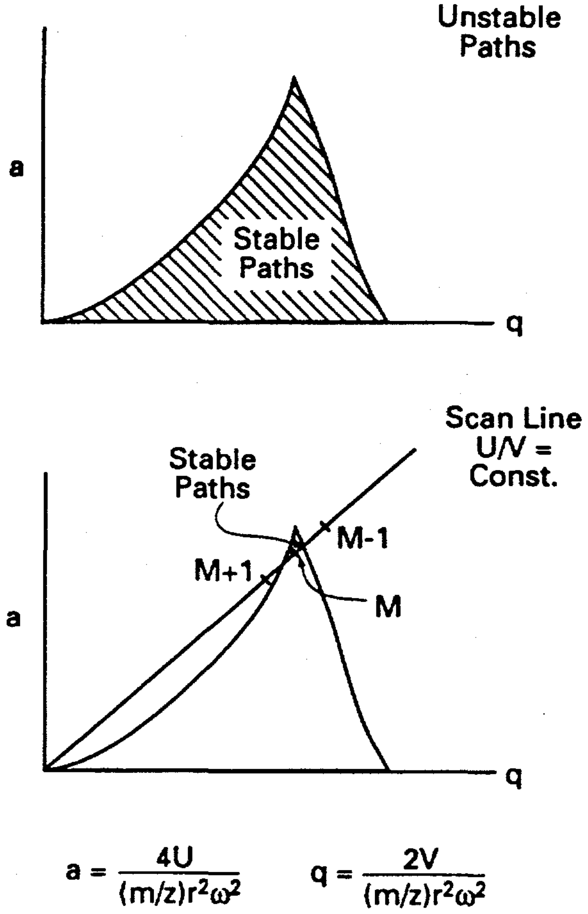

Figure 2. The a-q stability diagram [63].

$$a = 4zU/m\omega^2 r_0^2 \quad (1)$$

$$q = 2zV/m\omega^2 r_0^2 \quad (2)$$

U is the applied dc voltage, V is the applied rf amplitude and m is the mass of the ion. For a given set of rods, ω (angular frequency 2πf) and r_0 (distance from the central axis to the rod) are constant, hence, a ~ U/(m/z) and q ~ V/(m/z). As seen from Figure 2, varying the slope of the scan line (changing the a/q ratio) changes the resolution, whereas changing the values of U and V while keeping the a/q ratio constant transmits ions of different m/z value. Scanning or changing the resolution is done solely electronically.

Other mass spectrometers have also been employed with ICP such as the ion trap [78, 79], time of flight [80, 81], double focusing magnetic sector [82-86], and the ion cyclotron resonance cell [87].

Ion detection

Different types of detectors have been used for ion detection in ICP-MS such as the Daly detector [88], Channeltron electron multiplier [89], and now the discrete dynode type detectors [90] are used in most instruments. The Faraday cup detector with no gain mechanism has also been used [91]. Recently, the active film discrete dynode electron multiplier is becoming popular for its better gain stability and longer operating life, larger linear dynamic range, and tolerance to higher-pressure environment than the other detectors [92].

Analytical applications of ICP-MS

ICP-MS offers outstanding linear dynamic range (at least 6 to 8 orders of magnitude), low concentration detection limits (sub-part per trillion) for a wide range of elements, fast multi-elemental determination, and good precision in isotope ratio measurements [63]. The analytical application of ICP-MS is immense, especially in trace and ultra-trace analyses of geological, clinical, environmental samples, as the result of the striking features of the technique. There are hundreds of publications using ICP-MS as an analytical tool for routine elemental determinations and isotopic analyses. It is not feasible to name them all, only a few representative reviews are included in the references at the end of this dissertation [63, 93, 94].

Problems of ICP-MS

Despite the numerous advantages offered by ICP-MS, it is somewhat susceptible to spectral interference and non-spectral interference or matrix effects.

Spectral interference

Spectral interference arises from the spectral overlap of two or more ions sharing the same nominal mass. For examples, $^{40}Ar^+$, $^{40}K^+$, and $^{24}Mg^{16}O^+$ interfere with $^{40}Ca^+$; $^{40}Ar^{35}Cl^+$, $^{150}Nd^{+2}$, $^{150}Sm^{+2}$ interfere with $^{75}As^+$; $^{40}Ar^{16}O^+$, $^{40}Ca^{16}O^+$ interfere with $^{56}Fe^+$. A complete list of possible spectral interferences can be found in references 95-97.

Methods for attenuating the formation of metal oxide (one of the interference species) include altering the plasma operating conditions (forward power, aerosol gas flow rate, sampling position, etc.) [63, 98, 99], and adding certain molecular (N_2, H_2) or noble gases (Xe) to the central channel [100, 101].

Another method includes the use of a high-resolution mass spectrometer to solve the spectral interference problems. For example, Begerow and Dunemann used a high-resolution ICP-MS to determine Pd and Rh from human blood [102]. The determination of Pd and Rh using a quadrupole at standard operating conditions is impaired by spectral interferences of Cd^+, Pb^{+2}, RbO^+, SrO^+, $CuAr^+$, and $ZnAr^+$. However, even when the magnetic sector was operating at a resolution of $m/\Delta m = 7500$, it is still not sufficient to separate Pd and Rh from all spectral overlaps. Jiang et al. [103], Nonose [104], Sakata [105] and Tanner [106] have explored the possibility of operating the plasma under a "cooler" condition to attenuate some of the polyatomic interferences. When the plasma is kept cooled by increasing the aerosol gas flow rate and decreasing the rf power, troublesome background ions Ar^+, ArH^+ and ArO^+

are removed, the background mass spectrum is simplified dramatically [105]. However, polyatomic ions NO^+, O_2^+ and/or H_3O^+ still remain in the background spectrum. Other researchers have explored the use of helium plasma instead of argon plasma [107-109]. For some reason, though, the He ICP is much harder to operate than the Ar ICP. Cryogenic removal of organic solvents to attenuate certain polyatomic ions has also been performed [110]. Lastly, the best way to eliminate the problem is to employ chromatographic separation techniques to isolate the analyte from the interference species.

Non-spectral interference or matrix effects

Matrix effects arise from the change in analyte signal caused by the change in concentration of matrix elements. Normally, as the concentration of the matrix element increases, the analyte signal decreases. This interference is known as matrix-induced signal suppression [101-115]. In some cases, matrix induced analyte signal enhancement is also observed [116]. ICP mass spectrometrists have come a long way in studying and understanding matrix effects [117]. A common conclusion is that matrix effects are most severe when the analyte is light (such as lithium) and the matrix is heavy (such as lead). Researchers are now convinced that many aspects of the observed matrix effects phenomenon are attributed mainly to the space charge effect. Space charge effect is the coulombic repulsion of like charges when the ion current exceeds the space charge limiting current [73, 98, 118, 119]. The contribution of space charge to matrix effects was first proposed by Gillson et al. in 1988 [118] and later by Tanner [98], Li et al. [119], and Chen and Houk [117]. Matrix effects have been studied by spatial-resolved and time-resolved methods. The former one involves the deposition of ions onto a target [117, 120], and the later involves using monodisperse dried microparticlutate injector and laser fluorescence techniques to observe the temporal signal of the light analyte (Li^+) in the present of a heavy matrix (Pb^+) [48-50]. Allen et al. has also used the same sample introduction technique to study the matrix effect in their ICP-twin quadrupole system [121]. There are numerous ways to attenuate or correct for matrix effects. These are optimization of instrument parameters and ion optics, internal standardization, isotope dilution, other calibration methods, chromatographic separation of analyte from the matrix elements, and chemical vapor generation.

Optimization of instrumental parameters and ion optics

Wang and Caruso et al. [122, 123], and Marshall and Frank [124] successfully alleviated matrix effects by optimizing instrumental parameter such as rf forward power, aerosol gas flow rate, ICP sampling position, and etc. Evans and Caruso [125], Hu and Houk [126], Ross and Hieftje [127], Vaughan and Horlick [128], Chen and Houk [117], and Tanner [98] alleviated matrix effects by manipulating the ion lenses potentials. Beauchemin et al. added N_2 gas to the outer plasma flow to reduce the matrix suppression [129-131].

Internal standardization

An internal standard can compensate for matrix effects, instrumental instability and signal drift. Munro et al. first applied the technique to ICP-MS [132]. A variety of internal standard elements that span the whole periodic table are often employed [133-137]. For example, ^{9}Be, ^{59}Co, ^{115}In, and ^{205}Tl are common internal standards. This method requires the properties (both atomic mass and ionization potential) of the internal standard to closely match that of the analyte. This is often the limiting factor, which leads to a more sophisticated type of calibration method, which is discussed below.

Isotope dilution

Isotope dilution is the most powerful calibration method for elemental quantitative determination in conjunction with ICP-MS [138]. It is the ideal form of the internal standardization method. This technique is often used in the nuclear industry and isotope geology [139]. In 1969, the National Bureau of Standards (now the National Institute of Standards and Technology) first applied isotope dilution mass spectrometry (ID-MS) to certify a Standard Reference Material and to resolve a discrepancy between two analytical methods [140].

The isotope dilution method is based on the measurement of the change in ratio of two selected isotopes after one was enriched with a known amount of standard. If the element to be determined in the sample has two or more isotopes (e.g. vanadium-50 and vanadium-51), the more abundant isotope is often chosen to be the reference isotope (^{51}V), and the less abundant isotope is used as the spiked isotope (^{50}V). Assuming the vanadium in the sample is at natural abundance, then the ratio of ^{50}V / ^{51}V in the original unspiked sample is the same as the natural abundance ratio (0.0025 / 0.9975). After the sample is spiked with a standard of known weight and known concentration of vanadium as well as known ^{50}V / ^{51}V ratio (e.g. 0.56468) or known atom fraction, the altered ^{50}V / ^{51}V ratio is measured by ICP-MS. The measured $^{50}V/^{51}V$ ratio can be expressed mathematically in terms of the weight (W), atom fraction (A for ^{50}V, B for ^{51}V), and concentration of the original (C_x) and spiked samples (C_s).

$$R_m = (A_xC_xW_x + A_sC_sW_s) / (B_xC_xW_x + B_sC_sW_s)$$

Since every term in the expression is known except C_x, the original concentration of vanadium can be calculated.

The advantages of isotope dilution are: First, it can compensate for partial loss of the analyte during sample preparation. Second, it is immune to a wide variety of physical and chemical interferences because the two selected isotopes are from the same element. Any physical or chemical interference on one will have to same effect on the other, causing the effects to be cancelled out in the ratio measurement.

The disadvantages of isotope dilution are: First, the method is not applicable to mono-isotopic elements. Second, not all of the enriched isotopes are available. Third, the enriched isotope is usually costly. Fourth, the method is very time consuming for routine analysis.

Other calibration methods

Chen and Houk utilized the strong polyatomic ions as internal standards for matrix corrections in ICP-MS [117]. This is based on the fact that the signals of the polyatomic ions are suppressed by the matrix to the same extent as the analyte ion signals at nearby m/z values. For example, $^{14}N_2^+$ can be used as internal standard for $^{24}Mg^+$, and similarly, $^{35}Cl^{16}O^+$ for $^{55}Mn^+$, $^{40}Ar^{16}O^+$ for $^{59}Co^+$, $^{32}S^{16}O^+$ for $^{45}Sc^+$, $^{40}Ar_2^+$ for $^{75}As^+$, and $^{89}Y^{16}O^+$ for $^{103}Rh^+$. Traditionally, the standard addition method has been used to overcome the matrix effects in ICP-MS [141-143].

Chromatographic separation of analyte from the matrix elements

This is by far the most effective way to separate the analyte from the matrix interferences in order to obtain accurate and precise quantitative information of the elements. Much of the emphasis has been placed on rapid and direct analysis with minimal sample preparation. The analytical capability of the ICP-MS is degraded as a result of untreated samples with high solid contents. Chemical separations can indeed improve the detection limit of the system. It can also improve the precision and accuracy of the measurements

[144-147]. The following are some examples of sample cleanup with the aid of liquid chromatography.

Beauchemin and McLaren et al. [141, 146, 148], and Orians [149] utilized silica-immobilized 8-hydroxyquinoline to preconcentrate trace metals from sea water to remove matrix interferences and improve the detection limit as great as 7-fold. Miyazaki used Chelex-100 to preconcentrate lead for isotope ratio determination [150]. Jarvis et al. employed a commercially available ion chelation system (cellulose-immobilized ethylenediaminetriacetic acid) for the separation of many transition and rare earth metals from a sea water matrix [151].

Size exclusion chromatography has also been used to separate proteins containing metals [152]. HPLC and supercritical fluid chromatography can also be coupled with ICP-MS to separate organometalic compounds [153-160]. Recently, online capillary electrophoresis (CE) coupled to the ICP-MS has been explored [161, 162]. The high resolution of CE offers significant advantages for the determination of chemical speciation in complex mixtures. Finally, cryofocusing GC-ICP-MS systems have been described by many workers for the determination of selenite, organometallic and organometalloid species in river and harbor sediments [163].

Chemical vapor generation

Hydride generation continues to dominate the field of chemical vaporization. Environmental analysis appears to be the primary motivation for the continuing development of hydride generation. Geological applications are the second motivation. Hydride generation is a very effective method to separate the analyte from the matrix components.

There are many publications in the field, but only some representative papers are given in the references [164-166].

Signal drift and instrumental instability in ICP-MS

Signal drift with time is another problem of the ICP-MS system at least for now. Several causes have been identified, which include temperature dependent fluctuations in the quadrupole power supply [93] and gradual solid deposition on the sampler orifice. By limiting the total solute levels < 0.1% the latter problem should be minimized. However, the former problem still remains. This indeed limits the analytical precision of the quadrupole instrument. Other factors limiting the precision are associated with the noise of the ICP-MS system. Because of instrumental instability and signal drift, fast peak hopping is preferred than mass scanning for isotope ratio measurements. Although the peak can be hopped for a very short time scale (~ 1 ms), the precision of isotope ratio measurement is still limited to ~ 0.05% to 0.1% relative standard deviation (RSD%) in the single quadrupole system.

Improving precision by simultaneous measurement of two signals

Warren and co-workers constructed an instrument several years ago called the inductively coupled plasma twin quadrupole mass spectrometer (ICP-TQMS) mainly for improving precision during isotope ratio measurement [167]. The extracted ion beam is split into two parts by an ion beam splitter (discussed in greater details in Chapter 3). Each part of the beam is sent to its own quadrupole for m/z filtering and its own detector. In this fashion, both beams experience nearly the same level of noise at any given time. Thus, the noise on both sides is cancelled when the ratio of the two-m/z value is taken [168, 169]. In comparison with thermal ionization mass spectrometry (TIMS) [170-175], the long time champion in isotope ratio measurement, ICP-TQMS is attractive with its low operating cost, fast semi-quantitative multi-elemental determinations, and no time dependent isotopic fractionation. However, the peak profile generated by the quadrupole is far from "flat top" shape, which is always the case with the magnetic sector when operating at low resolution, whereas the problem associated with the TIMS method [165-170] mainly comes from the ionization source. Walder and co-workers has combined the best feature of ICP-TQMS and TIMS to generate a new instrument known as the multi-collector (MC) ICP-MS [83-85]. This instrument is capable of performing isotope ratio measurement with ultra high precision. In comparison with MC-ICP-MS, ICP-TQMS has the unique ability of measuring two m/z values far apart.

Dissertation objectives and organization

This dissertation consists of five chapters: The general introduction, three research manuscripts either published in or ready to submit to scientific journals, and the general conclusions. Each research manuscript has its own abstract, introduction, conclusion, acknowledgment, and references. The tables and figures in the manuscripts have their own numbering system. The references are formatted according to specifications of the corresponding journal.

Chapter 2 is a published scientific paper. This paper describes a novel chromatographic separation technique for the isolation and recovery of ultra-trace amounts of molybdenum from human blood plasma for the quantitative concentration determination of Mo using isotope dilution ICP-MS. This method can be used for isotope tracer study and Mo in complex organic matrixes can be quantified.

Chapter 3 introduces to the world a future alternative analytical method to gas chromatography combustion mass spectrometry for carbon isotope ratio determination in non-volatile bio-organic molecules (amino acid, protein, and carbohydrate) with an inductively coupled plasma twin quadrupole mass spectrometer device. To the best of our knowledge, we are the pioneers in determining the ^{13}C / ^{12}C ratio using ICP as the ionization source. Chapter 3 also presents the ion collection efficiency of our home-built ICP-TQMS device which can be increased 14-fold if the first ion extraction lens is positioned ~ 1/2 to 2/3 from the skimmer orifice to the onset of the second Mach disk.

Chapter 4 provides several instrumental approaches for improving the ion collection efficiency of our elemental ICP-TQMS system. This includes designing a new ion beam splitter, the component responsible for splitting the ion beam into two parts to achieve simultaneous measurement of two individual m/z values. This chapter also contains the first detailed statistical report on the relationship between dwell time and total ion collection time and their influences on the precision of isotope ratio measurements of the ICP-TQMS system.

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CHAPTER 5. GENERAL CONCLUSIONS

Methods for quantitative determination of ultra-trace level of molybdenum (Mo) in biological samples are limited because of the high solute content of matrices and the resultant spectral and non-spectral intereferences. Chromatographic isolation of Mo from the million-fold more concentrated sodium, chloride, sulfate, phosphate, etc. in aliquots of blood plasma is successfully accomplished using anion exchange microcolumns of alumina (made in house) with Mo uptake from 0.01 moles L^{-1} HNO_3 and elution with 3.75 moles L^{-1} NH_4OH. Sensitive and selective inductively coupled plasma mass spectrometry (ICP-MS) with isotope dilution is utilized for accurate ultra-trace concentration determination of Mo in the purified blood samples. This new level of detection and quantification has re-opened the door for many scientists in the field of biology, biochemistry, and clinical nutrition for their studies of molybdenum metabolism, functions, and distribution in plants, animals, and microorganisms.

Inductively coupled plasma with a twin quadrupole mass spectrometer (ICP-TQMS) has been demonstrated for high precision carbon isotope ratio measurements in amino acids, proteins, and carbohydrates. The ICP-TQMS system could also measure carbon ratio from gaseous or solid samples with proper sample introduction techniques. Carbon isotopic analysis is an important tool in geology [1], environmental science [2], biology [3], and medicine [4]. In biological and medical studies, analysis of isotopic ^{13}C / ^{12}C ratios enable the study of metabolic pathways in living systems. At present, either the radioactive tracer ^{14}C or stable ^{13}C isotope is used for such studies. The used of ^{13}C as a tracer for diagnostic purposes is a rapidly growing field [5]. Study using radioactive tracer is rapidly declined due to potentially harmful radiation effects and problems of radioactive waste disposal. In geological studies, such as the sedimentation of carbonates, or in atmospheric studies, such as polar ice core and oceanic isotope ratio measurements, measuring $^{13}CO_2$ / $^{12}CO_2$ ratios enable the determination of the cycle of CO_2 production and absorption throughout history. In comparison with the current well-established gas chromatography combustion isotope ratio mass spectrometry (GC-combustion-IRMS) for determination of carbon isotope ratios from non-volatile organic compounds, the ICP-TQMS system is attractive for its speed in analyses, less severe memory effect, no isotopic fractionation, and no need for ^{17}O correction. The ICP twin quadrupole mass spectrometer utilizes two detectors for ion detection. This design greatly facilitates the task in simultaneous measurements of two isotopes or two ions with very large small or very large ratios (can be as great as 1 to 132).

The original ICP-TQMS interface design has been modified with a new skimmer interface. The combination of the new interface and the new arrangement of the ion optics result in a substantial improvement of signal to noise ratio. Further improvement of the system can be achieved with a new ion beam splitter design. Precision in isotope ratio measurement can further be improved by changing the dwell time and total ion collection time.

Overcoming instrumental drift and reaching for ultra-high precision in isotope ratio measurements with the current ICP-TQMS device continues to be a challenge. The greatest challenge is perhaps the utilization of as many ions as possible that are created in the ICP. This requires substantial modification of the ICP-MS instrument as a whole.

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