Application Note 30300

Sulfur Isotope Measurement in Human Serum

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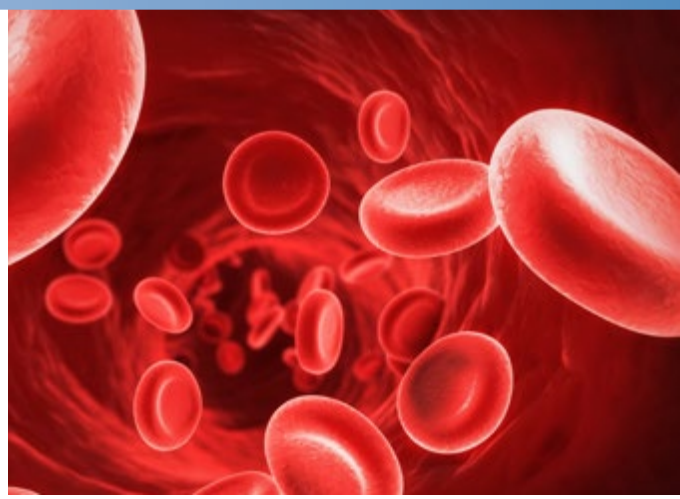
Keywords
Sulfur Isotopes, Multicollector ICP-MS, Human Serum, High Resolution

Goal

The major purpose of this study is to evaluate the potential of Multicollector ICP-MS for the analysis of sulfur isotopes in biomedical applications, which may help overcome particular problems with the commonly used EA-IRMS technique.



Introduction

The use of natural stable isotopes to study human biology has greatly expanded over the last decade and is currently providing a novel and informative perception of how metals are involved in disease relative to the more traditional analyses of element concentrations. In order to understand the metabolic cycling of the elements in normal and pathological pathways, isotopic fractionations have previously been measured in organs, body fluids and biological material for elements such as Fe, Cu, Zn and Ca (Walcyk and von Blanckenburg, 2002; Krayenbuehl et al., 2005; Albarède et al., 2011; Morgan et al., 2011; Jaouen et al., 2012; Balter et al., 2013; Tacail et al., 2014; von Blanckenburg et al., 2014).

Sulfur is a major element in the human body (0.3%). It plays a functional role in all living organisms. It takes part in the composition of two major amino-acids, cysteine and methionine, that compose many proteins, notably albumin. The concentration of sulfur is about 1200 mg/L in the human serum, about 300 mg/L in the plasma and 1700 mg/L in the whole blood.

Typically the sulfur isotope composition of geological materials is measured using elemental analysis coupled to isotope ratio mass spectrometry (EA-IRMS), in which S is converted to SO_2 gas and subsequently analyzed by the mass spectrometer (Giesemann et al., 1994; Grassineau, 2001, de Groot, 2008). During the years, the method has been improved and can be considered a routine-base technique (Grassineau et al., 2006; de Groot, 2008). Sample preparation is minimal, with little or no need for chemical separation. Precision on replicates of mineral isotopic standards is in the order of ±0.2‰ (2σ), which requires the extraction of a few mg of elemental sulfur (Grassineau et al., 2006). It has only been in the last ten years that multicollector inductively coupled plasma mass spectrometry (MC-ICP-MS) has been used to measure the isotopic composition of sulfur (Clough et al., 2006, Craddock et al., 2008) providing better precision than gas spectrometry with only a few ng of elemental sulfur. Recently, Paris et al. (2013) have shown that, working at high mass resolution mode, $\delta^{34}S$ values of natural samples could be measured with a typical reproducibility of 0.08–0.15‰ (2sd) on 5 to 40 nmol sulfur introduced into the Thermo Scientific™ Neptune Plus™ multicollector ICP-MS.

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One purpose of the present study is to evaluate the potential of MC-ICP-MS method for sulfur isotopes analysis for biomedical applications, which may help overcome some problems with the EA-IRMS technique. Blood samples (serum, plasma…) contain high Na and K contents and it is observed that, although Na does not interfere with the measurement, K is known to form ash with the tin background present in EA-IRMS, which leads to flow restrictions, poor combustion and biased sulfur isotope compositions.

For this particular study, we measured the isotopic compositions of sulfur in human serum and plasma on a Neptune Plus MC-ICP-MS in high resolution mode. The accuracy of the method is consistent with the work of Paris et al. (2013) and was validated by measurements of the standard reference materials IAEA-S1, S2, S3 and S4. The typical reproducibility obtained on standard and sample solutions is similar to the one achieved by the IRMS method (Grassineau et al. 2006), but the minimal amount of sulfur required for such measurement is one to two orders of magnitude lower than for the IRMS technique. In this work, we have also tested whether sulfur chemistry purification could be coupled to Cu, Zn and Fe separations in order to determine the isotopic compositions of the four elements on a singular sample of only 200 µL of serum or plasma.

Chemical Purification

All biological samples were digested in a mixture of concentrated HNO_3 and H_2O_2 in closed vessel on a hot plate (130 °C) for 12 hours. After evaporation to dryness the digestion was repeated to insure the full breakdown of organic matter. The four IAEA isotopic standards were dissolved in concentrated HNO_3. Sulfur was purified in one column chemistry step on a strongly anion-exchange resin according to a rather standard protocol (Karalova and Shibaeva, 1964; Menegario etal.,1998). We preferred this method to cation-exchange procedures (Cradock et al., 2008) because of the high sulfur blank caused by the sulfonic acid functional groups of the cation-exchange resin. After digestion, the dried residues were taken up in diluted HNO_3 0.03N and put through Bio-Rad™ columns filled with 0.8 ml of 200–400 mesh AG1-X8 anion-exchange resin in chloride form. The resin was initially washed with 10 mL of distilled water, 20 mL of 6N HCI and rinsed with 40 mL of distilled water. After loading in HNO_3 0.03N media, the matrix was removed with 10 ml of distilled water then sulfur eluted with 5 mL of HNO_3 0.5 N. The recovery of sulfur was calculated to be ~98 ± 2 %. The total procedural blank was ~15 ng.

Measurements and Data Acquisition

Sulfur isotopic compositions were measured on a Neptune Plus MC-ICP-MS with a Cetac Aridus II desolvator. Typical settings are described in Table 1. Sulfur has four isotopes (^{32}S, ^{33}S, ^{34}S and ^{36}S) that are all affected by polyatomic isobaric interferences at higher mass than the isotopes of interest. Because of the major interference created by ^{36}Ar, ^{36}S could not be measured. Significant interferences by O_2^+ occur at all sulfur masses with a relative mass separation of M/ΔM of 1801, 1460 and 1296 relative to ^{32}S, ^{33}S and ^{34}S respectively. Resolving the main interference $^{32}S^1H$ peak from the ^{33}S peak requires a resolution of 3908. Isotope ratios were therefore measured in high-resolution mode (R~9000). The central cup was positioned on the interference free plateau (Figure 1).

Table 1. Neptune Plus parameters.

Mass Spectrometer Settings	
RF power	1200 W
Cool gas	15 µL/min
Aux gas	~0.9 µL/min
Sample gas	~1.1 µL/min
Aridus Settings	
Spray chamber temp.	110 °C
Desolvating membrane temp.	160 °C
Ar flow	9 L/min
N2 flow	5 mL/min
Nebulizer	Savillex 100 µL/min
Cones	Std sampler+ H skimmer
Measurements Settings	
Cup configuration	^{32}S (L4), ^{33}S (C), ^{34}S (H4)
Resolution mode	High
Acquisition	40 cycles
Integration time	4 s
Uptake time	90 s
Quick wash	HNO_3 0.05 N
Quick wash time	30 s
Blank substration	yes

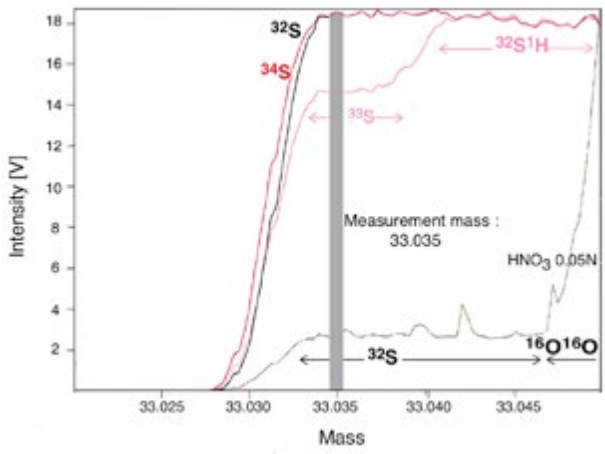

Figure 1. Peak shapes for ^{32}S, ^{33}S and ^{34}S in high-resolution mode. The shaded bar represents the interference-free plateau on which isotopic ratios were measured.

Background interferences were evaluated and subtracted using the 'on-peak zero' protocol by measuring signal intensities (8 ppb background equivalent, i.e. 0.020 V for a 2.5 V signal) on sulfur masses using a HNO_3 0.05N solution before each standard or sample analysis. Standard-sample-standard bracketing was used to correct for instrumental mass bias using an elemental Alfa Aesar ammonium sulfate standard solution. Two recent studies have shown that, using a desolvator system (DSN-100 or Aridus) coupled with a MC-ICP-MS, addition of a cation to the purified fraction of sulfur was critical for SO_4^{2} transmission through the desolvation process. Paris et al. (2013) added Na^+, while Han et al. (2013) chose to add Ag^+. Here we opted for NH_4^+, in order to reduce the mineral load of the plasma. We therefore added Suprapur™ ammonia solution in the stoichiometric proportions required to balance the charges of NH_4 and SO_4. The concentration of sulfur and the matrix charge due to ammonium ion in sample solutions were matched with those of the bracketing standard. We analyzed solutions at sulfur concentration of 8 mg/L with standard cones. Using the 'quick-wash' function of the Aridus II a throughput of 48 samples in 12 hours was achieved.

Sulfur isotope compositions were first measured as ^{34}S with respect to the in-house Alfa Aesar standard solution, with $\delta^{34}S_{AA}=[(^{34}S/^{32}S)_{sample}/(^{34}S/^{32}S)_{AA}-1]$. The $\delta^{34}S_{AA}$ of the reference materials IAEA S1, S2, S3 and S4 gives by difference the $\delta^{34}S$ value of the Alfa Aesar standard solution relative to the Vienna Cañon Diablo Troilite (V-CDT) (-4.88 ± 0.14), which finally allows the isotope compositions of each sample to be expressed relative to this international standard. The complete method was used to run the Fetal Bovine Serum (FBS) standard and samples of human serum and plasma.

Performances

Under the conditions of the present study, the typical transmission is 2.5 V/ppm with standard cones in HR configuration. The two-sigma external dispersion of $\delta^{34}S$ and $\delta^{33}S$ for 25 replicates of the Alfa Aesar standard solution at 8 mg/L is ±0.1‰ and ±0.15‰ (2σ), respectively (Figure 2). The lower reproducibility of $\delta^{33}S$ relative to $\delta^{34}S$ is due to the small $\delta^{33}S$ beam (~160 mV) and to the narrow interference-free plateau. The reproducibility of biological samples is also affected by the presence of remaining organic matter (Figure 2).

Resolution mode efficiency was evaluated by correlating the $^{33}S/^{32}S$ with $^{34}S/^{32}S$ ratios of samples and standard solutions, expressed in logarithm scale (Figure 3). The good agreement between the theorical slope predicted by the linear approximation of the mass dependent fractionation law and the calculated slope from the analyzed solutions indicates that measured intensities are free of interferences.

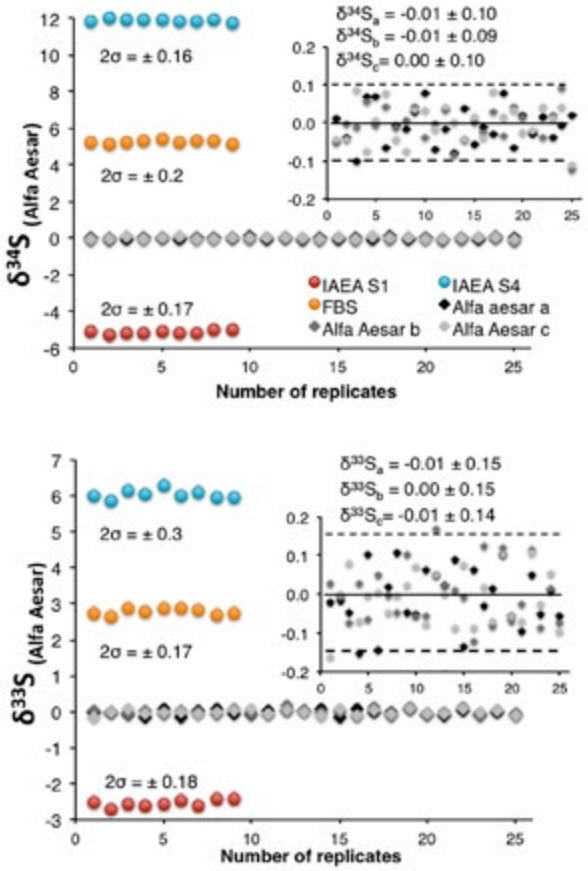

Figure 2. Two-sigma reproducibility for the $\delta^{34}S$ and $\delta^{33}S$ of Alfa Aesar standard solutions (grey symbols) over independent analytical sessions a, b and c. The reproducibility for the reference materials IAEA S1 and S4 anf for the Fetal Bovine Serum (FBS) is calculated from two separate chemical procedures over multiple and different analytical sessions.

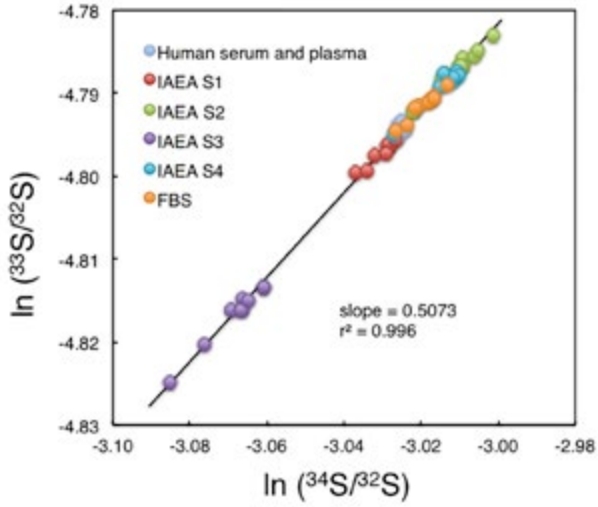

Figure 3. Sulfur isotope ratios of biological samples and IAEA standard solutions measured in this study, expressed in logarithm scale. The slope of the linear regression line is 0.5073, which is in good agreement with the theoretical slope of 0.5 derived from the exponential approximation of mass-dependent fractionation.

We also investigated the performances of the technique when Jet cones are mounted for sulfur-poor sample applications. With these cones, the sensitivity dramatically increases to 20V/ppm so that the minimal amount required for a significant S isotope measurement drops to 0.375 µg of sulfur (12 nmol). A 0.1‰ spread of $\delta^{34}S$ values obtained on standard solutions at 0.5 mg/L with the Jet cones is identical to that achieved with standard cones at 8 mg/L. We also confirmed that the isotope compositions of reference materials IAEA S1, S2, S3 and S4 measured with the Jet cones and standard cones are indistinguishable. We nevertheless observed that the production of $^{32}S^{1}H$ is higher with the Jet cones, so that the high-resolution mode is compulsory when Jet cones are mounted. The samples analyzed in the present work are concentrated enough to be run with standard cones, but the method can be applied on sulfur-poor samples, such as cell cultures or even cell membranes, using the Jet cones with similar analytical performances.

Results

All measured isotope compositions of serum, plasma and IAEA standards are shown in Table 2. For bovine serum, human serum and plasma, the $\delta^{34}S$ values were determined on 200 µL of material only. The sample denoted "mat FBS" refers to FBS after column purification applied on remaining matrix fraction of Cu, Zn and Fe chemistry separation procedure done on 200 µL Fetal Bovine Serum. The sulfur isotopic composition of mat FBS is indistinguishable, within uncertainty, from the FBS composition. Chemical processing of the samples for Cu, Zn and Fe, prior to isotopic analysis, therefore does not significant contamination or isotope fractionation of sulfur. This demonstrates that the isotope composition of these four elements can be measured on a single 200 µL aliquot of organic material.

Table 2. Isotopic composition of reference materials and samples of this study expressed in $\delta^{34}S_{V\text{-}CDT}$. External precisions are calculated from replicate analyses. The $\delta^{34}S_{V\text{-}CDT}$ of each IAEA refrence material is a compilation of measurements from two separate chemical procedures.

Name	Sample type	Number of replicates N*	$\delta^{34}S_{V\text{-}CDT}$	2σ	Published data**
Reference Materials					
IAEA S1	Ag2S	14	-0.25	0.17	-0.3 ± 0.24
IAEA S2	Ag2S	14	22.56	0.17	22.67 ± 0.18
IAEA S3	Ag2S	12	-32.11	0.35	-32.55 ± 0.20
IAEA S4	Soufre de Lacq	11	16.75	0.16	16.9 ± 0.24
Bovine Serum					
FBS	Fetal Bovine Serum	9	10.16	0.20	
FBS-2	Fetal Bovine Serum	3	10.10	0.11	
mat FBS	FBS post Cu chemistry	3	10.30	0.31	
Human Serum and Plasma					
M serum	Man 50 years	3	5.33	0.07	
M plasma	Man 50 years	1	5.26	0.10	
M membrane cell	Man 50 years	2	4.78	0.06	
M free sulfur	Man 50 years	2	0.87	0.03	
F serum	Woman 40 years	3	6.25	0.18	
F plasma	Woman 40 years	3	6.32	0.27	
F membrane cell	Woman 40 years	1	5.12	0.10	
F free sulfur	Woman 40 years	1	0.23	0.10	

* When N=1, the errors are the 2σ of the standard solution.

** Errors are given at 2σ uncertinity. Coplen and Krouse, (1998); Ding et al., (2001); Qi and Coplen, (2003).

We analyzed the sulfur isotopic composition of serum and plasma of two healthy donors, a man and a woman. From the plasma, we separated the free sulfur from the sulfur of the proteins using an ultrafiltration technique of serial filtration through decreasing molecular weight cutoff until 3 kDa. This low molecular weight fraction referred to as 'free sulfur', contains peptides, free amino acids and inorganic sulfate. We also subjected the red blood cells remaining from the dissociation with the plasma to an osmotic shock in order to isolate the membrane of the cells for isotopic analysis. As shown in Table 2 and Figure 4, the isotope compositions of sulfur in serum and plasma are identical within uncertainty, but vary from on person to another. The free-sulfur fraction of the plasma and the red blood cell membrane fraction are enriched in light isotopes with respect to the whole plasma.

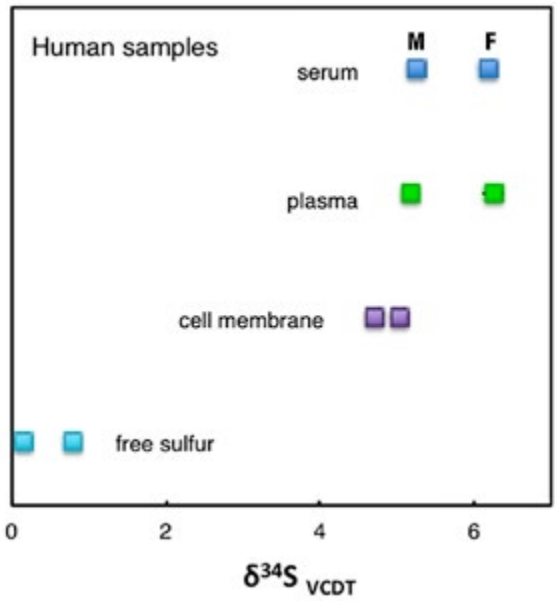

Figure 4. Isotopic compisition of sulfur, expressed in $\delta^{34}S$, of serum, plasma, red blood cell membrane and free sulfur of human samples.

Conclusion

The present note describes a method allowing for precise and accurate sulfur isotope measurements on small aliquots of human serum and plasma. This method can also be applied to a broad range of biological samples, such as whole blood, urine or organs. Due to the high sensitivity of the technique, smaller constituents of body fluids, such as membrane cells or the free-sulfur fraction of the plasma, can be analyzed after ultrafiltration. For applications in biomedicine, the advantage of MC-ICP-MS over IRMS stems from the small amount of sulfur required for isotopic analysis and from the possibility of measuring the isotope compositions of Fe, Cu, Zn, and S on a single aliquot of serum collected during blood tests. Finally, the variability of sulfur isotope compositions in the multiple samples of body fluids analyzed in this preliminary work points out the strong potential of sulfur isotope studies in a wide range of investigations needed for understanding human biological processes in normal and pathological pathways.

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