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RESEARCH ARTICLE

STUDY OF THE ISOTOPIC COMPOSITION OF MERCURY: EXPERIMENTAL APPROACH

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ABSTRACT

The main objective of this work was to study experimentally the isotopic fractionation of mercury Hg, in derivatization processes i.e. Ethylation and Propylation. The multi-collector inductively coupled plasma mass spectrometry (MC-ICPMS) technique makes it possible to measure isotopic signatures of mercury stable elements efficiently in extremely compact i.e. 1ppb and 10ppb concentration systems. This device made it possible to study the evolution of mass independent fractionation (MIF) and mass dependent fractionation (MDF) under different conditions. According to our results, the MIF or the so-called anomalies are observed only for the odd isotopic mercury (^{199}Hg and ^{201}Hg). The study conducted from the samples, reveals the presence of a maximum anomaly for hydrochloric acid (HCl) 3% in concentration equal to +1.2 ‰ for Δ^{201}Hg, but also the presence of a negative anomaly, equal to -0.43 ‰ for Δ^{201}Hg at 10% concentration of HCl. As derivatization procedure, direct aqueous phase methylations and propylation were tested on the sample. The different methods of derivatization are compared.

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INTRODUCTION

For several years, mercury has been the subject of numerous studies by scientific researchers. Mercury is the only liquid metallic element under normal conditions of temperature and pressure. Mercury is a chemical element with symbol Hg and atomic number 80 and electronic configuration is [Xe] $4f^{14}5d^{10}6s^2$. Mercury element is composed of seven stable isotopes ^{196}Hg (0.16%), ^{198}Hg (10.00%), ^{199}Hg (16.9%), ^{200}Hg (23.1%), ^{201}Hg (13.2%), ^{202}Hg (29.7%) and ^{204}Hg (6.8%).The element mercury has many stable isotopes ranging from mass 196 to 204; however, only the two with odd mass number are known to have a nonzero nuclear magnetic moment associated with a nuclear spin: mass 199 and 201. ^{200}Hg and ^{202}Hg, have neither nuclear spin nor magnetic moment. The isotopes, ^{199}Hg and ^{201}Hg, have nuclear spins respectively 1/2 and 3/2 and strong magnetic moments of the order of +0.5029μβ and -0.5602μβ respectively (Buchachenko *et al*., 2004). Knowledge of the chemical behavior of mercury is important because it is extremely polluting and toxic, leading to serious effects on the health of populations.

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It has long been used industrially in the extraction of precious metals and its use persists in artisanal operations, particularly in the Amazon (Carmouze *et al*., 2000). It is also a catalyst for many organic syntheses. Mercury is also used in many fields, such as medicine, or it is used as an ointment against skin diseases such as gall. It is also found in cosmetics and some instruments such as the thermometer and batteries. However, these applications undergo controls due to the toxicity of various mercury species. Mercury exists in three oxidation states (0), (I+) and (II+). The forms Hg (I) and Hg (II) are present in several environmental compartments, complexed with anions such as chlorides, sulphides, hydroxides, bromides, iodides, etc. Under certain conditions, the CH_3Hg^+ (monomethylmercury) cation, dimethylmercury, may form and complex with these bases to form monomethylmercury salts such as CH_3HgCl, CH_3HgS, CH_3HgI, etc. The toxicity of these elementary molecular species is now proven. The molecular form that currently raises the most questions is monomethylmercury (MeHg), which could be the main species involved in the biogeochemical and neurotoxic cycle and is present in biotic compartments and in aquatic systems where it is bioaccumulated: in this case, we speak of a process of biomagnification, a phenomenon which is not without consequences on the food chain in which man is involved

(Bergquist, 2007). The main source of mercury is undoubtedly linked to human activities such as industrial activities, mining, the combustion of fossil fuels such as coal combustion, releasing a large quantity of inorganic Hg into the atmosphere, or as a gag- Hg(0), either in the form of highly reactive species of (Hg^{2+}, X^-) type, naturally transforming into methylmercury (MeHg). For confirmation, pathology was clearly diagnosed in 1956 in the Japanese village of Minamata, later called "Minamata Disease". It results from a massive methylmercury poisoning, caused by the consumption of fish and seafood. These products caught in the bay were found to be contaminated by mercury releases from a nearby plant using this compound for the production of acetaldehyde (Fain and Ferrari, 2003). Bergquist *et al*, have also shown that populations with high per capita fish consumption are the most at risk, but this risk also applies to areas where environmental pollution has increased significantly in recent decades. However, the risks of ingestion of the MeHg form also exist even though per capita fish consumption and average levels of mercury in fish are relatively low. A follow-up of mercury speciation is therefore necessary, particularly with the help of experimental studies on the electronic and nuclear properties of the various mercury isotopes. In our study, the choice of isotopic fractionation was made.

By definition, isotopic fractionation is the phenomenon that characterizes the isotopic composition of a chemical element as it moves from one physical state, or one chemical composition, to another. In our work, we will mainly study the evolution of isotopic fractionation of mercury in a biogeochemical cycle in the aquatic environment. As isotopic fractionation can also be defined as the phenomenon quantifying the variations of the abundances of each isotope within a specific sample, to a given physicochemical or biological process, this will allow us to better understand and understand the natural process of mercury evolution in nature: for example, studies in lakes Michigan, Mame aiguillat and Dolt and studies at IPREM laboratories reveal the presence of high levels of mercury (Hg) in fish and its risk of exposure to the human population (Bergquist, 2007). Two types of isotopic fractionations are: (i) mass independent fractionation (MIF) and (ii) mass dependent fractionation (MDF).The study of MIF today touches fields of application such as cosmo-chemistry, paleoclimatology, physical chemistry, atmospheric chemistry and bio-geochemistry concerning all types of atoms (Bergquist, 2007).

In particular, the MIF is now well known for the element 'heavy' mercury and in particular on the two odd isotopes ^{199}Hg and ^{201}Hg involved in radical or photochemical reactions on the surface of the water, where we observe photo-reduction of Hg (II) and photodemethylation of monomethylmercury. Although these isotopic abnormalities, $\Delta^{199}Hg$ and $\Delta^{201}Hg$, are now commonly accepted, it was not until 2007 that the MIF hypothesis (Bergquist and Blum, 2007), (Biswas *et al.,* 2000), and (Bridget *et al*., 2009). Concerning the mass dependent fractionation (MDF), the experimental measurements and the theoretical predictions are not yet able to exploit this property, in particular because of notions still not understood concerning the behavior of the nuclear spin of the isotope considered. To determine the composition of a sample, researchers have different spectroscopic methods to access the composition and structure of the material. In the case of the study of mercury isotopes, several mass spectrometry techniques have been used: Inductively Coupled Plasma Source Mass Spectrometry (ICPMS), Thermo-Ionization Mass Spectrometry (TIMS) and Spectrometry of Secondary Ionization Mass (SIMS). These three techniques constitute a set of analysis techniques that can detect, but also finely identify, either the elements or the molecules (Sabine *et al.,* 2000). Concerning the study of elements that are difficult to ionize, Walder and Furuta (Halliday and Lee, 1995) demonstrated that the ICP-MS technique is the only one that is sufficiently fast and accurate for isotopic analyzes of environmental samples. The most commonly used technique for the study of mercury fractionation is multi-collector inductively coupled plasma-source mass spectrometry, the MC-ICP-MS (Multi Collector) technique. Lee and Hall-Ideal have argued that the MC-ICP-MS has the best accuracy in measuring isotopes. For example they have determined with this device an accuracy of 0.006% of 182W/183W (Sabine *et al*., 2000). According to (Albaréde *et al*., 2004), the MC-ICPMS was born about ten years ago. The capabilities of this technique have been demonstrated in studies of many metals, such as iron, zinc, copper or cadmium. This technique is today the most efficient and it allows the analysis of very low levels of concentration: the limit of detection is currently 0.18pg/g (Fain and Ferrari, 2003). MC-ICPMS has the advantage of much simpler sample preparation and shorter measurement time (Sergei *et al*., 2006). Nevertheless, it is a heavy and very expensive method of use. The accuracy that we are now entitled to achieve using this technique is of the order of 0.002% (Foucher and Hintelmann, 2006). Lauretta *et al*, were the first researchers to use the MC-ICPMS technique to study mercury isotopes in environmental matrice (Run-Sheng *et al*., 2010). These technical advances made it possible to analyze the seven stable isotopes of mercury Hg in low concentration samples. As part of our study, the experimental technique used for the analysis of mercury samples is "Nu instruments", "Nu Plasma HR" model, coupled to multi-collector Plasma Inductive Plasma Mass Spectrometry (MC-ICPMS). Cold Steam Generator (CVG) and Desolvation Nebulizer System (DSN-100).

Figure 1. Nu Plasma HR MC-ICPMS (LCABIE)

MATERIAL AND METHODS

Synthesis of materials: Preparation of raw materials is an important step in the experimental stud. Obtaining a good quality samples four steps are necessary. Details of the experimental work are available in table 1. However, the results of this study with the MC-ICPMS method are presented in Table 2 below.

STEP ONE: Calibration solution	1ppm Hg: Take a volume of 2 ml of the standard solution of mercury (Hg^{2+}) of 5ppm concentration that is introduced into a bottle. Add 8 ml of Milli-Q water (18 ΩM) to the vial to obtain the desired 10 ml volume and homogenize the solution.
	0.1ppm Hg: Take a volume of 0.2 ml of the standard solution of mercury (Hg^{2+}) of concentration 5 ppm that is introduced into a bottle. Add 9.8 ml of Milli-Q water (18 ΩM) to the vial to obtain the 10 ml volume and homogenize the solution.
STEP TWO: Sample preparation	1ppb 0.01%HCl: Take 0.05ml (50µl) of the solution at 0.1ppm mercury Hg and place in a bottle. Add a volume of 0.001315 (1.315µl) of the HCl solution. Add 4.95ml of Milli-Q water (18ΩM) (necessary to reach a final volume of 5ml). Close the bottle and shake the solution well. 10ppb 0.01%HCl: Take 0.05 ml (50µl) of the solution of 1 ppm mercury Hg and place in a bottle. Add a volume of 0.001315ml (1.315µl) of the HCl solution. Add 4.95ml of Milli-Q water (18ΩM) (necessary to reach a final volume of 5ml). Close the bottle and shake the solution well.

Table 1. Summary of the preparations of the different experimental solutions

C.1ppb/10ppb	Ligand	VL. Ligand	[Ligand]%	VL.1ppm/10ppb	VL.0.1ppm/1ppb	VL.MQ(18MQ)
1ppb	HCl	0.001315	0.1		0.05	4.95
1ppb	HCl	0.003947	0.03		0.05	4.95
1ppb	HCl	0.013	0.1		0.05	4.93
1ppb	HCl	0.0394	0.3		0.05	4.91
1ppb	HCl	0.13	1		0.05	4.82
1ppb	HCl	0.394	3		0.05	4.55
1ppb	HCl	1.3157	10		0.05	3.6343
10ppb	HCl	0.001315	0.01	0.05		4.95
10ppb	HCl	0.001315	0.03	0.05		4.95
10ppb	HCl	0.013	0.1	0.05		4.93
10ppb	HCl	0.0339	0.3	0.05		4.91
10ppb	HCl	0.13	1	0.05		4.83
10ppb	HCl	0.394	3	0.05		4.55
10ppb	HCl	1.3157	10	0.05		3.6343
1ppb	HNO_3	0.00714	0.1		0.05	4.236
1ppb	HNO_3	0.071	1		0.05	4.879
1ppb	HNO_3	0.714	10		0.05	4.9426
10ppb	HNO_3	0.00714	0.1	0.05		4.236
10ppb	HNO_3	0.071	1	0.05		4.879
10ppb	HNO_3	0.714	10	0.05		4.9426
1ppb	H_2SO_4	0.005	0.1		0.05	4.45
1ppb	H_2SO_4	0.05	1		0.05	4.9
1ppb	H_2SO_4	0.5	10		0.05	4.945
10ppb	H_2SO_4	0.005	0.1	0.05		4.45
10ppb	H_2SO_4	0.05	1	0.05		4.9
10ppb	H_2SO_4	0.5	10	0.05		4.945
1ppb	CH_3CO_2H	0.005	0.1		0.05	4.45
1ppb	CH_3CO_2H	0.05	1		0.05	4.9
1ppb	CH_3CO_2H	0.5	10		0.05	4.945
10ppb	CH_3CO_2H	0.005	0.1	0.05		4.45
10ppb	CH_3CO_2H	0.05	1	0.05		4.9
10ppb	CH_3CO_2H	0.5	10	0.05		4.945
1ppb	NH_4OH	0.005	0.1		0.05	4.45
1ppb	NH_4OH	0.05	1		0.05	4.9
1ppb	NH_4OH	0.5	10		0.05	4.945
10ppb	NH_4OH	0.005	0.1	0.05		4.45
10ppb	NH_4OH	0.05	1	0.05		4.9
10ppb	NH_4OH	0.5	10	0.05		4.945

The study of isotopic fractionation during the alkylation reactions for the different solutions described above requires a derivatization protocol. For this, a derivatization of the chemical forms is carried out beforehand, in order to obtain only alkylated forms of mercury, more stable at high temperature and more volatile. This derivatization is generally carried out by ethylation and / or propylation of the compounds, that is to say that the anionic radicals which have substituents (Cl^-, OH^-...) are replaced by ethyl or propyl groups derived from tetraethylborate sodium $NaBEt_4$ or sodium tetrapropylborate $NaBPr_4$.

STEP THREE: Derivatization: Derivatization must result in volatile and thermally stable compounds,which must be unique and unambiguously related to the species in the environmental matrix. In this work different derivatization techniques have been compared.

Ethylation: The sodium tetraethylborate $NaBEt_4$, first used by Rapsomaniki et al in 1986 by the derivatization of organo-lead species was proposed in 1989 by Bloom as a derivative of MeHg. It has since been preferred to tetraalkylborate and has been widely applied successfully on aqueous samples (Carlier-Pinasseau and al., 1997).

Propylation: The sodium tetrapropylborate $NaBPr_4$, recently synthesized for the purpose of the derivatization of mercury, proves more effective than sodium tetraethylborate $NaBEt_4$ from the point of view of the derivatization yield. It is estimated that the detection limits are generally less than 1 pg / l, for both mercury and methylmercury (MeHg), when using $NaBPr_4$ (Bravo-Sanchez and al., 2004). A summary of the two derivatization procedures described is given below.

STEP FOUR: Preparation of samples before analysis: The samples are placed in polystyrene tubes, avoiding losses as much as possible, in which 100 µl and 50 µl of $BrCl_3$solution are added to each tube of the organic phase and the aqueous phase, respectively for to oxidize all the mercury, the solution is then stirred manually.

Derivatization	Samples(10ppb&1ppb)	Operating mode
Ethylation	10ppb	All bottles each contain 2.5 ml in solution. A volume of 1000 µl of iso-actane (IOA) and then 250 µl of sodium tetraethyl borate $NaBEt_4$ at 2% are rapidly added to each vial. The bottles are immediately capped and rigorously shaken by hand for 5 minutes. The organic and aqueous phases are then separated and the organic phase is then recovered in small 2 ml borosilicate glass flasks. The organic extract obtained is left to stand, corks open for about 48 hours. After evaporation, 5 ml of the matrix (10% HNO_3, 2% HCl) are added.
	1ppb	All bottles each contain 5ml in solution. A volume of 1000 µl of isooctane (IOA) and then 400 µl of sodium tetraethyl borate $NaBEt_4$ at 2% are rapidly added to each vial. The bottles are immediately capped and rigorously shaken by hand for 5 minutes. The organic and aqueous phases are then separated and the organic phase is then recovered in small 2 ml borosilicate glass flasks. The organic extract obtained is left to stand, corks open for about 48 hours. After evaporation, 5 ml of the matrix (10% HNO_3, 2% HCl) are added. Hg^{2+} +2$NaBEt_4$$Et_2Hg$+2Na+2$BEt_3$
Propylation	10ppb	All bottles each contain 2.5 ml in solution. A volume of 100 µl of isooctane (IOA) and then 250 µl of 2% $NaBPr_4$ sodium tetrapropylborate are rapidly added to each bottle. The bottles are immediately closed and rigorously shaken by hand for 5 minutes. The organic and aqueous phases are then separated and the organic phase is then recovered in small 2 ml borosilicate glass flasks. The organic extract obtained is left to stand, corks open for about 48 hours. After evaporation, 5 ml of the matrix (10% HNO3, 2% HCl) are added. Hg^{2+} + 2$NaBPr_4$$Pr_2Hg$+2$Na^+$ + 2BPr_3

After one hour, 50 µl and 25 µl of the hydroxylamine solution (30%) are added respectively into the organic and aqueous phase. This solution plays the role of a pre-reducer to neutralize the excess of BrCl, visible by the disappearance of the yellow color.

RESULTS AND DISCUSSIONS

Calculation of the value of δ^{xxx}Hg (‰): The delta notation "δ" is used to denote the mass dependent fractionation (MDF). The so-called "standard technical bracketing" method is now commonly used to calculate the δ (‰) fractionation for each isotopic ratio, using the mass of the ^{198}Hg isotope as the reference mass (Blum and Bergquist, 2007). We thus find the evaluation equation of MDF.

$$\delta^{xxx/198}Hg = \left[\left(\left({}^{xxx}Hg/{}^{198}Hg\right)_{sample}/\left({}^{xxx}Hg/{}^{198}Hg\right)_{NIST\ \ SRM\ \ 3133}\right)-1\right]\times 1000 \quad (1)$$

Where xxx represents the mass of mercury isotopes other than element 198. To apply this method, the standard NIST SRM 3133 sample (Mercury standard solution) or UM-Almadèn and the sample should be of the same mercury concentration and placed in the same acid matrix. This will bring the mercury behavior in the standard closer to that of the mercury in the sample, in order to appreciate the accuracy of the analysis.

Calculation of the value of Δ^{xxx}Hg (‰): The mass independent fractionation (MIF) represented by the notation "Δ", also called anomaly, is calculated by the difference between the isotopic ratios $\delta^{xxx}/^{198}$ measured and calculated theoretically, assuming that it is due solely to a dependent fractionation mass of Hg isotopes (Blum and Bergquist, 2007).Thus equation (2) gives the analytical expression for evaluating the anomaly due to a mass-independent fractionation.

$$\Delta^{xxx}Hg = \delta^{xxx}Hg - \delta^{202}Hg \times {}^{xxx}\beta \quad (2)$$

Where xxx is the mass of Hg isotopes between 199 and 204 amu and β is the fractionation factor depending on the mass of Hg isotopes and is given by equation (3).

$$\beta = \frac{\frac{1}{197.966752}-\frac{1}{mx}}{\frac{1}{197.966752}-\frac{1}{mz}} \quad (3)$$

In this equation mx and mz represent the masses of the isotopes x and z respectively. The value of β depends on each isotope. The equations below are used to calculate the value of the mass-independent fractionation (MIF) for the different mercury isotopes. In this case, the ratio ^{202}Hg/^{198}Hg (i.e. δ^{202}Hg) is used to determine the following theoretical values: Δ^{199}, Δ^{200}, Δ^{201} and Δ^{202}; by applying the kinetic law of mass dependent fractionation (MDF), established by Bigeleine in 1949 and reconsidered, as follows, by Young et al, in 2002 (Sonke, 2011).

$$\Delta^{199}Hg = 1000\times\left(\left\{\ln\left[\left(\delta^{199}Hg/1000\right)+1\right]\right\}-0.2520\times\left\{\ln\left[\left(\delta^{202}Hg/1000\right)+1\right]\right\}\right) \quad (4)$$

$$\Delta^{200}Hg = 1000\times\left(\left\{\ln\left[\left(\delta^{200}Hg/1000\right)+1\right]\right\}-0.5024\times\left\{\ln\left[\left(\delta^{202}Hg/1000\right)+1\right]\right\}\right) \quad (5)$$

$$\Delta^{201}Hg = 1000\times\left(\left\{\ln\left[\left(\delta^{201}Hg/1000\right)+1\right]\right\}-0.7520\times\left\{\ln\left[\left(\delta^{202}Hg/1000\right)+1\right]\right\}\right) \quad (6)$$

$$\Delta^{204}Hg = 1000\times\left(\left\{\ln\left[\left(\delta^{204}Hg/1000\right)+1\right]\right\}-1.493\times\left\{\ln\left[\left(\delta^{202}Hg/1000\right)+1\right]\right\}\right) \quad (7)$$

For values of δ^{xxx}Hg(‰) much lower than 10‰, the values of Δ^{xxx}Hg(‰) can be approximated by the following equations (Blum and Bergquist, 2007).

$$\Delta^{199}Hg = \delta^{199}Hg - \left(\delta^{202}Hg\times 0.2520\right) \quad (4')$$

$$\Delta^{200}Hg = \delta^{200}Hg - \left(\delta^{202}Hg\times 0.5024\right) \quad (5')$$

$$\Delta^{201}Hg = \delta^{201}Hg - \left(\delta^{202}Hg\times 0.7520\right) \quad (6')$$

$$\Delta^{204}Hg = \delta^{204}Hg - \left(\delta^{202}Hg\times 1.493\right) \quad (7')$$

In Table 2 below is listed the isotopic composition of Hg (‰) found in our study.

Table 2. Isotopic composition of Hg (‰)

[C.]ppb	Ligand	[Lig.]%	$\delta^{204}Hg$	$\delta^{202}Hg$	$\delta^{201}Hg$	$\delta^{200}Hg$	$\delta^{199}Hg$	$\Delta^{201}Hg$	$\Delta^{199}Hg$	$\Delta^{200}Hg$
1	HCl 1-8E(a)	0.3	2.49	0.95	2.9	0.32	2.47	2.19	2.22	-0.16
1	HCl 1-10E(a)	10	-1.05	-0.82	-0.45	-0.49	-0.07	0.17	0.14	-0.08
1	HCl 1-11E(a)	3	0.98	0.48	4.16	0.1	4.71	3.8	4.58	-0.14
1	HCl 1-13E(a)	1	0.38	0.22	1.07	-0.25	2.33	0.91	2.27	-0.36
10	HCl 10-3E(a)	0.1	-1.38	-0.61	-0.53	-0.41	-0.25	-0.07	-0.09	-0.10
10	HCl 10-13E(a)	0.3	-0.33	-0.29	0.07	-0.24	0.37	0.29	0.44	-0.10
10	HCl 10-1P(a)	0.01	-1.79	-0.82	-1.08	-0.54	8.64	-0.47	8.85	-0.13
10	HCl 10-3P(a)	0.03	-0.39	-0.51	-0.4	-0.19	1.3	-0.01	1.43	0.06
10	HCl 10-5P(a)	0.1	-0.87	0.06	0.07	0.03	0.2	0.02	0.19	0.00
10	HCl 10-7P(a)	0.3	0.41	0.12	0.12	0.09	0.21	0.03	0.18	0.03
10	HCl 10-9P(a)	1	0.45	0.27	1.36	0.04	1.56	1.16	1.49	-0.09
10	HCl 10-11P(a)	3	0.73	0.67	1.68	0.42	1.53	1.18	1.36	0.09
10	HCl 10-13P(a)	10	0.37	0.25	-0.24	0.05	-0.07	-0.43	-0.13	-0.08
1	HNO_3 1-5E(a)	10	-25.55	-23.82	-17.1	10.95	1004.05	0.85	1010.1	22.9
10	HNO_3 10-3E(a)	1	-2.29	-2.25	-1.03	1.63	98.36	0.67	98.93	2.76
10	HNO_3 10-5E(a)	10	0.04	-0.03	0.56	0.15	-0.47	0.58	-0.47	0.16
1	H_2SO_4 1-1E(a)	0.1	-29.76	-27.28	-19.25	12.05	1136.08	1.33	1143.01	25.75
10	H_2SO_4 10-1E(a)	0.1	0.06	-0.02	0.2	-0.02	0.58	0.21	0.59	-0.01
10	H_2SO_4 10-3E(a)	1	-0.01	0.01	0.09	0.06	0.03	0.08	0.02	0.05
10	H_2SO_4 10-5E(a)	10	-0.05	0.18	-0.07	-0.05	0.13	-0.2	0.08	-0.15
10	H_2SO_4 10-7P(a)	0.1	-0.14	0.31	0.03	0.13	0.05	-0.2	-0.03	-0.02
10	H_2SO_4 10-9P(a)	1	0.73	0.35	0.34	0.28	1.01	0.08	0.92	0.1
10	H_2SO_4 10-11P(a)	10	-0.37	-0.03	0.09	0.14	0.12	0.11	0.13	0.15
10	CH_3CO_2H 10-5P(a)	10	-0.57	-0.73	-048	-0.4	-0.7	0.07	-0.51	-0.03
10	CH_3CO_2H 10-9E(a)	1	-1.81	-0.62	0.00	-0.12	-0.5	0.47	-0.34	0.19
10	NH_4OH 10-1E (a)	0.1	0.11	0.2	0.1	0.11	0.39	-0.05	0.34	0.01
10	NH_4OH 10-7P (a)	0.1	0.52	0.29	0.21	0.15	-0.1	-0.01	-0.17	0.00
10	NH_4OH 10-9P (a)	1	1.46	0.86	0.91	0.59	0.27	0.27	0.02	0.16
10	NH_4OH 10-11P (a)	10	0.73	0.67	0.4	0.34	0.64	-0.1	0.47	0.01
1	HCl 1-7E(o)	0.3	-1.03	-0.29	-0.22	-0.16	1.48	0.00	1.56	0.01
10	HCl 10-12E(o)	0.01	0.1	0.08	0.09	0.00	-1.3	0.03	-1.32	-0.04
10	HCl 10-14P(o)	10	-91.11	-84.13	-60.91	37.57	3461.13	2.53	3482.5	79.8
10	HNO_3 10-4 E(o)	1	-2.19	-1.82	-1.12	1.19	86.13	0.25	86.59	2.1
10	HNO_3 10-6 E(o)	10	0.56	0.33	-0.38	0.01	-0.68	-0.63	-0.76	-0.16
10	HNO_3 10-10 P(o)	1	-25.7	-23.74	-16.5	10.87	994.43	1.4	1000.46	22.79
10	H_2SO_4 10-4 E(o)	1	0.19	-0.23	-0.33	0.54	-0.01	-0.16	0.05	-0.42
10	H_2SO_4 10-6E(o)	10	0.33	-0.18	-0.24	0.01	0.33	-0.1	0.38	0.1
10	CH_3CO_2H 10-8E(o)	0.1	0.21	0.14	0.17	0.23	0.26	0.07	0.23	0.16
10	CH_3CO_2H 10-10E(o)	1	-0.7	0.35	0.51	0.37	3.52	0.25	3.43	0.19
10	CH_3CO_2H 10-12E(o)	10	0.16	-0.16	0.05	0.01	0.3	0.17	0.34	0.09

Nomenclature: E-Ethylation process; P-Propylation process; (a)-aqueous phase and (o)-organic phase

Table 3. UM-Almaden: Mean isotopic signatures (‰) (Mean±2*σ)

Isotope	$\delta^{204}Hg$	$\delta^{202}Hg$	$\delta^{201}Hg$	$\delta^{200}Hg$	$\delta^{199}Hg$	$\Delta^{201}Hg$	$\Delta^{199}Hg$	$\Delta^{200}Hg$
UM (we)	-0.95±0.25	-0.60±0.10	-0.49±0.12	-0.30±0.07	-0.17±0.10	-0.03±0.11	-0.01±0.08	-0.00±0.010
UM-Estrade	--------	-0.51±0.15	-0.41±0.11	-0.26±0.10	-0.14±0.09	-0.03±0.04	-0.01±0.07	-0.01±0.06
UM-Bergquist	-0.83±0.11	-0.54±0.08	-0.44±0.07	-0.27±0.04	-0.14±0.06	-0.04±0.04	-0.01±0.02	-0.00±0.02

Table 4. Comparison table of MDF and MIF

Ligands/Isotopes	$\delta^{202}Hg$(‰) ±2*σ	$\delta^{201}Hg$(‰) ±2*σ	$\Delta^{201}Hg$(‰) ±2*σ
HCl	0.42±0.10	1.3±0.12	0.98±0.11
HNO_3	-0.03±0.10	0.56±0.12	0.58±0.11
H_2SO_4	0.18±0.10	-0.07±0.12	-0.2±0.11
CH_3CO_2H	-0.73±0.10	-0.48±0.12	0.07±0.11
NH_4OH	0.67±0.10	0.4±0.12	-0.1±0.11

Table 5. Comparison of the two derivatization processes: Propylation and Ethylation

Ligand	[C]. Ligand	$\delta^{200}Hg$ (‰)		$\delta^{201}Hg$(‰)		$\Delta^{201}Hg$(‰)	
		Propylation	Ethylation	Propylation	Ethylation	Propylation	Ethylation
HCl	0.01	-0.82	----------	-1.08	----------	-0.47	---------
HCl	0.03	-0.51	----------	-0.4	----------	-0.01	----------
HCl	0.1	0.06	-0.61	0.07	-0.53	0.02	-0.07
HCl	0.3	0.12	-0.29	0.12	0.07	0.03	0.29
HCl	1	0.27	0.41	1.36	1.33	1.16	1.02
HCl	3	0.67	0.20	1.68	0.20	1.18	1.37
HCl	10	0.25	0.42	-0.24	1.3	-0.43	0.98

DISCUSSIONS

The average isotopic signatures: The reference material we used was synthesized from liquid Hg(0) from the Almadén mines (Spain), UM-Almadén. This work should allow us to validate our analyses. Table 3 shows our measurements of average isotopic signatures (‰) on all reference materials. We can see that our values compare very favourably with those obtained by (Bergquist and Blum, 2007)and (Estrade and al., 2009). The differences between works are small. Isotope compositions were referenced to the bracketing Hg standard (NIST 3133 solution). Compositions were reported in per mil (‰) as either mass-dependent fractionation (denoted as "del" or δ^{xxx}Hg) or mass-independent fractionation (denoted as "delta" or Δ^{xxx}Hg).

Comparison of the ligands used: As shown in Table 1, the concentration of the ligands studied is between 0.01% and 10%. The results in Table 4, taken from about 10% concentration measurements for 10ppb Hg ligands in the aqueous phase. Nearly all observed isotopic fractionation in mass dependent and independent fractionation. The two splits, dependent and independent of mass, are observed for all ligands with isotopic signatures that vary greatly depending on the ligand. As can be seen in the table below, these ligands have MDF with slight isotopic variations. There are, however, some exceptions where the value of fractionation (MDF) of HCl sample is superior of +1‰ (exactly +1.3‰ for δ^{201}Hg). So we also notethat acetic acid has the lowest MDF, equal to -0.73‰ for δ^{202}Hg and -0.48‰ for δ^{201}Hg.Similarly, mass-independent fractionation (MIF) is only the majority in the case of HCl and HNO_3 for the ^{201}Hg isotope. Hydrochloric acid and nitric acid induce positive anomalies in the aqueous phase of +0.98 (‰) and +0.58 (‰) respectively, while negative anomalies are observed for sulphuric acid and ammonium mixtures of -0.2‰ and -0.1‰ respectively for the same isotope. The anomalies observed for the last three samples (i.e. H_2SO_4, CH_3CO_2H, NH_4OH) can be considered negligible.

Comparison of the two derivatization processes: Ethylation and Propylation: Table 5 allows us to compare the results obtained for the two derivatization processes performed, for the HCl ligand at 10ppb in the aqueous phase. For comparison, the results reported in Table 5 confirm the hypothesis that derivatization with $NaBPr_4$ (Propylation) would be more effective than with $NaBEt_4$ (Ethylation) (Sonke, 2011). We noted above that the MDF values (δ^{202}Hg and δ^{201}Hg) for Propylation are higher than those obtained by the Ethylation process, with the exception of HCl at 10% concentration. In addition to the fact that the propylation protocol is more effective for derivatization, it is worth noting a significant difference in efficiency between these processes on the MIF values for the isotope ^{201}Hg i.e. +1.37‰ at 3%. Slight anomalies noted for HCl concentrations below 1%.In propylation we note values between -0.47‰ and 0.03‰ and in ethylation it varies from -0.07‰ to 0.29‰ for Δ^{201}Hg. This table also shows that between 1% and 3% in HCl concentration, the MDF and MIF values are all positive. It is also noted that between 0.01% and 3% HCl concentration, all isotopic signatures obtained in propylation increase with HCl concentration, which is not observed in the ethylation process.

Influence of HCl concentration on MDF: Figure 2 shows we have plotted the spectra of MFD measured for propylation process. In this study, we show that HCl samples exhibit a similar behaviour towards the isotopic variations observed in Figure 2.a and 2.b. However, three main trends appear to be emerging for compounds with concentrations in the:

-[0.01-0.1]: the isotopic signatures of mercury Hg are almost zero;
- [0.1-3]: the evolution of the isotopic signature observed is increasing;
- [3-10]: a progressive decrease in the isotopic signature for δ^{202}Hg (‰) and a sudden decrease in the variation of δ^{201}Hg (‰), up to a negative value at 10% in HCl concentration, are observed.

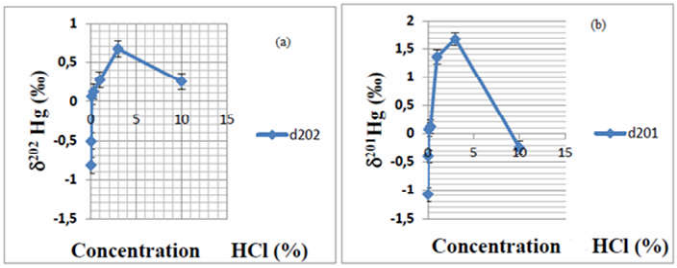

Figure 2. Evolution of MDF as a function of HCl concentration: (a) δ^{202}Hg (‰) vs[HCl]% and (b) δ^{201}Hg (‰) vs [HCl]%

These results show that the variations of δ^{202}Hg(‰) are not significantly different from those observed for δ^{201}Hg(‰) depending on the HCl concentration. Indeed, the isotopic signature δ^{202}Hg (‰) has an important MDF, from +1.8‰ to 3%[HCl]. In comparison to these two figures, it is important to note that the values of δ^{202}Hg(‰) are much lower than those of δ^{201}Hg(‰). We can therefore conclude with certainty that the different MDF, δ^{202}Hg(‰) and δ^{201}Hg(‰), observed are practically identical and that the variation in isotopic signatures does indeed depend on the species concentration.

Influence of HCl concentration on MIF: From the results found and illustrated in the figure below, we notice a low MIF, observed for 0.01%<[HCl]<0.3% and ranging from[-0.4‰< Δ^{201}Hg(‰)<+0.03‰] to a high MIF between 1% <[HCl]<3% corresponding to fractionation values equal to +1.15‰ for the 1%[HCl] and +1.2‰ for the 3%[HCl] solution. Above 3% in HCl concentration, there is a gradual decrease in the value from Δ^{201}Hg (‰) to a negative MIF, equal to -0.43‰ to 10%[HCl].

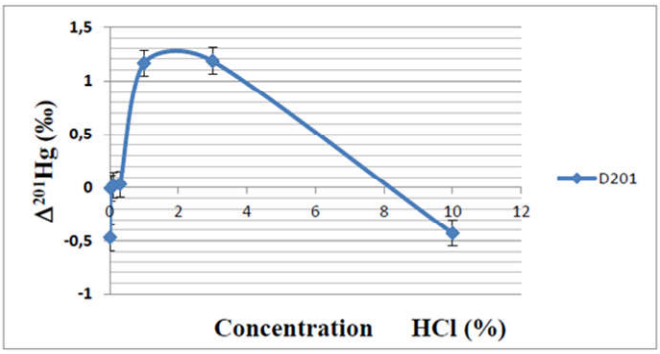

Figure 3. Evolution of the MIF as a function of the concentration of [HCl]

According to the literature, two main mechanisms have been advanced to explain the effect of Hg's MIF, i.e. the nuclear volume effect [mechanism established by Bigeliesen (1996) and Schauble (2007) and the magnetic isotopic effect

highlighted by Buchachenko et al (1976), Turro and Kraeutler (1978), Turro (1993), Buchachenko (1995), Buchachenko (2001)] (Yin and al., 2010). It now seems certain that the magnetic isotopic effect is the main cause of MIF observed in photochemical reactions involving mercury.

MIF and MDF as a function of MDF-line: In this part of the interpretation, both Figure 4-a and 4-b present similar scenarios, supported by the presence of a relationship between MDF.

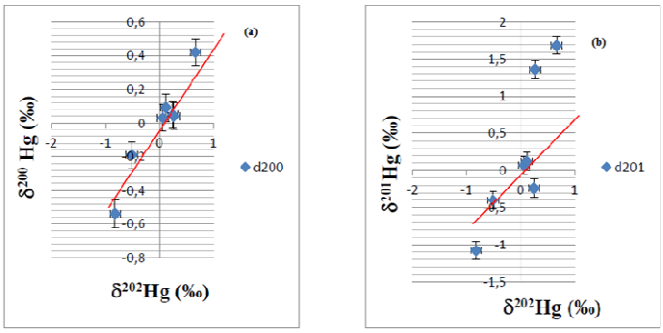

Figure 4. Relation between: (a) δ^{200}Hg(‰) vs δ^{202}Hg(‰) (b) δ^{201}Hg(‰) vs δ^{202}Hg(‰)

Indeed, in the representations showing the evolution of δ^{201}Hg as a function of δ^{202}Hg, and the evolution of δ^{200}Hg as a function of δ^{202}Hg, a linear function called MDF-line (red line) allows to clearly observe the behaviour of Δ^{201}Hg as compared to δ^{200}Hg. Values above the MDF-line function will be considered negative. From Figure 4, it can be seen that the anomaly Δ^{201}Hg increases with δ^{202}Hg. An identical scenario is observed for the evolution of δ^{200}Hg according to δ^{202}Hg. A mass dependent fractionation (MDF) is observed with very low isotopic signatures, which vary between -0.58‰ and +0.43‰ for δ^{200}Hg; similarly, mass independent fractionations (MIF), between -1.2‰ and +1.7‰ are observed for Δ^{201}Hg. Equally important positive anomalies are observed i.e. +1.7‰ and +1.35‰ for Δ^{201}Hg, while an MDF equal to +0.45‰ is observed for δ^{200}Hg. Similarly, low isotopic variations are observed i.e. -1.2‰ and -0.25‰ for Δ^{201}Hg as well as an MDF equal to -0.58‰ for δ^{200}Hg. These negative anomalies are significant for samples of the HCl ligand, compared to the uncertainties calculated (2σ) and reported in Table 3. The results of the analyses of the HCl samples show that there is a significant difference in the evolution of isotopic variations for δ^{200}Hg and Δ^{201}Hg, these variations being much smaller for δ^{200}Hg than for Δ^{201}Hg.

Conclusion

In this work, we test two techniques (propylation and ethylation) to determine mercury isotopic. We have studied in detail the two types of isotopic fractionation, dependent (MDF) and mass independent (MIF). The technique used was chosen with the aim of achieving the maximum possible experimental precision, estimated at 0.1-0.3% (Klaue and Blum, 2000). Compared to the literature, our study showed that the statement by several authors that the propylation process was much more effective than ethylation was not always verified for some samples, such as HCl type samples with a concentration of 10%. This therefore requires a thorough study on the influence of these two processes. The study revealed that no isotopic signature was observed in the majority of samples of the organic phase, this could be explained by the evaporation time.

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