Hg speciation in petroleum hydrocarbons with emphasis on the reactivity of Hg particles

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ABSTRACT: Particulate HgS is generally thought to be the most abundant Hg species in stored petroleum hydrocarbons stocked on-shore. It is also assumed that due to its high stability constant, HgS is chemically inert. However, results from the current study would suggest otherwise. Firstly, a comparison study of the analytical performance of ICP-MS and CV-AFS with respect to matrix effect, showed no significant difference in the measured Hg concentrations in studied samples when CV-AFS is used in the alkali mode. Subsequently, the suitability of three quantification methods during Hg speciation was investigated. Both external calibration and standard addition methods resulted troublesome with the former showing matrix dependence and the latter being hindered by the formation of an emulsion during the derivatisation step. Results from species-specific isotope dilution (SS-ID-GC-ICP-MS), on the other hand, performed at different equilibration times, showed a random variation in the calculated Hg^{2+} concentration (RSD 32%),

suggesting that factors independent of equilibration time cause the observed variation. Further sedimentation of Hg particulate by means of ultracentrifugation improved the precision of SS-ID-GC-ICP-MS by 10-fold. These results would suggest that Hg particles in petroleum products are reactive at low temperature during Grignard alkylation.

1. INTRODUCTION

Fossil fuels including coal, petroleum and natural gas, shale oil and other bituminous fuels, are formed by anaerobic decomposition of organic material remains. Crude oils, belonging to the group of petroleum liquids, consist predominantly of hydrocarbons with minor amounts of impurities, including organic and inorganic metal complexes.[1] While some metal complexes, mainly focusing on nickel and vanadium porphyrins have been extensively studied, the geochemical origin of other metal impurities such as mercury is still not well understood.[2] One possible explanation is the ancient atmospheric deposition of Hg from volcanic eruptions resulting in global impacts, including Hg impacts to hydrocarbons.[3] However, as the Hg concentration varies with different geographical origin by several orders of magnitude; it is believed that secondary geological processes may play an important role. Considering the presence of Hg belts within the Earth crust, it is assumed that high pressures and temperatures could mobilise Hg from the source rock leading to Hg migration and re-deposition in the reservoir.[4]

The 1973 Skikda plant explosion in Algeria raised awareness of the potential corrosion impacts on metal from Hg-containing hydrocarbons. The Skikda explosion was attributed to Hg facilitated corrosion. There are two types of Hg corrosion; amalgamation and liquid metal embrittlement (LME). Almost all metals are

susceptible to mercury amalgamation, which can be triggered by defects in the oxide layer, thus potentially compromising the properties of the metal. On the other hand, LME is caused by diffusion of liquid Hg into the grain boundaries of the alloy potentially resulting in the loss of ductility and formation of cracks along the grain boundaries.[5] Both types of Hg corrosion require close monitoring if one considers that operations on the petrochemical plants are carried out under high pressure and temperature.

Due to the potential impact that Hg may have on the refining processes, several monitoring controls are followed to determine its concentration. The analytical methods for detection of total Hg in hydrocarbons vary significantly; the most common methods include acid digestion,[6] oxidative extraction,[7,8] combustion/trap[9] and vaporisation/ICP-MS.[10] Each method requires different sample preparation. Depending on the concentration of Hg in the sample, different instrumentation is employed. Acid digestion is typically employed for matrix decomposition and chemical oxidation of mercury species to Hg^{2+} form. For the concentrations in ng/kg range cold vapor atomic fluorescence spectroscopy (CV-AFS) with a gold trap showed excellent results.[11] Additionally, for the µg/kg range acidic digest can be analysed by inductively coupled plasma (ICP) followed by mass spectrometry (MS)[6] or atomic emission spectrometry (AES).[12] However, acid digestion is prone to sample contamination as laborious sample preparation is needed. On the contrary, the combustion/trap technique has much simpler sample preparation, as the actual sample is introduced into the combustion chamber where the entire matrix with analyte is vaporised. The vapor containing Hg is trapped via amalgamation on gold and thermally desorbed and detected by CV-AFS. While this technique eliminates the

possible contamination problem, its efficiency is limited by the sulfur content in the sample.[11]

Hg speciation may assist with Hg removal during refining processes. Individual Hg species exhibit different chemical and physical properties and could accumulate in different production streams. Speciation consists of multiple and sequential analysis of various Hg species to fulfill the requirements of the mass balance equation below (Eq. 1).[13]

$$Hg_{Total} = Hg^0 + R_2Hg + RHg^+ + Hg^{2+} + Hg_{particulate} \qquad \text{(Eq. 1)}$$
R = alkyl group
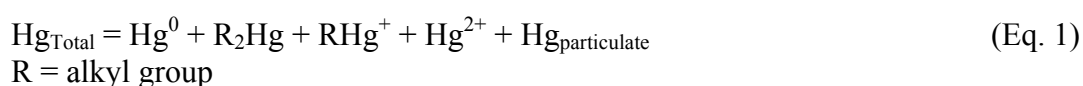
The chromatographic technique is used to detect and separate individual Hg species prior to Hg measurement. Both gas chromatography (GC) and high performance liquid chromatography (HPLC) coupled to ICP-MS have been applied successfully.[14,15] Due to the intrinsic volatility of some Hg species or the ability to convert them into volatile compounds through derivatisation, GC seems to be a very attractive separation technique. In this sense, the typical protocol for Hg speciation using GC consists of three steps. First, the sample is directly injected into the GC for determination of Hg^0 and any R_2Hg species. Both species are retained on the column and sequentially released through a suitable temperature program. Second, any ionic and monoalkyl species (Hg^{2+} and RHg^+) are alkylated, most commonly by Grignard reagent (R-MgCl), and the derivatised sample is injected into the GC, which allows species separation. Third, the particulate Hg is defined as a difference between the Hg_{Total} and the sum of Hg^0 and alkylated species (Eq. 1). Alternatively, particulate Hg can be separated through filtration process for further characterisation. Depending on

the method of quantification, the detector used can be AFS or ICP-MS if the isotopic information is required.

In this study, we examine the challenges of Hg quantification in the complex organic matrices represented by petroleum hydrocarbon samples with careful attention paid to Hg speciation and the mass balance. The quantification of total Hg in the digested samples was compared between two detection techniques, CV-AFS and ICP-MS. We also highlighted the limitations of three quantification methods for Hg speciation: external calibration, standard addition and species-specific isotope dilution mass spectrometry (SS-ID-GC-ICP-MS). Additionally, an insight into the reactivity towards derivatisation of nano-particulate Hg present in the samples is addressed for the first time.

2. EXPERIMENTAL

Reagents and standards. All chemicals used were of analytical reagent grade unless stated otherwise. For the sample digestion 70% HNO_3 was purchased from VWR International, (UK). For the sample derivatisation, 2M butylmagnesium chloride solution in THF and *o*-xylene was purchased from Sigma-Aldrich, (UK). For the CV-AFS analysis, sodium hydroxide (NaOH) and 98% tin(II)chloride dehydrate were obtained from Sigma-Aldrich, (UK). Stock standard solution of 1000 ± 3 mg/L Hg^{2+} in 12% nitric acid (HNO_3) was purchased from Sigma-Aldrich, (UK). MeHgCl was purchased from Sigma-Aldrich, (UK) and the working standard solution of 10 g/L of $MeHg^+$ was prepared in methanol from VWR International (UK). Mercury oxide (^{199}HgO) standard of 994 mg/kg as $^{199}Hg^{2+}$ was purchased from Oak Ridge National Laboratory, (USA). All stock standard solutions were stored in the dark at -20 °C. Working standards were prepared daily prior to experimental analysis. Double

distilled water, which was used throughout the experiments, was purified using an Aquatron A4000D, (UK).

Samples. Five petroleum hydrocarbon samples from different geographical locations having following compositions: sulfur (100 – 10,000 mg/kg), nitrogen (100 – 1000 mg/kg), viscosity at 20 °C (0.80 – 1.30 cSt) were provided by Chevron, USA and stored in 200 mL amber bottles in the dark at room temperature. Prior to subsampling of the hydrocarbon petroleum samples, they were warmed up to ca. 30 °C in a water bath and were placed on the automatic shaker for 30 min. Subsamples were aliquoted into 15 mL vials with septum lids and aliquots for the analysis were taken using syringe without opening of the vials.

Analytical methods. Samples digestion for total Hg determination. For total Hg determination the samples were warmed up to ca. 30 °C in a water bath and left on the automatic shaker for 30 min to homogenise. Accurately weighed amounts of approximately 0.3 g of a hydrocarbon petroleum sample was mixed with 5 mL of HNO_3 (70%) and digested in the closed vessels using the MARS 5 (Microwave Accelerated Reaction System, CEM Corporation, UK). The microwave program was as follows: step 1, room temperature to 180 °C, 30 min; step 2, 180 °C, 150 min. The resulting digest was allowed to cool to room temperature, transferred into plastic vials and stored at 4 °C until further use.

Total Hg determination by ICP-MS and CV-AFS. Total Hg measurement was performed on an ICP-MS Agilent 7500c (Agilent Technologies, Santa Clara, CA, USA) and CV-AFS (Millennium Merlin, 10.023, PS Analytical Ltd, Kent, UK). CV-AFS was equipped with a low-pressure mercury vapor discharge lamp, a collimator, a reflectance filter and a photomultiplier tube placed at 90° to the incident light used. The measurements were performed in the ratio mode, allowing for internal baseline

correction. The sample was first mixed with the blank solution (5% HNO_3), followed by reaction with reductant (2% $SnCl_2$) in the sample valve. This reaction resulted in elemental Hg vapor generation, which was purged with argon gas from the gas-liquid separator (0.3 L/min) through the dryer (2.5 L/min) into the atomiser. ICP-MS Agilent 7500c used forward power of 1240 W and reflected power of 1 W. Sample solutions were taken up into the Meinhard nebuliser by peristaltic pump into the Scott spray chamber cooled to 2 ˚C. Skimmer and sampler cones of nickel were used. Hg isotopes 200 and 202 were monitored together with ^{205}Tl as an internal standard.

Hg derivatisation for speciation analysis. Hg speciation was performed on accurately weighed petroleum hydrocarbon samples (approx. 1 g), which were diluted in 1 mL of *o*-xylene and derivatised immediately. First, 200 µL of 2 M Grignard reagent (BuMgCl in this work) was added into the sample and mixture was let to react for 5 min. Then, 10 mL of 0.5 M H_2SO_4 was added to stop the reaction. Finally, the entire mixture was centrifuged to facilitate separation of the organic and aqueous layer for 10 min at 3747 x g. The organic layer was transferred into GC amber vial and stored at -20°C until analysis.

Speciation analysis using SS-ID-GC-ICP-MS. Species specific isotope dilution mass spectromtetry is described in detail elsewhere.[16,17] Briefly, the natural isotopic ratio was altered by addition of enriched ^{199}Hg^{2+} (as ^{199}HgO in HCl) standards into the samples prior to the derivatisation step. Measured ratio between ^{199}Hg and ^{202}Hg was consequently used for calculation of Hg species concentration. Hg^{2+} and MeHg^{+} calibration standards were prepared from 1 mg/kg stock solution in *o*-xylene and derivatised in the same way as the samples. An HP 6890 GC (Agilent Technologies, Santa Clara, CA, USA) coupled with an ICP-MS Agilent 7500c (Agilent Technologies, Santa Clara, CA, USA) was used for mercury species separation and

detection. Both instruments were connected via a home-built transfer-line.[18] Injections were made by means of a HP 6890 series automatic sampler. The optimised parameters for the GC and ICP-MS are shown in Table 1.

Table 1. Optimised GC-ICP-MS parameters.

Parameter	Optimised value
GC parameters	*HP-6890 GC (Agilent Technologies, USA)*
Column	100% PDMS DB-1
	30 m × 0.59 mm × 1 μm
Injection type, volume	Splitless, 1 μL,
Injector temperature	200 °C
Temperature programme	50 °C (1 min); 50 °C/min up to 250 °C
Transfer line	Inner: MXT® guard column, 0.28 mm ID
	Outer: Silcosteel® 1 mm ID, 1/16" OD
Transfer line temp.	220 °C
Carrier gas flow	Helium: 15 mL/min
ICP-MS parameters	*7500c (Agilent Technologies, USA)*
RF power	1350 W
Plasma gas flow	16 L/min
Carrier gas flow	Argon: 0.7-0.8 L/min
Make-up gas flow	Argon; 0.3 – 0.35 L/min
Spray chamber	Micro-concentric
Nebuliser	Teflon PFA micro flow concentric
Internal standard	Continuous aspiration 0.3-0.4 mL/min of 10 μg/L Tl in 5% HNO_3

Monitored *m/z*	^{199}Hg, ^{200}Hg, ^{201}Hg, ^{202}Hg, ^{203}Tl, ^{205}Tl

Hg distribution imaging by LA-ICP-MS. For spatial distribution using LA-ICP-MS a drop of petroleum hydrocarbon sample was placed between two clean glass slides and smeared to create an event layer sample prior to laser ablation. The prepared sample was left to air-dry overnight. A laser ablation system (NWR-213, New Wave, Fremont, CA, USA) was coupled to the Agilent 7500c ICP-MS (Agilent Technologies, Santa Clara, CA, USA). Manual tuning of the ICP-MS was performed while ablating the CRM glass NIST 612 (National Institute of Standards and Technology, Gaithersburg, USA) with a spot size of 100 μm, at a scan speed of 5 μm/s, employing a frequency of 10 Hz and 65% laser energy resulting in a fluence of 9.17 – 10.11 J/cm^2. A carrier gas flow of 1.45 L/min at 1360 W RF power was used. Mass-to-charge ratios of ^{29}Si and ^{208}Pb were monitored. On the smeared drop of sample, 30 lines were ablated with the spot size of 8 μm, at a scan speed of 25 μm/s, employing a frequency of 10 Hz and 35% optimised laser energy resulting in a fluence of 0.45 - 0.6 J/cm^2. Before each line, the laser was warmed up for 20 s and after each series of ablations, the chamber was rinsed for 50 s. Mass-to-charge ratios of ^{34}S, ^{77}Se and ^{202}Hg were monitored.

Sedimentation of Hg particles. Sedimentation of particles was done using Optima MAX Ultracentrifuge (Beckman Coulter, UK) with an MLN-80 rotor. Eight mL polyallomer tubes were filled with sample aliquots ca 8 mL and centrifuged for 72 hours at 369,550 x g. Due to high samples viscosity only the sample A and B could be subjected to centrifugation.

3. RESULTS AND DISCUSSION

Total Hg analysis by ICP-MS and CV-AFS. The quantification of total Hg after acid digestion in the petroleum hydrocarbon samples was performed using two different instruments: ICP-MS and CV-AFS in order to validate the results. Total Hg concentration spans between 0.77 and 7.70 mg/kg with generally very good precision, RSD below 8% in almost all samples (Table 2). The greatest discrepancy between the compared analytical instrumentation was found for sample A with Hg concentration of 7.70 (± 0.47) mg/kg when analysed by ICP-MS in comparison to 8.63 (± 0.44) mg/kg obtained by CV-AFS but nonetheless, the results are comparable with a good confidence.

Table 2. Average total Hg concentration in petroleum hydrocarbon samples analysed by ICP-MS and CV-AFS after closed vessel digestion. CV-AFS was operated under alkali conditions. Average (Av.) value was calculated from triplicate sample digestion. SD stands for standard deviation and RSD for relative standard deviation

Sample ID	Hg (mg/kg) ICP-MS			Hg (mg/kg) AFS		
	Av.	SD	% RSD	Av.	SD	% RSD
A	7.70	0.47	6.2	8.63	0.44	5.1
B	1.52	0.09	5.7	1.62	0.08	5.0
C	0.769	0.05	6.1	0.765	0.01	1.2
D	2.32	0.17	7.4	2.37	0.05	2.0
E	3.32	0.41	12	3.25	0.13	4.0

Our previous study (data not published) showed underestimation of Hg most probably resulting from increased amount of sulfur in the digested samples, if CV-AFS is operated in the acidic mode, which can be mediated by preparing the reductant 2% $SnCl_2$ in HCl (1.1 M). The potential cause of Hg underestimation may lay in the reduction of sulfates into sulfides by $SnCl_2$ under the acidic conditions as it happens when Cr^{2+} is used as a reductant[19] and subsequently, Hg^{2+} reacts with S^{2-}. Thermodynamically reduction of sulfates to sulfides by $SnCl_2$ in the acidic conditions is feasible as the redox potential for sulfate is higher (E_h = +0.4 V)[20] than the redox potential of Sn^{2+} (E_h = +0.1 V).[21] Therefore, in order to overcome sulfate reduction while maintaining Hg reduction efficiency, the CV-AFS was operated under alkali conditions i.e. 2% $SnCl_2$ was prepared in NaOH (5 M) which improved the correlation of the results on average by 58%. Furthermore, comparison of the Hg concentration analysed by ICP-MS and CV-AFS operated in the alkali mode did not show significant difference (t-test, p = 0.90).

Previous studies showed that a high content of dissolved carbon in the sample digest might enhance or suppress the ICP-MS signal. This is greatly dependent on the amount of carbon entering the plasma and identity of the actual analyte.[22] Because the samples are mainly hydrocarbons with minor impurities from other elements, two calibration methods using ICP-MS were tested: external calibration and standard addition. Although, it could be expected that external calibration method will result in biased quantification due to matrix differences, the actual results in this study did not show a significant difference (t-test, p = 0.99) between Hg concentrations obtained by tested calibration methods most probably due to high dilution factor (Table 3).

Table 3. Comparison of total Hg concentration in petroleum hydrocarbon samples analysed by ICP-MS using standard addition and external calibration.
n = triplicate sample digestion.

Sample ID	Hg (mg/kg) ICP-MS					
	Standard addition			External calibration		
			%			%
	Av.	SD	RSD	Av.	SD	RSD
A	7.38	0.159	2.2	7.78	0.023	0.3
B	1.41	0.044	3.1	1.59	0.087	5.5
C	0.771	0.018	2.3	0.772	0.008	1.0
D	2.53	0.033	1.3	2.47	0.090	3.6
E	3.76	0.072	1.9	3.14	0.338	10

Variations in Hg concentration between the petroleum hydrocarbons are highly dependent on the geographical origin of the sample. For instance, Hg concentration range in the petroleum hydrocarbons originating from Thailand was reported to vary between 9 – 950 µg/kg[14] and trace amounts were found in Brazilian hydrocarbons, 0.4 – 0.9 µg/kg.[23] However, concentration levels found in petroleum hydrocarbons in North Germany were as high as 4,350 µg/kg[24] and value of 49,400 µg/kg was reported by Bloom *et al.* in the sample of unknown origin.[8] Our results support the previous finding and illustrate how Hg concentration vary and reinforce the needs for robust method for Hg analysis that could support reliably the petroleum hydrocarbons assay.

In general the total Hg concentrations in all analysed samples obtained by both instruments, ICP-MS and CV-AFS are in good agreement among them with the correlation coefficient of 1.00 (Table 2, Figure S1). The presented results demonstrated the capability of both techniques to cover a wide concentration range of Hg with a good performance and providing confidence in the data.

Hg species quantification by external calibration method. Various solvents for the preparation of calibration standards have been described in the open literature[25,26] and therefore isooctane, toluene and *o*-xylene were used in this study to assess the instrument response to the analyte in different matrices. The selection of these solvents is based on their inherent solubility with hydrocarbon and derivatives from petroleum. Calibration standards of $MeHg^+$ and Hg^{2+} with the same nominal concentrations were derivatised in the same way as the samples and in order to calculate the linear regression derivatised standards were injected to GC-ICP-MS.

Table 4. Slopes and correlation coefficients of the calibration lines for quantification of $MeHg^+$ and Hg^{2+} prepared in various solvents.

	MeHgBu		Bu$_2$Hg	
	slope/ng Hg	R^2	slope/ng Hg	R^2
isooctane	0.017	0.997	0.022	0.994
toluene	0.045	0.997	0.028	1.000
o-**xylene**	0.040	0.997	0.021	0.981

The slope of the calibration line of $MeHg^+$ standards prepared in toluene and *o*-xylene does not show significant difference, 0.045 and 0.040 respectively, which is

likely a result of similar molecular structure of these two solvents (Table 4, Figure SI 2). However, the magnitude of the slope is reduced to half when the calibration standards were prepared in isooctane. On the contrary, calibration standards for Hg^{2+} derivatised in isooctane showed very similar slope (0.022) to those in o-xylene (0.021) but lower than in toluene (0.028) (Table 4, Figure SI 3). The dependence of the slope magnitude on the solvent type is a direct result of modified plasma ionisation conditions by varying amounts of carbon arriving in the plasma. This was discussed in more detail elsewhere.[14] Ultimately, the results suggest that external calibration should not be used for the accurate quantification of organic Hg species in the petroleum hydrocarbon samples.

Hg species quantification by standard addition method. The potential of standard addition method for Hg species quantification in hydrocarbon petroleum samples such as crude oils and condensates has been previously demonstrated.[27,14] However, during the Grignard derivatisation of the studied samples, emulsification was observed after the addition of H_2SO_4. This resulted in the formation of 3 distinct layers after centrifugation of the samples. Emulsion separated the aqueous and organic layer of the mixture and was of dark brown colour. The texture resembled wax but the real identity has not been investigated. Interestingly, the amount of emulsion formed varied between replicates of the same samples.

The formation of the emulsion could be a major limitation of the application of standard addition for the quantification of Hg species in hydrocarbon samples; it seems that potential natural surfactant present in these types of petroleum hydrocarbons samples could be activated during the derivatisation and subsequently promote the formation of emulsion.[28] Other properties such as density, asphaltenes content and total acid number should be taken into consideration prior the use of this

technique. As spiked Hg species prior to derivatisation may be trapped within the formed emulsion layer, quantification would not be accurate. Therefore, it would be necessary to calculate the recovery of the standard added into the sample for which external calibration is needed.

Hg species quantification by species specific isotope dilution mass spectrometry (SS-ID-GC-ICP-MS). This quantification technique is generaly thought to be very accurate and precise and its principle is based on the alteration of Hg isotopic ratio in the studied samples by addition of an enriched standard.[16,17] Altered isotopic ratio is determined from the monitored signals of the respective isotopes by ICP-MS. Because the isotopic ratio in the sample is changed prior to derivatisation and/or extraction step, this technique is independent of the derivatisation and/or extraction efficiency during the sample preparation steps. Evenmore, as the enriched standard is spiked into the samples and no calibration curve is needed for calculation of the final Hg concentration, quantification is essentially matrix independent. However, one of the key parameters for successfully performing quantification by SS-ID-GC-ICP-MS is the equilibration time and stability of Hg species as their interconversion may result in biased quantification.

Effect of equilibration time. To address the first variable the evaluation of suitable equilibration times between the enriched and endogenous Hg species an equilibration test was carried out. Within this scope, portion of sample E was divided into 5 identical subsamples and each one was spiked with $^{199}Hg^{2+}$ standard in the same way. Each subsample was let to equilibrate for a different period of time; 15, 30, 60, 90 and 120 min after which it was derivatised. Using GC-ICP-MS for the analysis, Hg isotopes 199 and 202 were monitored (Figure 1). The peak area of both isotopes was corrected for the mass bias using isotopes ^{203}Tl and ^{205}Tl (SI Figure S2). Mass bias

corrected isotope ratio was used for the calculations of the actual concentration of Hg^{2+} in the samples in accordance with literature.[17,16]
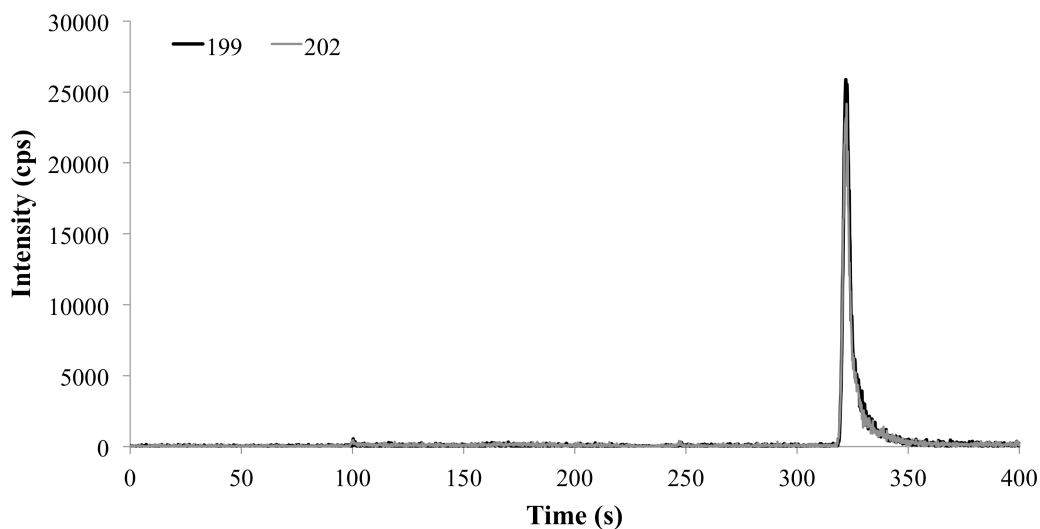

Figure 1. GC-ICP-MS chromatogram of petroleum hydrocarbon sample E spiked with $^{199}Hg^{2+}$ enriched standard. Peak at 321s corresponds to the elution of Hg^{2+} as Bu_2Hg.

   It could be expected that after the equilibrium between the enriched and endogenous Hg species is reached, the concentration in the subsamples will remain constant regardless the equilibration time. But interestingly, the determined concentration in the individual subsamples varied significantly, with RSD value of 32% (Figure 2).
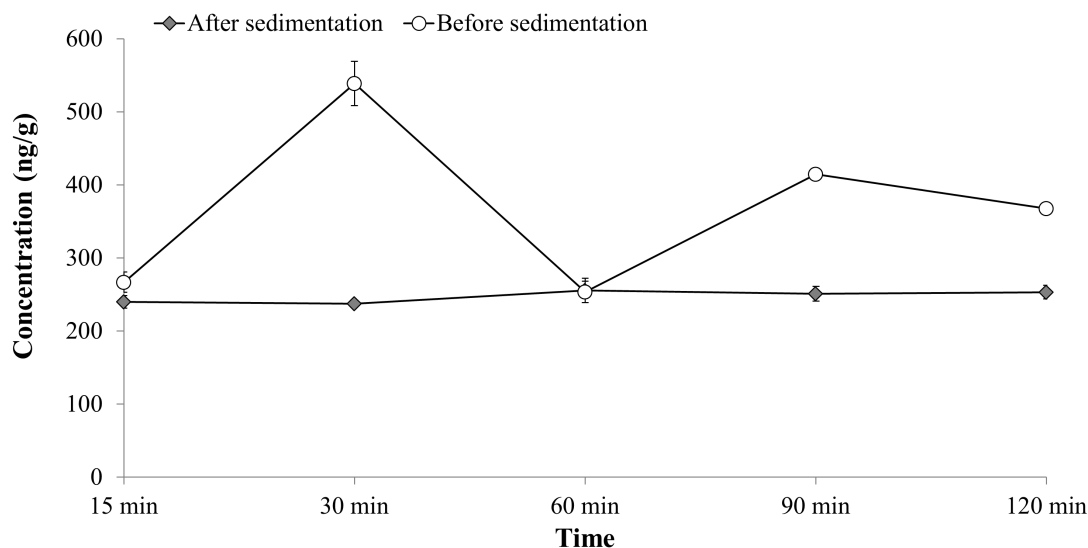
Figure 2. Equilibration time dependent variation in the calculated concentration of dissolved Hg^{2+} in sample E determined by SS-ID-MS before and after sedimentation of Hg particles by means of ultracentrifugation. The error bars represent standard deviation (SD) of triplicate injection.

The randomness of the variation suggests that a factor other than the equilibration time is causing this variation. A possible explanation may lay in the reactivity of the suspended Hg that could be in the domain of nanoparticles. It has been previously reported that Hg particulate in crude oil samples could be HgS, which is assumed to be the dominant species in the petroleum hydrocarbons stocked on-shore.[8] Non-homogeneous distribution of these particles within the samples may result in each sub-sample containing different amounts of this Hg species. Although, the high stability constant of HgS ($K_{sp} = 10^{52}$) predicts its low reactivity in the presence of other ligands it is possible that the activity may depend on particle size distribution or if it is present in bulk. Supporting this hypothesis, a recent study showed that by exposing sulfate-reducing bacteria to HgS in nano and micro-particulate form, the

overall methylmercury production in cultures exposed to nanoparticles was 6 times greater than in cultures containing micro-scale particles.[29]

The above could be supported considering that under macromolecular solid domain, surface atoms contribute with only relatively small fraction, which is available to interact with the substrate, whereas in nanoparticles domain practically all atoms are available. Then, such atoms have lower coordination numbers than in the bulk and as a consequence, which leads towards the main characteristics of nanoparticles: is their inherent reactivity due to the high surface area-to-mass ratio available in comparison with bulk which modified the surface chemistry.[30,31] Thus, there is a combined effect; first, greater accessibility to all constituent atoms on the surface concomitantly, enhanced activity because of the low coordination number they exhibit. Furthermore, nucleated nanoparticles possess high surface energy, which predicts their high reactivity and tendency towards formation of aggregates beyond the nanoscale.[32] However, interactions of nanoparticles with molecular structures like natural organic matter can mitigate the surface energy instability and enable the particles to persist in the nano-scale for longer period.[33] Consequently, the matrix in which the nanoparticles are being formed dictates the reactivity of suspended HgS. Therefore, owing to the non-homogeneous distribution of particulate Hg within the subsamples, random variation in the calculated concentrations will be observed even after complete equilibration as a result of some Hg particles' susceptibility to alkylation.

To visualise the homogeneity of Hg distribution, a drop of sample E was used for LA-ICP-MS analysis. Background corrected 2 D map obtained from the experiment confirmed not only non-homogeneous distribution of the analyte but also formation of large Hg containing aggregates (Figure 3). In a recent study Gaulier, et al.,[34] identified formed Hg aggregates as HgS particles using scanning electron microscope with

energy dispersive spectroscopy. The average size of the particles was found to be 1 μm in diameter and the particles formed aggregates of 5 – 10 μm in size.
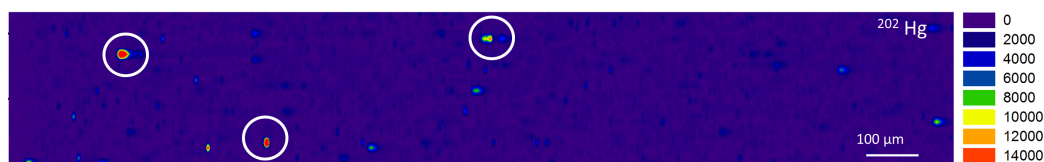

Figure 3. 2 D map of Hg spatial distribution in sample E. Hg hotspots (circled) confirmed the presence of Hg aggregates, most as micro-nanoparticles. The normalised intensities are scaled from the minimum (blue) to the maximum (red).

Consequently, the samples were subjected to ultracentrifugation to sediment any suspended particles and the equilibration test was repeated, spiking the centrifuged samples with $^{199}Hg^{2+}$ standard. Interestingly, the variation between calculated Hg concentrations in the individual subsamples was significantly reduced, with a 10-fold reduction of the RSD value (Figure 2). Although the size of the particles was not defined, their reactivity with respect to alkylation shows that the nature of Hg particulate is not as chemically inert as previously anticipated.

Subsequent Hg speciation by SS-ID-GC-ICP-MS method confirmed the presence of dissolved Hg^{2+} in all studied samples, however, any other Hg species were below the instrumental detection limit (40 ng/kg). Furthermore, the supernatant of the sedimented samples was digested and analysed for total Hg concentration which are in close agreement with the results from SS-ID-GC-ICP-MS confirming successful sedimentation of suspended Hg as well as the absence of other Hg species (Table 5). In order to close Hg mass balance in studied samples the concentration of Hg

particulate was operationally defined as [HgT]-[Hg^{2+}] and contributes to 98.3% and and 91.1% in the sample A and B, respectively.

Table 5. Hg speciation after sedimentaion of particulate Hg in the selected samples. Hg^{2+} was obtained by SS-ID-GC-ICP-MS after sedimentation of suspended Hg. Portion of the centrifuged fraction after sedimentation was also digested and analysed for total Hg concentration. The Hg particulate was operationally defined as [HgT]-[Hg^{2+}], based on HgT concentration analysed by ICP-MS.

Sample	Hg^{2+} as Bu$_2$Hg (mg/kg)			HgT in centrifuged fraction (mg/kg)		Hg particulate (mg/kg)		
ID	Av.	SD	% of HgT	Av.	SD	Av.	SD	% of HgT
A	0.130	0.006	1.7	0.135	0.015	7.57	0.475	98
B	0.134	0.004	8.9	0.128	0.007	1.38	0.086	91
C*	0.099	0.018	-	NA	-	NA	-	-
D*	0.423	0.059	-	NA	-	NA	-	-
E*	0.248	0.008	-	NA	-	NA	-	-

Asterisk (*) identifies samples, which were not subjected to sedimentation.
NA means not analysed.

Effect of GC inlet temperature. Furthermore, it was observed that with increasing GC inlet temperature the area of Hg^0 increases while Hg^{2+} remains constant (Figure 4). Effect of the GC inlet temperature on the decomposition of DEtHg and DBuHg and subsequent formation of monoalkyl-Hg was previously demonstrated[35] but sample used in our experiment contained only dissolved Hg^{2+} and particulate Hg.

Therefore, the observed formation of Hg^0 is most probably an analytical artifact from decomposition of HgS particles in the injector upon sample introduction to the GC. Although, experimentally defined temperature of HgS thermal desorption is 365 °C (range between 250 – 435 °C)[36] here, nano size HgS particles were used. It is expected that volatility of nano size HgS will differ from the commercially available bulk material. This observation further advocates the necessity of removal particulate Hg from the sample, prior to Hg speciation.
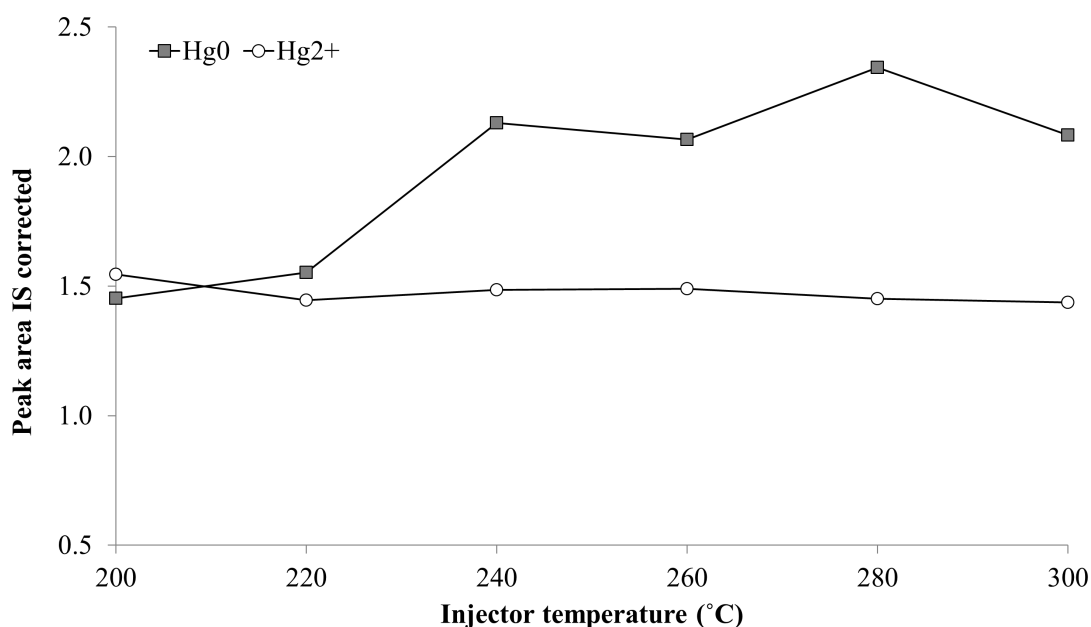

Figure 4. Internal standard corrected peak areas of Hg^0 and dissolved Hg^{2+} during multiple injections of sample E at different GC injector temperature.

4. CONCLUSION

Petroleum hydrocarbons contain a wide concentration range of Hg impurities, which may have potentially adverse effects on the refining and production processes. Low vapour pressure of some Hg species and the hydrocarbon matrix itself requires necessary precaution to be taken during the sampling and adequate storage to ensure

the authenticity of the analytical results. CV-AFS and ICP-MS are capable to accurately determine Hg concentration in the digested petroleum hydrocarbon samples; however, CV-AFS should be operated in the alkali mode to avoid Hg underestimation. Quantification of Hg species by external calibration and standard addition method was hindered by the complexity of the matrix and thus these methods are not recommended for Hg speciation in the sample with high carbon number. Although, SS-ID-GC-ICP-MS method has proved to be suitable for quantification of Hg species in petroleum hydrocarbons, it is critical that suspended Hg is removed by ultracentrifugation. The susceptibility of suspended Hg particles to alkylation during the derivatisation step points out the reactivity of these particles that contradicts their suspected chemical inertness and their presence can lead to overestimation of dissolved Hg species. Therefore, in order to achieve accurate Hg quantification in petroleum hydrocarbons, the identity of all Hg species present in the sample must be known.

ASSOCIATED CONTENT

Figures showing an example of SS-ID-GC-ICP-MS chromatogram of spiked petroleum hydrocarbon sample, spectra of individual alkylated Hg species with identified retention time and graphical representation of external calibration method for quantification of Hg species concentration. This material is available free of charge via the Internet at http://pubs.acs.org.

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