JYU DISSERTATIONS 725

Suvi Kulomäki

Preconcentration, Speciation, and Determination of Mercury in Natural Waters by Inductively Coupled Plasma Mass Spectrometry

UNIVERSITY OF JYVÄSKYLÄ
FACULTY OF MATHEMATICS
AND SCIENCE

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ABSTRACT

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Mercury (Hg) is one of the most toxic elements released into the environment from anthropogenic and natural sources. Once deposited, the biogeochemical processes can convert inorganic Hg (iHg) into methylmercury (MeHg). Poisonous MeHg accumulates within the food chain thus posing risks to the environment and human health. The aquatic environment is often the last receptor of contaminants, and therefore, monitoring low Hg levels in natural water is essential due to the extreme toxicity of Hg. This thesis presents highly sensitive analytical methods for the preconcentration and speciation of iHg and MeHg and the determination of total Hg concentrations in natural waters at low ng L^{-1} concentrations.

The first part of the thesis develops an inductively coupled plasma mass spectrometry (ICP-MS) analysis method for the determination of ultra-trace concentrations of iHg. This was achieved by applying 3D printed scavenger filters for iHg preconcentration. The procedure is based on the adsorption of iHg into a 3D printed metal scavenging filter followed by elution with acidic thiourea solution and ICP-MS determination.

Without using the developed preconcentration method, biased low Hg recoveries were obtained with the direct ICP-MS method. Therefore, an ICP-MS method for the determination of total Hg in humic-rich natural waters was developed. To release Hg species from the sample matrix and to stabilize them in solution, 0.12% thiourea and 3% HCl were added to all sample solutions. ICP-MS could then be used to accurately determine the total Hg concentrations in natural waters containing at least 2 ng L^{-1} of Hg. This approach also eliminates the Hg memory effect, a common problem with liquid sample introduction systems.

The final part of the thesis combines the advantages of the two developed methods with subtle modifications that allow the preconcentration and speciation of MeHg and iHg by using 3D printed scavenger filters followed by ICP-MS determination, whereas total Hg determination can be performed directly with ICP-MS without using any preconcentration procedures or hyphenated techniques.

Keywords: mercury, methylmercury, ICP-MS, thiourea, natural water, 3D printing

TIIVISTELMÄ

Kulomäki, Suvi
Elohopean esikonsentrointi, spesiaatio ja määritys luonnonvesistä induktiivisesti kytketyllä plasma-massaspektrometrillä
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Elohopea (Hg) on yksi toksisimmista alkuaineista, jota vapautuu ympäristöön sekä ihmisten toiminnan seurauksena että luonnollisista lähteistä. Biogeokemialliset prosessit voivat muuntaa epäorgaanisen elohopean (iHg) metyylielohopeaksi (MeHg). Myrkyllinen MeHg kerääntyy herkästi ravintoketjuun aiheuttaen riskejä niin ympäristölle kuin ihmisten terveydelle. Suurin osa saasteista, ja myös äärimmäisen myrkyllinen Hg, päätyy usein lopulta vesistöihin, joten luonnonvesien alhaisten Hg-pitoisuuksien seuranta on hyvin tärkeää. Tässä väitöskirjassa esitellään erittäin herkät analyysimenetelmät iHg:n ja MeHg:n esikonsentrointiin ja spesiaatioon sekä kokonaiselohopean määritykseen luonnonvesistä nanogrammapitoisuustasolla.

Väitöskirjatyön ensimmäinen tavoite oli kehittää analyysimenetelmä Hg:n erittäin pienten pitoisuuksien analysoimiseksi luonnonvesistä induktiivisesti kytketyllä plasma-massaspektrometrillä (ICP-MS). Tämä saavutettiin hyödyntämällä 3D-tulostettuja metallisieppareita iHg:n esikonsentrointiin. Menetelmässä iHg adsorboidaan 3D-tulostettuun metallisiepparіin, josta se eluoidaan happamalla tiourealiuoksella. Lopulta Hg-pitoisuudet määritetään ICP-MS:llä.

Ilman esikonsentrointia Hg:lle saatiin toistuvasti liian alhaisia saantoja suoralla ICP-MS-menetelmällä, joten seuraavaksi kehitettiin menetelmä kokonaiselohopeapitoisuuksien analysoimiseksi humuspitoisista luonnonvesistä. Kehitetyssä menetelmässä kaikkiin näytteisiin lisätään 0,12 % tioureaa ja 3 % vetykloridihappoa, jotta Hg saadaan vapautettua näytematriisista ja stabiloitua liuokseen. Kehitetyllä ICP-MS-menetelmällä voidaan analysoida näytteitä, joissa Hg:aa on vähintään 2 ng L^{-1}. Samalla saadaan poistettua myös Hg:n muistiefekti, joka on hyvin yleinen ongelma nestemäisissä näytteensyöttöjärjestelmissä.

Väitöskirjan viimeisessä osassa yhdistetään kahden edellä kehitetyn menetelmän edut ja laajennetaan se myös MeHg:lle. Tällöin on mahdollista konsentroida ja erottaa MeHg ja iHg toisistaan 3D-tulostettujen metallisieppareiden avulla, jonka jälkeen niiden pitoisuudet määritetään ICP-MS:llä. Kokonaiselohopea voidaan puolestaan määrittää suoraan ICP-MS:llä ilman esikonsentrointimenetelmiä tai yhteen liitettäviä tekniikoita.

Asiasanat: elohopea, metyylielohopea, ICP-MS, tiourea, luonnonvesi, 3D-tulostus

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PREFACE

The research presented in this dissertation was conducted at the Department of Chemistry, University of Jyväskylä, during the years 2017–2023. The work was funded by the Jenny and Antti Wihuri Foundation and the University of Jyväskylä Graduate School for Doctoral Studies. I am truly grateful for all the funding that I have received, including a travel grant given by the Alfred Kordelin Foundation.

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A special thanks to the former and present members of Team Väisänen and other colleagues at the Department of Chemistry. It was a great pleasure to get to know you all. Thank you for the enjoyable coffee break conversations, including the virtual ones during the COVID-19 pandemic.

In spring 2021, I moved on to new challenges and continued writing the last manuscript and the summarizing report of this thesis alongside a new career. I am extremely grateful for the opportunity to continue authoring this thesis alongside my new job at Metso.

Thanks to my friends and family for the endless support during all these years. Finally, my greatest gratitude I owe to my well-beloved fiancé Anton. Thank you for always being there for me. This journey would not have been possible without your love, encouragement, and support.

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The author's contribution
The author of the thesis is the primary author and performed the drafting of all manuscripts, excluding the 3D printing and material characterization sections in publication **I**. The author performed all experimental work in publications **I**–**III**, excluding 3D printing in publication **I**. A more detailed description of the author's contribution is included in each publication in the section of author contributions or CRediT authorship contribution statement.

CONTENTS

ORIGINAL PAPERS

ABBREVIATIONS

3D	three-dimensional
AAS	atomic absorption spectrometry
AFS	atomic fluorescence spectrometry
APDC	ammonium pyrrolidinedithiocarbamate
CE	capillary electrophoresis
CPE	cloud point extraction
cps	counts per second
CRM	certified reference material
CV	cold vapor
CVG	chemical vapor generation
DC	direct current
DMeHg	dimethylmercury
EDTA	ethylenediaminetetraacetic acid
EFSA	European Food Safety Authority
ERM	European reference material
ESI	Elemental Scientific
EU	European Union
GC	gas chromatography
HPLC	high performance liquid chromatography
IC	ion chromatography
ICP	inductively coupled plasma
ICP-MS	inductively coupled plasma mass spectrometry
ICP-OES	inductively coupled plasma optical emission spectrometry
IDL	instrument detection limit
iHg	inorganic mercury
LLE	liquid-liquid extraction
LOD	limit of detection
LOQ	limit of quantification
LPME	liquid-phase microextraction
m/z	mass to charge ratio
MAC-EQS	maximum allowable concentration environmental quality standards
MDL	method detection limit
MeHg	methylmercury
MS	mass spectrometry
MSPE	magnetic solid-phase extraction
PA11	polyamide-11
PA12	polyamide-12
PET	polyethylene terephthalate
PP	polypropylene
ppb	parts per billion
ppm	parts per million

PTFE	polytetrafluoroethylene
QC	quality control
QID	quadrupole ion deflector
RF	radio frequency
RSD	relative standard deviation
SLS	selective laser sintering
SPE	solid-phase extraction
SPME	solid-phase microextraction
TDS	total dissolved solids
Thiol	thiol-functionalized silica
TOF	time-of-flight
TWI	tolerable weekly intake
US EPA	United States Environmental Protection Agency
v/v	volume to volume ratio
w/v	weight to volume ratio
WHO	World Health Organization

1 INTRODUCTION

Mercury (Hg) is a naturally occurring, extremely toxic element that circulates between the atmosphere, oceans, and land for centuries to millennia. It is eventually removed from the system as it becomes buried in ocean and lake sediments and subsurface soils. The environment naturally releases and cycles a certain amount of Hg, for example, during a volcanic eruption and weathering of Hg-containing rocks. However, Hg can also be released into the environment through human activities such as artisanal mining, combustion of coal, metal smelting, and cement manufacturing. For hundreds of years, Hg has been used in several products and processes due to its unique properties, and total atmospheric concentrations of Hg have risen to approximately 450% above natural levels.[1–4]

Hg occurs in different chemical forms in the environment, of which elemental mercury (Hg^0), inorganic mercury (iHg), and organic mercury are the main species. As Hg cycles through the environment, it undergoes a variety of chemical and physical changes and may be deposited and re-emitted several times before exiting the global cycle.[1] Especially in aquatic systems, iHg can be converted into methylmercury (MeHg) through microbial action and then concentrate up the food chain. MeHg is one of the most toxic Hg species with the highest bioaccumulation factors.[5] Hg concentrations vary widely by species, but, for example, top predatory fishes can contain up to 10^6 times higher Hg concentration than the surrounding water, and up to 95% of this Hg can be in the methylated form.[6]

Dietary consumption of fish, shellfish, and marine mammals contaminated with MeHg is the major source of human exposure to Hg. In some communities, the source of Hg exposure may be derived from rice-growing regions heavily contaminated with Hg, primarily in mining sites.[1,4,7] Human exposure to Hg can arise mainly occupationally, in which case exposure occurs through inhalation of Hg vapor or via contact with Hg-containing products. Exposure to Hg^0 and iHg compounds may mainly affect the nervous system and kidneys. MeHg, in turn, adversely affects brain development, especially in fetuses.[1] Overall, Hg and its compounds have a range of severe health effects, depending on the form of Hg, the concentration level, and the exposure pathway, affecting the central nervous system, immune systems, digestive systems, lungs, kidneys, skin, and eyes. It also causes neurological and behavioral disorders, and the effects are often recognized only when it is too late.[2,8,9]

The worst historical case of Hg poisoning, one of the first tragedies of its type, occurred around Minamata Bay in Japan in the 1950s. Patients were reported to suffer from neurological symptoms of an unknown cause. Ultimately, the cause was revealed to be a MeHg-induced central nervous system disease that had developed among residents who routinely consumed considerable amounts of fish and shellfish contaminated with MeHg. The MeHg was generated as a by-product in the acetaldehyde manufacturing process, where iHg compounds were used as a catalyst. The chemical plant discharged the wastewater without treatment into Minamata Bay over 35 years, and MeHg accumulated at high concentrations by food chains. More than 1,700 people with Minamata disease, named after the place where it was first observed, have died after MeHg poisoning, and thousands more were affected.[10,11]

Unfortunately, Minamata is not the only example of a devastating incident of Hg poisoning. Another large-scale disaster took place in rural Iraq in the 1970s.[12] Through several unfortunate events, 459 people died after consuming homemade bread prepared from seed grains treated with organomercury fungicide. According to epidemiological monitoring, up to 40,000 people may have been poisoned.[13] The discovery of biomethylation and bioaccumulation has aroused strong interest in the fate of environmental Hg and routes of human exposure.[13] Finally, the world woke up to the dangers of Hg and the fact that Hg is a global problem that requires joint global actions to effectively protect human health and ecosystems. Awareness of Hg pollution and its impacts has led to the control of emissions.[2,9]

Significant efforts have been undertaken over the last decades to reduce Hg pollution through the implementation of laws and regulations on Hg emissions and uses. At long last, on August 16, 2017, the Minamata Convention came into force to protect human health and the environment from the harmful effects of Hg and its compounds throughout its life cycle. One hundred forty-seven parties from all over the world have worked together to disrupt trade, increase public awareness, strengthen institutional capacity, and produce Hg-free products. The Convention describes a set of measures to ban new Hg mines and control releases to water, land, and air to make Hg history.[2,9]

There is an essential need for the determination of Hg and its species in natural waters to meet the regulations on the presence of Hg in aquatic systems, assess Hg contamination, and monitor the effectiveness of the measures taken under the Minamata Convention.[14] Advances in Hg analysis could help to provide more reliable and widespread data on Hg concentrations in the environment to fully understand its impacts on health and ecosystems.[9] Therefore, the present thesis aims to develop sensitive and selective analytical techniques for iHg, MeHg, and total Hg determination.

2 MERCURY IN THE ENVIRONMENT

2.1 Properties, occurrence, and uses

Mercury, also called quicksilver because it is liquid at room temperature, has been known since ancient times. It is a dense, silvery-white, shiny transition metal belonging to Group 12 of the periodic table and has an atomic number of 80 and atomic weight of 200.59 g mol^{-1}.[15–17] Hg has seven naturally occurring isotopes with ^{202}Hg being the most abundant (29.74%) followed by ^{200}Hg (23.14%), ^{199}Hg (16.94%), ^{201}Hg (13.17), ^{198}Hg (10.04%), ^{204}Hg (6.82%), and ^{196}Hg (0.155%).[18] It exists in the oxidation states 0 (metallic), +1 (mercurous), and +2 (mercuric), and the latter two oxidation states form numerous inorganic and organic chemical compounds. Hg forms monovalent and divalent compounds with fluorine, chlorine, iodine, and sulfur.[16]

The terrestrial abundance of Hg is about 50 parts per billion (ppb) with significant local variations.[17] Relatively low-grade Hg ores are located close to the earth's surface, with an Hg content from 0.1 to over 2%.[16,17] Vermillion color mineral cinnabar (HgS) is the most important ore for Hg extraction and was used as a coloring agent for several thousands of years.[19] Other minerals found in Hg deposits, such as corderoite ($Hg_3S_2Cl_2$), livingstonite ($HgSb_4S_8$), montroydite (HgO), terlinguaite (Hg_2OCl), calomel (Hg_2Cl_2), and metacinnabar (a black form of HgS) are rare.[17] Many economically valuable minerals, especially non-ferrous metals, contain Hg as an impurity.[9] Quantitative estimates of reserves of Hg are not available, but the estimated world resources of Hg are 600,000 metric tons, while the world mine production of Hg was estimated to be 4,000 tons in 2019.[20]

Humans have released Hg into the atmosphere for millennia. Historically, Hg was used in the extraction of gold, amalgamation with other metals, cosmetics, as a contraceptive, and for religious ceremonies. The cinnabar powder has been part of traditional Chinese and Indian Ayurvedic medicines for 2,000 years.[17,21] Around the 16th century, Hg was used in the treatment of syphilis and for the manufacturing of mirrors by using the Hg-foiling process. After the invention of the barometer in the 1640s, the use of Hg increased. Then, throughout the 19th and 20th centuries, Hg was found in applications such as biocides, dental amalgam, batteries, pharmaceuticals, paints and pigments, organic synthesis, mining, chlorine and caustic soda manufacturing, light switches, and explosives.[17] Currently, the use of Hg has declined worldwide, except for the use of Hg in artisanal gold mining.[17] Hg is still found in several electrical devices and instruments (different kinds of switches, relays, sensors, and thermometers), batteries, paints, dental amalgam, energy-efficient fluorescent light bulbs, cosmetics, and some pesticides and fungicides, although Hg-free alternatives are replacing these on a large scale.[9]

2.2 Natural and anthropogenic releases

The current anthropogenic Hg emissions and the anthropogenic releases of Hg emitted and deposited historically and still circulating in the biosphere, i.e., legacy Hg, are the major contributors to increased Hg levels and exposure. The increased Hg emissions are a concern for both ecological and human health, even in remote locations, as Hg is transported around the globe.[1–3] Natural sources of Hg include geothermal activities, volcanic eruptions, emissions from the ocean, forest fires, and weathering of Hg-containing rocks, but Hg can also be released into the environment by human activity.[2,9] Currently, artisanal and small-scale gold mining are the major sources of anthropogenic emissions, followed by coal combustion and operations that process ores or produce cement. The rest of the emissions consist of the disposal of mercury-added product waste (lamps, batteries, and dental fillings), stationary combustion of fuels other than coal, ferrous-metal production, and other sources (**Fig. 1**).[4]

Almost all Hg emitted into the atmosphere is in the form of iHg (chemical symbols of Hg(II) or Hg^{2+}), as gaseous Hg^0, and oxidized species in particulate and gaseous forms.[4] In 2015, the amount of anthropogenic emissions of Hg into the atmosphere was estimated to be 2,220 metric tons and were approximately 20% higher compared to estimates for 2010.[1,3,4] Emissions are anticipated to remain high in the early 2000s, at around 2,000-2,500 metric tons per year, giving rise to global pollution.[1] However, there are numerous uncertainties in model physics and chemistry concerning model inputs and measurements used for evaluating current and historical Hg emissions from diverse sources, and the influence of climate change and legacy Hg complicates the estimates. Nevertheless, the models have progressed to the point where useful policy-relevant information on the relationship between source and receptor can be derived. Models can also be used regionally and globally to provide the first estimates of the effects of emission reductions on Hg deposition.[4]

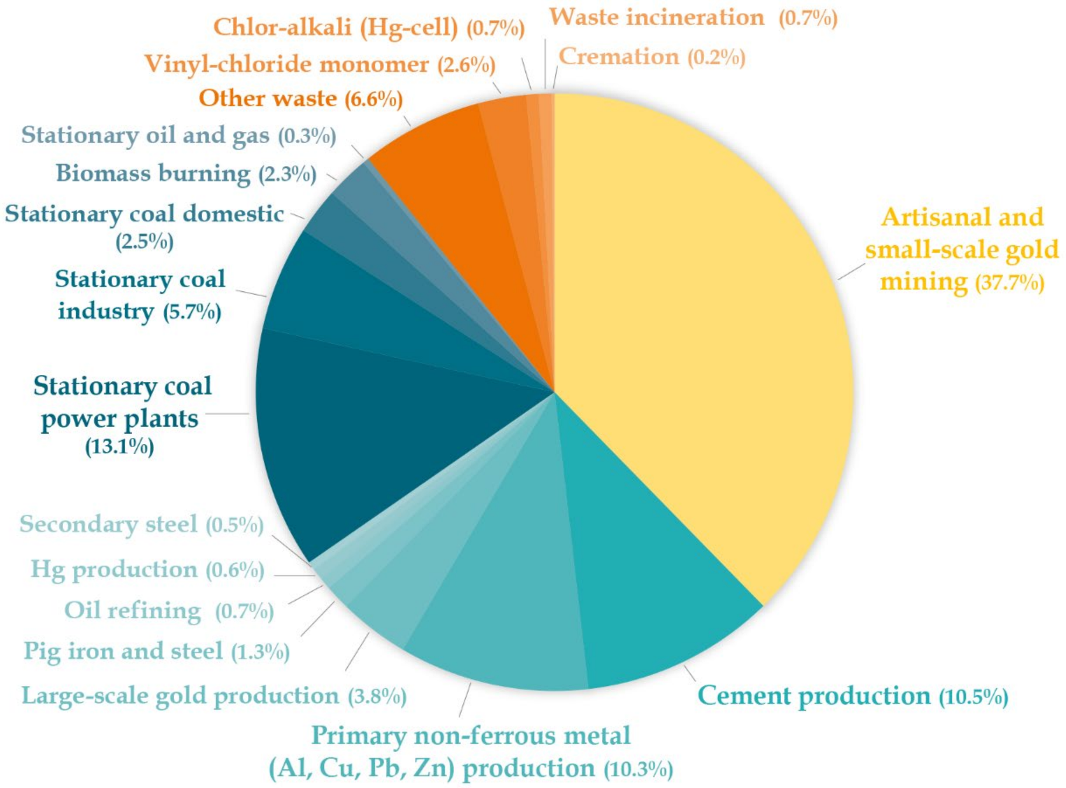

FIGURE 1 Proportions of global Hg emission to air (2,220 metric tons in total) from different anthropogenic sources in 2015.[4]

According to the United Nations Environment Programme, information regarding global releases of Hg to aquatic systems is a work in progress, and it is a crucial step towards filling the gap in inventories of anthropogenic releases of Hg into the environment. Releases of Hg into the water can occur from various areas of human activity in addition to natural sources. Ore mining and processing (42%), waste treatment (42%), and the energy sector (16%), currently releasing around 580 metric tons per year, are the major sectors accounting for most of the anthropogenic releases to aquatic systems. The artisanal and small-scale gold mining input to water and land is approximately 1,220 metric tons. Hg releases to soils are estimated at roughly 7,000 to 8,000 metric tons, which is a potential secondary source of Hg to water and the atmosphere.[1,4]

2.3 Cycling and bioaccumulation of Hg

The chemistry and cycling of Hg are inherently complex; therefore, the behavior of Hg in the environment is challenging to predict. The conceptual model of the Hg cycle is depicted in **Fig. 2**, although the dominant controls on the biogeochemical cycle of Hg are oversimplified in this depiction. Once released, gaseous Hg^0 can remain in the atmosphere for 0.5–1 year, undergo various atmospheric redox reactions, and become widely dispersed. Divalent Hg (Hg(II)) and Hg associated with the particulate matter have a much briefer lifespan than Hg^0, just a few days to a few weeks. These are also more soluble in water than Hg^0 and can be deposited to watersheds, lakes, and terrestrial surfaces from the atmosphere through precipitation, i.e., wet deposition, or they can settle on the surfaces as dry deposition without precipitation. Atmospheric iHg deposited on surfaces can be reduced to Hg^0 and may then volatilize back into the atmosphere.[4,22]

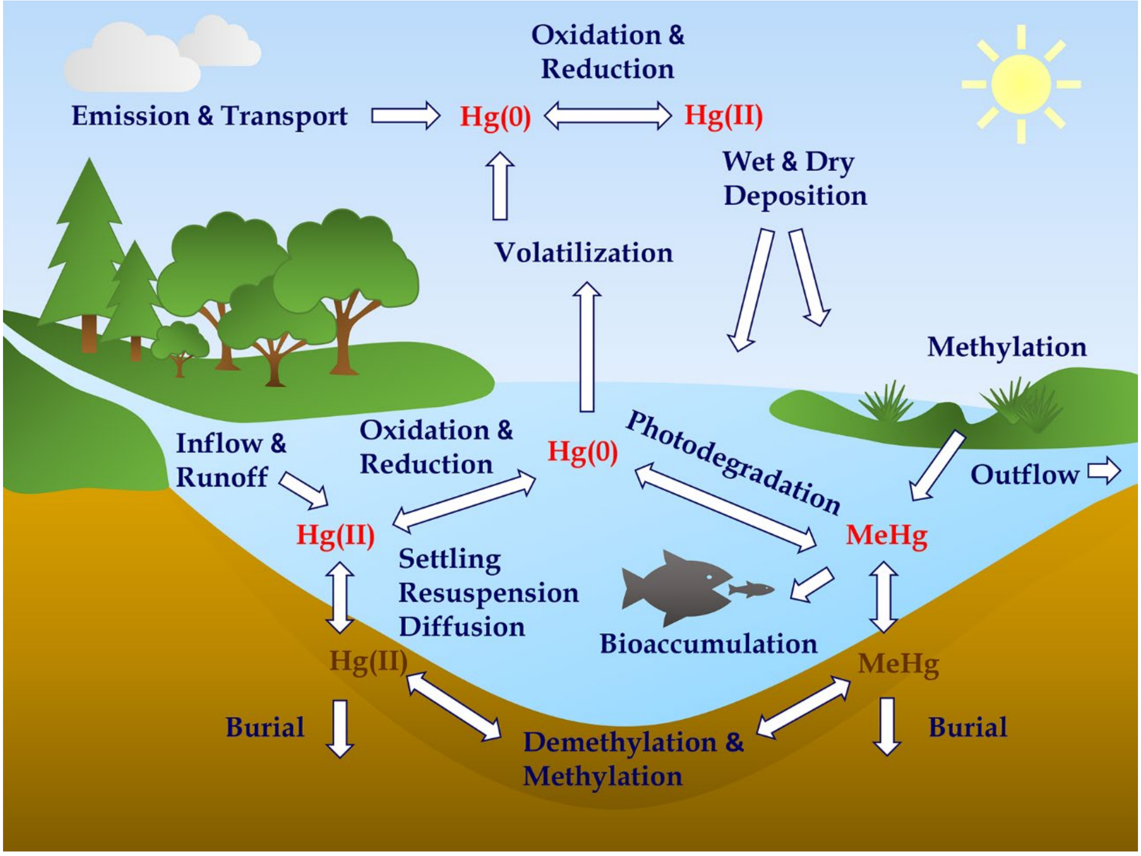

FIGURE 2 Conceptual model of Hg cycling in freshwater lakes.[23–25]

Hg deposited in water is predominantly in the form of iHg and, to a lesser extent, liquid Hg^0.[3] The main dissolved Hg species include Hg^0, complexes of Hg(II) with several inorganic and organic ligands, and organic Hg species, primarily MeHg and dimethylmercury (DMeHg).[26] Site-specific environmental conditions and variables, many of which are interrelated, will largely determine the fate of Hg once entered into the aquatic system. The Hg cycle is complex due to the interconversion of Hg compounds. They can be released from sediments into the water bodies, incorporated into aquatic biota, released into the air, or transported to previously uncontaminated locations.[3,4,26] The pH and redox conditions and concentrations of complexing agents greatly affect the chemical form of Hg in aquatic systems.[26]

Both iHg and MeHg tend to form complexes, especially with sulfur.[26] In addition to sulfur or sulfides, Hg(II) can form complexes with halide ions, hydroxides, amines, humic acids, and fulvic acids.[27] Hg(I) is stable only as a dimer (Hg_2^{2+}) in aqueous solution and is not commonly found in natural water samples as it readily decomposes into Hg^0 and Hg^{2+}. However, it may be found in experimental media or soils.[26,27] Hg^0 is volatile and usually a small fraction of the total Hg but is not usually present in water at measurable concentrations. It does not strongly associate with the molecules that complex Hg^{2+} in an aqueous solution. Volatile DMeHg also does not form complexes with ligands and is mainly detectable in seawater.[27]

A small part of the deposited iHg is converted to MeHg, which is the most common organomercurial in freshwaters.[22,27] Hg in oceans and soils remains a major source of iHg to be converted into MeHg. Methylation is favored in anoxic environments, and many competing formations, transformation, and demethylation processes affect the final concentration of MeHg in the aquatic environment.[1,4] The production rates generally depend on the presence and productivity of methylating bacteria and archaea, including fermentative bacteria, methanogens, and sulfate- and iron-reducing bacteria, and the uptake of iHg to these microbes.[28,29] Additionally, the sulfur cycle, ecosystem pH, the redox potential of the environment, and the presence of organic matter affect the methylating process, which is a topic of continuing research.[22,30]

Typical Hg concentrations in natural waters are at the ng L^{-1} level, approximately 0.2–100 ng L^{-1}. MeHg concentrations are even lower, from less than 1% up to 30–40% of the total Hg concentrations, typically around 0.05 ng L^{-1}.[31–33] The highest Hg concentrations can be found in humic-rich waters where they are up to 20 ng L^{-1}.[33] MeHg is the only form of Hg that biomagnifies in food chains, and top predators carry a far greater load of Hg compared to those at the bottom of the food chain.[4,9] Also, zooplankton often contain many hundred times more MeHg than the ambient water.[34]

The most common pathway of human exposure to Hg is through the consumption of marine fish and mammals, thus involving humans in the environmental Hg cycle.[4] In European countries, tuna, swordfish, cod, whiting, pike, and hake are the major sources of MeHg exposure.[35] MeHg is extremely toxic because it is easily absorbed in the digestive tract and is transported freely throughout the body to concentrations of toxicological concern.[1,9] The symptoms of Hg poisoning are well known through the Minamata and Iraq poisonings and are irreversible, but they do not usually worsen without new exposure.[9]

2.4 Mercury policy

In recent decades, the use of Hg has been restricted or banned, and Hg emissions have been regulated as Hg poses a significant risk to both the global environment and human health. The Minamata Convention on Mercury is intended to control, decrease, and eventually eliminate Hg emissions into the environment.[9,34] In general, policy changes resulting from the Minamata Convention include bans on new Hg mines and phasing out existing ones, air emissions control measures, and international regulations on the informal sector of small-scale and artisanal gold mining.[9] At the moment, the Minamata Convention has 147 parties and 128 signatories.[2] If the Convention is properly implemented, it should have genuinely global effects.[9] However, if emissions from anthropogenic sources stopped immediately, it would take several centuries for ambient Hg levels to decline to pre-industrial levels.[34]

Currently, European Hg legislation is more stringent than the Convention requires. The European Union (EU) Hg regulation[36] (*Regulation (EU) 2017/852*) prohibits new uses of Hg in industry and products within the EU unless they demonstrate considerable environmental and health benefits and provided that technically practicable Hg-free alternatives are not available.[9] New regulations also set out measures to reduce amalgam use and pollution, as it is the largest use of Hg in the EU. In 2010, the EU accounted for about 4.5% of global anthropogenic Hg emissions into the atmosphere, and it is estimated that 40–80% of the Hg deposited in the EU comes from outside of the EU.[3,36]

Over the last few decades, EU legislation has prohibited the use of Hg in most products.[9] For example, the Hg content of batteries can be no more than 0.0005% by weight if they are to be placed on the EU market in accordance with *Directive 2006/66/EC*. The directive on the restriction of the use of certain hazardous substances in electrical and electronic equipment (*RoHS Directive 2011/65/EU*) restricts the use of Hg in electrical and electronic equipment. The directive on waste electrical and electronic equipment (*WEEE Directive 2012/19/EU*) requires removing Hg-containing equipment and components from any separately collected WEEE. All Hg-containing waste must also be safely disposed of (e.g., *Industrial Emissions Directive 2010/75/EU, WEEE Directive, and Waste Framework Directive 2008/98/EC*).[9] The best available techniques are also required to decrease emissions from all applicable industrial activities, such as manufacturing and smelting metals and cement production.[37]

Hg and its compounds have been identified as priority hazardous substances, and environmental quality standards have been set for Hg in both surface water and biota to protect top predators from secondary poisoning via bioaccumulation (*Directive 2013/39/EU*).[9] The maximum allowable concentration environmental quality standards (MAC-EQS) for Hg and its compounds in inland and other surface waters have been set at 0.07 $\mu g\ L^{-1}$, and EQS for biota has been set at 20 $\mu g\ kg^{-1}$ wet weight. In the EU, the biota matrix is preferred for the measurement of Hg. The findings have shown that 41% of Europe's water bodies have failed to achieve good chemical status for Hg and exceeded the EQS.[9,38]

To protect human health, European and national food safety authorities guide the consumption of fish. In addition to providing energy and protein, fish contains important nutrients, including omega-3 polyunsaturated fatty acids. Consuming approximately one to two servings of seafood per week and up to three to four servings per week during pregnancy is associated with improved functional neurodevelopment outcomes in children and a reduced risk of death from coronary heart disease in adults.[39] However, only a few servings (<1–2) of species with high MeHg content can be consumed before the tolerable weekly intake (TWI) is reached.[40] The European Food Safety Authority (EFSA) has defined TWI for MeHg and iHg at 1.3 and 4 $\mu g\ kg^{-1}$ body weight per week, respectively.[35] Commission Regulation (EC) No 1881/2006 sets maximum levels of Hg at 0.5 $mg\ kg^{-1}$ wet weight for fish in general and 1.0 $mg\ kg^{-1}$ wet weight for some larger predatory species.

In drinking water, Hg is of concern only in the inorganic form, as MeHg has significantly lower water solubility and is seldom encountered in drinking water in the EU.[41] According to Annex I of the Drinking Water Directive, "*Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption,*" <1.0 $\mu g\ L^{-1}$ of Hg is a minimum requirement for Hg in drinking water, although the World Health Organization (WHO) recommended the removal of Hg from Annex I due to its low occurrence.[41,42] However, Hg was kept in Annex I due to health reasons and because a binding value had to be set at the EU level.

3 ICP-MS INSTRUMENTATION AND PRINCIPLE OF OPERATION

ICP-MS is a widely used analytical technique for trace element analysis that can measure most of the elements of the periodic table and the individual isotopes of these elements. A major advantage of ICP-MS is its multi-element capability, allowing simultaneous determination of multiple elements in a single analysis even at the ng L^{-1} range. Most samples analyzed by ICP-MS are liquids that are pumped through a sample introduction system consisting of a spray chamber and a nebulizer. The generated aerosol is then directed to a high-temperature plasma (6000–7000 K) via a sample injector. Sample droplets are dried, vaporized, atomized, and ionized. Positively charged ions generated within the plasma are directed into the mass spectrometer through the interface region, separated according to the mass-to-charge ratio (m/z), and finally, the detector counts the individual ions. **Fig. 3** shows an example illustration of an ICP-MS instrument.

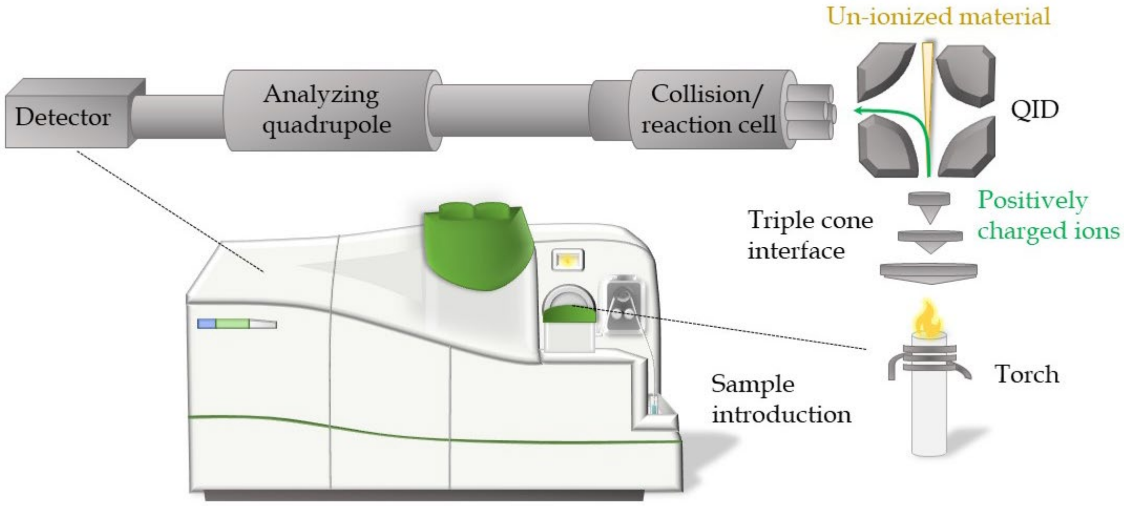

FIGURE 3 Example illustration of an ICP-MS instrument with a triple cone interface and QID.

The commercial ICP-MS instruments can vary quite significantly in the design of the instrument, mainly in the design of the interface region, the mass-analyzer, which operates at a high vacuum, and how the ion beam is focused on the mass-analyzer. The ion beam is focused through a cone interface consisting of two to three cones. The orifice opening of the cones allows the ion beam to pass into the vacuum region. It is important to achieve an adequate reduction in pressure between plasma and the mass-analyzer to minimize the divergence of the ion beam.[43] After entering the vacuum region, photons and neutrals are separated from analyte ions by either ion optics or a quadrupole ion deflector (QID), depending on the manufacturer.

After focusing the ion beam via ion optics or QID, it is directed into a mass separation device. The mass separation device separates the analyte ions from the matrix, solvent, and argon-based ions according to their m/z ratio.[44] The mass analyzer can be based on a quadrupole, double-focusing magnetic sector, or time-of-flight (TOF) technology.[45] The aforementioned technology is the most traditional, and it utilizes four metallic rods of equal diameter and length in mass separation.[45] At the entrance of the four rods of the quadrupole, specific radio frequency (RF) and direct current (DC) potentials are applied to the opposing rods of each set, allowing analyte ions of a specific m/z to pass through the rods and reach the detector.

Collision/reaction cell technology has enhanced the performance of the quadrupole mass analyzers used in ICP-MS. The collision/reaction cell is located before the analyzer quadrupole and is used to remove interferences with collision/reaction gas such as ammonia, helium, or hydrogen.[45] The mass analyzer can also be a double-focusing magnetic sector instrument that is a high-resolution mass spectrometer or a TOF instrument with the unique capability to sample all the ions generated in the plasma at once.[44] Although there are differences between mass analyzer technologies, they all perform very well in trace analysis, and their function is to sort the singly charged analyte ions by their m/z ratio.

The ions exiting the mass analyzer are converted into a measurable electronic signal with a detector, usually an electron multiplier detector. As an ion strikes the active surface of the detector at the first dynode, electrons are released. The released electrons then strike the next dynode, and more electrons are released. The amplification process is continued at each dynode until a measurable pulse is created. Next, a pulse of electrons is captured by the anode or multiplier collector.[45] By comparing the intensities of the measured pulse to those from calibration curve standards, the software determines the concentration of the element.[43,46]

3.1 Spectral interferences

The interferences encountered with ICP-MS include spectral and non-spectral interferences, also known as matrix effects.[47] Spectral interferences are caused when interfering species carry an m/z ratio that is the same as that of the analyte ion and cannot be separated due to the limited resolution of the mass spectrometer. For example, ^{204}Hg suffers an isobaric overlap from ^{204}Pb, which means that they have isotopes with a common mass, and ^{204}Pb can interfere with the determination of ^{204}Hg. Two or more isotopes from different elements may also combine and have the same nominal mass as the analyte, causing a polyatomic interference. Polyatomic interferences of tungsten oxides or hydroxides ($^{183}W^{16}OH^+$, $^{184}W^{16}O^+$, $^{184}W^{17}O^+$, and $^{186}W^{16}O^+$) may interfere with Hg determination if present at high concentrations.[48] Additional spectral overlaps in ICP-MS can occur from doubly charged ions formed in addition to singly charged ions. Thus, an analyte at a normal single charge and particular mass is indistinguishable from a double-charged ion that produces an isotopic peak at half its mass.[47] For example, rare earth and alkaline elements form doubly charged ions to a larger degree than other elements. The interfering species arise from ions generated from the plasma, the sample, or a combination of these.[43,47,49]

3.2 Non-spectral interferences

Non-spectral interferences refer to the effect of the matrix on signal intensity. In ICP-MS, these are mainly due to the effect of the sample on the ionization conditions of the plasma discharge and the sample transport effect.[47] Changes in the physical properties of the sample (surface tension, viscosity, density, and volatility) and the composition of the matrix can cause significant signal suppression or enhancement.[47,49,50] For example, higher concentrations of acid can result in severe suppression of the analyte signal.[47] In the sample transport effect, acid concentration or dissolved solids in the sample impact the formation of droplets in the nebulizer or droplet size selection in the spray chamber, causing a physical suppression or enhancement of the analyte signal.[51] Total dissolved solids (TDS) can also be deposited on the nebulizer or cones. Therefore, it is recommended to keep them below 0.2% (2 g L^{-1}) to reduce the occurrence of these events.[47]

3.3 Correction of non-spectral interferences

Internal standardization, matrix-matching, and calibration by standard addition can be used to compensate for physical interferences caused by a matrix.[47,52] Other correction techniques include the addition of surfactants or an ionization buffer.[50]

Internal standardization adds an internal standard in equal concentration to all samples and calibration solutions before analysis. The signal variation of the internal standard is used to correct the response for the analyte. Ideally, the internal standard should be as similar as possible to the analyte in terms of atomic mass, first ionization potential, and chemical behavior.[52] The internal standard should not be present in the sample in measurable quantities. Hg has an extremely high ionization energy, so it is quite difficult to find elements with similar ionization potential. However, iridium has an ionization potential of 9.1 eV, which is quite close to that of Hg (10.4 eV). Additionally, ^{193}Ir has a mass similar to Hg (Ir has an atomic mass of 192.22 g mol^{-1}, and the atomic mass of Hg is 200.59 g mol^{-1}) and has been used successfully as an internal standard for Hg.[53] The internal standard can also correct for instrument drift resulting from matrix components that gradually glogg the cone orifices.

Non-spectral interferences can also be corrected using a technique called matrix matching. The purpose of this technique is to adjust the matrix of the sample and calibration standards as close to each other as possible to reduce interferences.[50] Samples can be diluted to better mimic the calibration standards. Alternatively, the acid matrix, digestion reagents, and the main components in the matrix can be added to the calibration standards.

In the standard addition method, also known as calibration with analyte addition, the intensity is measured before and after the addition of known quantities of the analyte into the sample. The calibration is executed separately for each sample, making the method time-consuming but at the same time allowing optimal correction for non-spectral interferences.[50]

4 DETERMINATION OF ULTRA-TRACE CONCENTRATIONS OF MERCURY IN WATER SAMPLES

Various analytical techniques have been applied to monitor low concentrations of Hg in different matrices. The most common methods for Hg determination in aqueous media include atomic absorption spectrometry (AAS), atomic fluorescence spectrometry (AFS), and inductively coupled plasma mass spectrometry (ICP-MS).[6,54–57] These techniques are often combined with chemical vapor generation (CVG) to enhance the sensitivity of these methods or chromatographic techniques to separate Hg species. The accurate determination of Hg at ultra-low concentrations is still considered challenging mainly due to the low sensitivity of instruments, instability of Hg in solutions, and contamination during sample collection, stabilization, transportation, and storage.[58,59]

Most of the United States Environmental Protection Agency (US EPA) methods have adapted cold vapor atomic absorption spectroscopy (CVAAS) or cold vapor atomic fluorescence spectroscopy (CVAFS) for total Hg determination. For example, the EPA Method 245.1, "*Determination of Mercury in Water by Cold Vapor Atomic Absorption Spectrometry,*" has a working range of 0.2–10 µg L^{-1} of Hg, while the EPA Method 1631, "*Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry,*" is suitable for the determination of Hg between 0.5 and 100 ng L^{-1}.[60,61] However, the method detection limit (MDL) as low as 0.05 ng L^{-1} can be achieved with a larger sample volume used for preconcentration and with particular caution in the sample and reagent handling.[61]

ICP-MS is used in EPA Methods 200.8, "*Determination of Trace Elements in Waters and Wastes by Inductively Coupled Plasma-Mass Spectrometry,*" and SW-846 Method 6020B, "*Inductively Coupled Plasma-Mass Spectrometry, part of Test Methods for Evaluating Solid Waste, Physical/Chemical Methods,*" for Hg determination, both having an estimated detection limit of 0.2 µg L^{-1} of Hg.[62,63] The estimated detection limit is considerably higher than for EPA Method 1631. The sensitivity and selectivity of ICP-MS can also be enhanced with CV generation.

4.1 Sampling, preservation, and storage

Clean sampling and applicable sample pre-treatment are very critical phases when determining Hg in natural waters, without forgetting the importance of highly selective detection methods.[6,64] Before sampling, precautions should be taken to avoid Hg contamination, prevent loss of analyte, and in the case of speciation analyses, eliminate interconversion between Hg species.[6] All materials used should be determined to be free of Hg, including the containers, reagents, and laboratory wear to be used to eliminate possible sources of contamination. The sample must also not be exposed to any condition that may result in contamination from airborne Hg.[60,65]

The choice of the sample container material is important to minimize the adsorption losses of Hg. Sample containers made of polyethylene terephthalate (PET), polytetrafluoroethylene (PTFE), and glass have been reported to be suitable materials for Hg collection and storage in natural waters.[6] Reuse of PTFE and glass containers requires rigorous cleaning procedures, while PET containers are typically single-use due to their low cost. Borosilicate or quartz glass bottles reduce the loss of iHg and are also suitable for storing MeHg solutions.[66] New polypropylene (PP) tubes have been reported to be suitable for Hg samples and standards if single used.[59]

It is generally accepted that keeping Hg in solution requires low pH and high ionic strength to limit the adsorption on vessel walls, while complexing and oxidizing conditions help to keep Hg in a divalent state.[67] Typically, acids (e.g., HCl or HNO_3) or oxidants (e.g., BrCl) are used for the preservation of total Hg. For MeHg, the acidification is done with HCl, or the sample can be stored deep-frozen.[6,64] Many studies[61,68–70] demonstrate that borosilicate glass or Teflon containers with HCl preservation are suitable for total Hg analysis.[69] Organic complexing agents, such as cysteine, have also been reported to be good preservatives of Hg in natural water samples.[71] However, chemicals used for preservation are typically the major sources of Hg contamination, so it is ideal to keep the used reagents to a minimum.[59]

4.2 Hg determination by ICP-MS

Hg is a challenging element to be analyzed by ICP-MS, especially at low concentrations. The problems arise from the volatility of Hg, and it can be difficult to keep in solution. In liquid nebulization, the sample introduction efficiency is low; typically, 1–2% of the sample enters the plasma.[47] Also, Hg has a high first ionization potential (10.4 eV), leading to poor ionization and low sensitivity. The seven stable isotopes of Hg also have relatively low abundance, all less than 30%, further reducing sensitivity. Usually, ^{202}Hg is the preferred isotope for ICP-MS as it is the most abundant one and is also recommended in EPA Method 200.8. EPA Method 6020B recommends ^{200}Hg, and in some cases,

^{201}Hg is selected as it has a relatively lower tungsten-based interference.[72] However, the individual Hg isotopes can be summed together to improve signal precision with a higher count rate.[53,73]

4.2.1 Matrix compatibility

Traditionally, HNO_3 matrices are preferred in ICP analyses, and HNO_3 is often used in different sample pretreatment procedures. Most elements are relatively stable or soluble in this matrix. When using HNO_3, interferences are reduced compared to chlorine, sulfur, fluorine, or phosphorous-containing acids.[49] However, low-level Hg in aquatic samples is lost within a few days using HNO_3 preservation.[49,59,74,75] To avoid Hg loss in HNO_3 matrices, adding a trace amount (0.2–1 parts per million, ppm) of gold salt to the HNO_3 solution is recommended to stabilize Hg at ppb concentrations.[49,74,76] The gold ion acts as a strong oxidizer and preserves all forms of Hg.[74] Unfortunately, the addition of gold as a stabilizing agent is not a possible option if gold is also analyzed at a trace level with the same ICP-MS instrument. Also, gold may build up in the sample introduction system, on cones and in the interface, increasing maintenance frequency.[53]

The use of the hydrochloric acid medium is not as appealing with ICP-MS as the nitric acid medium. If HCl is used in the sample preparation, it can form, e.g., polyatomic interferences and thus interfere with the determination of some analytes. HCl is also volatile and can corrode the instrument and its electronics.[49] Nevertheless, the use of HCl is inevitable in the Hg analysis by ICP-MS if the use of HNO_3 and gold as a stabilizer is not an option.

4.2.2 Memory effect

One of the most frequent problems in Hg analysis by ICP-MS is the so-called memory effect or carryover effect. Even at fairly low concentrations, Hg can stick to the walls of the sample introduction system and be released during subsequent analyses, extending washout times.[74] Memory effect is observed as non-linear calibration graphs, decreased sensitivity with time, poor accuracy, and low reliability.[77–79] As discussed above, for example, gold chloride ($AuCl_3$) can be added to the HNO_3 solution to preserve Hg. This also prevents the memory effect as Hg is kept in the solution. Other reagents, such as ammonium pyrrolidinedithiocarbamate (APDC), 2-mercaptoethanol, potassium ferricyanide, thiourea, L-cysteine, and ethylenediaminetetraacetic acid (EDTA), have also been used for the removal of the memory effect of Hg in inductively coupled plasma optical emission spectrometry (ICP-OES) and ICP-MS techniques.[53,77–83]

4.2.3 Cold vapor generation

The detection limits obtained when using conventional liquid nebulization have typically been insufficient for the determination of Hg in natural waters.[84] To enhance the sensitivity and selectivity of ICP-MS for Hg determination, a cold vapor generation system is commonly used as it can provide essentially 100% analyte transport efficiency.[85] It can provide more than 10-fold improvement in the sensitivity of ICP-MS for the detection of Hg.[86] However, an oxidation step is required before analysis when using the CV technique. All compounds of Hg from the sample matrix need to be released into reducible Hg, and this is usually done with a strong oxidant, e.g., potassium bromate or potassium bromide, which can also contain Hg impurities and thus increase blank levels.[87] The excess bromine is destroyed in a pre-reduction step (e.g., with $NH_2OH \cdot HCl$). After pre-reduction, Hg(II) is reduced to Hg^0 vapor (called cold vapor) with a reducing agent such, as sodium borohydride ($NaBH_4$) or tin(II) chloride ($SnCl_2$), and finally transported to the detector.[6,84,87] However, the use of modern ICP-MS provides extremely sensitive element-specific detection capabilities and can also eliminate the need for vapor generation.[88]

5 MERCURY SPECIATION

In speciation analysis, the quantities of one or more individual chemical species are identified or determined in a sample.[89] Element speciation has become one of the most essential topics in trace metal analysis as the bioavailability and toxicity of an element are greatly dependent on its species rather than its total concentration. Without the species information, the evaluation of metal toxicity, metabolism, or bioavailability is incomplete and may even be misleading.[90] As discussed previously, organic Hg species tend to be more toxic than the corresponding inorganic Hg species. The speciation of Hg is important to evaluate Hg pollution and the biological risks, especially due to the toxicity of MeHg.[85]

It is essential to maintain the original species composition throughout the speciation analysis at each stage of sampling, treatment, and detection. Factors affecting the stability of Hg in solutions include, e.g., the concentration of Hg and its compounds, the chemistry of the sample, the redox conditions, the material of the sample bottle used, the cleaning and pretreatment of the sampling equipment, and the reagents used for preservation.[67,91] Hg species may be lost or transformed during storage and pretreatment. In this case, the concentration determined does not represent that of the original sample.[92] Therefore, each step should be carried out cautiously to prevent these events and maintain species integrity. Certified reference materials can be utilized for accuracy verification and quality assurance purposes, provided that they are available in comparable matrix and concentration of the species of interest.[64,92]

Current EPA methods for Hg speciation in the water matrix require separate analyses for MeHg and total Hg. The concentration of iHg can then be calculated by the difference in total Hg and MeHg concentrations.[93] EPA method 1631, "*Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry,*" is used for total Hg determination, while the EPA Method 1630, *"Methyl Mercury in Water by Distillation, Aqueous Ethylation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry,"* is for MeHg determination in the range of 0.02–5 ng L^{-1}.[65] The method has been described as difficult, tedious, and time-consuming, which limits its use.[27,94,95]

The speciation analysis of Hg is most often conducted by coupling a separation technique with an element-specific detection, such as ICP-MS, AFS, or AAS. The principal chromatographic methods for Hg speciation include gas chromatography (GC), high-performance liquid chromatography (HPLC), ion chromatography (IC), and capillary electrophoresis (CE).[6,54,58,96] Before separation, Hg species are typically isolated, derivatized, or complexed to form non-polar or volatile species and then separated by liquid or gas chromatography.[6] In these methods, the different Hg species can be identified based on retention time and comparison with standards due to the different elution behaviors.[97] These methods are most commonly used for Hg speciation and have been reported and reviewed thoroughly elsewhere,[6,54,64,96,98–103] so they are not discussed further in this thesis. Besides chromatographic methods, speciation can be conducted using non-chromatographic methods based on different chemical and physical properties of different Hg species.[6,104] This approach was used in paper **III** and will be discussed in more detail in the next chapter.

5.1 Non-chromatographic speciation methods of Hg

Chromatographic methods are generally more popular than non-chromatographic methods for speciation analysis. Nevertheless, simple non-chromatographic approaches can be used to determine, for example, the most toxic species as a screening parameter or to distinguish between organic and inorganic compounds, especially in the case of iHg and MeHg.[90,102,105]

Non-chromatographic approaches are more accessible than chromatographic techniques because they use simpler equipment. They are less costly than chromatographic methods and can even offer better sensitivity and shorter time of analysis.[105] Nowadays, non-chromatographic methods present competitive limits of detection and are a commonly acknowledged method of speciation analysis for several elements.[105,106] Non-chromatographic methods for speciation analysis of Hg have recently been reviewed by Yang *et al.*[104]

Of the many non-chromatographic methods available for speciation analysis of Hg, selective oxidation via chemical vapor generation is the most used method for Hg speciation analysis. In this approach, iHg species are first reduced to generate Hg^0 (CV generation, see also chapter 4.2.3) and then detected with an appropriate detection technique (e.g., AAS, AFS, or ICP-MS). Organic Hg species (including MeHg) must be decomposed before CV generation to obtain total Hg concentration. MeHg is not directly determined as its concentration is obtained by the difference between total Hg and iHg.[104]

Other non-chromatographic methods utilize e.g., applications of extraction (including noble metal amalgamation,[107–111] co-precipitation,[30,112–114] solid-phase extraction (SPE),[115–120] solid-phase microextraction (SPME),[121–123] magnetic solid-phase extraction (MSPE),[58,124–128] liquid-liquid extraction (LLE),[129–131] liquid-phase microextraction (LPME),[132–138] and cloud point extraction (CPE)[139–141]), applications of selective atomization, and photochemical vapor generation.[104] Of the techniques utilizing the application of extraction, solid and liquid phase extraction are most often employed in non-chromatographic speciation procedures.[142] SPE scheme was also used in this thesis and is examined in more detail in the next section.

5.2 Solid-phase extraction

SPE is a sample preparation procedure used to concentrate or purify analytes for analysis. Target analytes from the aqueous phase are transferred to the active sites of the solid material. After adsorption, elution is performed with a suitable solvent to desorb the retained analytes from solid material.[54,143] The technique is often used due to its simple operation, solid phase stability and reusability, and high availability of adsorbents. It provides clean extracts with a high preconcentration factor. Additionally, it can be connected to different detection techniques in either off-line or online modes with negligible changes in Hg species.[125,126,144–150] Frequently described limitations are that column pressure in SPE is relatively high, and adsorbent recovery is difficult in dispersed SPE operation.[125,126]

As discussed before, direct determination of Hg and its compounds at extremely low concentrations of ng L^{-1} or less is challenging, especially if speciation analysis is to be performed. The interference of the complicated sample matrix can further complicate the analysis. SPE can also be utilized as a preconcentration step and to increase sensitivity.[151,152] The preconcentration step increases the Hg available for analysis and can separate the analyte from most of the matrix. Consequently, possible interferences are minimized and lower detection limits are achieved.[27,152]

One of the most critical issues in SPE is the choice of the adsorbent. This is crucial because it determines the extraction efficiency and selectivity of the method.[125] Materials with a large surface area and selective functional groups are highly desirable. The higher the adsorption capacity, the less adsorbent is needed to concentrate the analytes from each sample.[153–155] Solid supports, e.g., silica gel, can be pretreated with functional groups with high affinity for Hg.[54] Especially sulfur-containing ligands, such as thiol, dithizone, and dithiocarbamate, are effective due to the ability of Hg to coordinate with sulfur ligands.[94,154,156–160] To improve selectivity, new adsorbents, such as modified silica,[149,161] nanometer-sized materials,[127,145,148,162,163] magnetic nanoparticles,[58,125,127] ion exchange resins,[119,155,159,164–166] and molecularly or ionic imprinted polymers,[127,144] have been developed.

The use of sulfhydryl cotton fiber adsorbent for MeHg is a good example of a non-chromatographic approach. It has been proposed since the late 1980s,[167,168] and its use has also been adapted in EPA Method 3200 "*SW-846 Test Method 3200: Mercury Species Fractionation and Quantification by Microwave Assisted Extraction, Selective Solvent Extraction and/or Solid Phase Extraction*".[169] In this method, SPE is used to separate extractable Hg fractions into iHg and organic Hg fractions from soil or sediment samples by using sulfhydryl cotton fiber as an SPE medium.[169] Organic Hg species can then be eluted with 1 M HCl and 1 M NaCl, followed by water. The extractable iHg fraction is eluted from the sulfhydryl cotton fiber medium with 6 M HCl, saturated NaCl, and 0.1% $CuCl_2 \cdot H_2O$. Individual species can also be separated and determined with HPLC or other suitable separation devices connected to a suitable detector.[169]

In addition to the EPA's procedure, where iHg is separated from the organic fraction with selective elution or chromatographic separation after preconcentration, another approach is to use selective sorption and elution of iHg alone, while MeHg is not absorbed and is determined directly.[170] If MeHg is not determined directly, its concentration is commonly calculated as the difference between the total Hg and the iHg concentration.[171] This subtraction approach is a typical indirect speciation approach found in the literature.

6 AIMS OF THE STUDY

The focus of this thesis is to develop simple, sensitive, and selective analytical techniques for the determination of iHg, MeHg, and total Hg at ultra-trace concentrations in natural waters. If simple methods for Hg determination and speciation are developed, they would greatly ease the analytics. Thus, this study aims to:

- Develop a preconcentration method for Hg by using 3D printed scavenger filters.
- Develop a rapid and reliable ICP-MS method for the determination of ultra-trace concentrations of total Hg in natural waters.
- Develop a sensitive method for the determination of MeHg and iHg at an ultra-trace level in natural water samples.

7 EXPERIMENTAL

7.1 Sampling and sample pretreatment

In this study, samples of natural waters were collected from Central Finland, South Savo, and Pirkanmaa in thoroughly cleaned glass bottles, preferably amber-colored bottles (papers **II–III**). The sample bottle was triple rinsed with sample water, filled as full as possible, closed tightly, and sealed in clean plastic bags. Whenever possible, samples were pretreated after sample collection; otherwise, they were stored overnight at 4 °C (papers **I–II**).

7.1.1 Determination of total Hg

To determine of total Hg (paper **II**), unfiltered samples were adjusted to contain 0.12% thiourea and 3% HCl and prepared to a volume of 20 mL (by weight), followed by Hg determination with ICP-MS. Samples were prepared directly into new 50 mL PP centrifuge tubes, and a test tube shaker was used for mixing. For the preparation solutions of iHg (5 or 20 ng L^{-1}) or MeHg (10 ng L^{-1}) used in the recovery tests, adequate volumes of stock solution were added, and the matrix of all sample solutions was adjusted to 0.12% (w/v) thiourea and 3% HCl. HCl was added first, followed by iHg, and then thiourea. The samples were prepared to the volume using the sample water. If samples were spiked with MeHg, this was done in a fume hood due to its high toxicity.

7.1.2 Preconcentration of Hg

In paper **I**, samples were filtered if solids were present. Samples were then acidified to obtain a 3% HCl concentration. For the recovery tests, the matrix of samples was adjusted to 3% HCl in a volume of 45 mL. From this, 5 mL was set aside for the determination of total Hg concentration by ICP-MS. For the analysis of natural water samples, 5 mL of the solution was saved for total Hg determination, and the remaining 495 mL was used for the preconcentration procedure.

7.1.3 Speciation of Hg

To enable both adsorption and elution of iHg and MeHg, as well as the total Hg determination, the matrix of sample solutions was adjusted to 0.01% thiourea before the adsorption step (paper **III**). For the recovery tests, samples were prepared in duplicate to a volume of 45 mL. Natural water samples were made in a volume of 500 mL in triplicate. 5 mL of these solutions were saved for ICP-MS for the determination of total Hg. The matrix of these sample solutions was adjusted to 0.18% thiourea and 3% HCl, followed by ICP-MS determination.

7.2 Preparation of 3D printed scavenger filters

Selective Laser Sintering (SLS) 3D printing was used to prepare the 3D printed metal scavenging filters utilized in the preconcentration and speciation procedures in papers **I** and **III**. FreeCAD software was used to design the objects, a slicer program was used to generate the G-code from the object, and a Sharebot SnowWhite SLS 3D printer was used in the 3D printing process.[172] The building materials used in this study were made by mixing polyamide-12 (PA12) or polyamide-11 (PA11) powder with thiol-functionalized silica, i.e., Thiol. The polyamide was used as a chemically passive carrying polymer, and Thiol was used as a chemically active additive. The printer parameters, such as the laser power, laser speed, and printing temperature, were adjusted to obtain the desired physical characteristics. The unsintered powder was cleaned off from the filter, and the filter was placed in a 10 mL syringe (**Fig. 4**). Finally, filters were washed with high-purity water.

FIGURE 4 Image of the 3D printed metal scavenger filters in a 10 mL syringe.

7.3 Preconcentration procedure (paper I)

After the sample pretreatment, the sample solution was delivered by a peristaltic pump through the syringe containing the 3D printed metal scavenger, i.e., PA12-Thiol, in this case. The flow rate of the peristaltic pump was ca. 5 mL min^{-1}. After iHg was adsorbed into PA12-Thiol, the peristaltic pump was detached from the syringe. An eluent containing 2 mL of 0.3% (w/v) thiourea in 8% (v/v) HCl solution was added to the syringe. The first drop of the elution solution was gently pushed through the PA12-Thiol filter with the syringe plunger. After this, the remaining eluent was let to flow freely through the PA12-Thiol filter. The flow of the elution was counter-current to adsorption. Then, the PA12-Thiol was rinsed with 3.3 mL of ultrapure water, and at the same time, a more moderate concentration of 3% HCl was obtained. These solutions were then analyzed by ICP-MS using thiourea and HCl matrix-matched calibration.

7.4 Speciation procedure (paper III)

In the optimized speciation procedure, two PA11-Thiol filters were placed in the syringe. About 10 mL of high-purity water was used to condition the filters. Similarly, as in the preconcentration procedure, the adsorption step was performed with a peristaltic pump at a flow rate of 5 mL min^{-1} for the pretreated sample solution. At first, MeHg was eluted at a flow rate of 1 mL min^{-1} from the filters with two 5 mL portions of eluent containing 0.1% thiourea in 3.5% HCl. Then, 1 mL of high-purity water was pushed through the filters. After each elution phase, the maximum speed of the peristaltic pump was used for a few seconds to recover all of the sample solution from the filters.

After MeHg had been eluted from the PA11 filters, 2 mL of 0.3% thiourea in 8% HCl was used to elute iHg species. This was performed without a peristaltic pump in the counter-current direction; then, the filters were washed with 2 mL of high-purity water. The matrix of all sample solutions containing either MeHg or iHg species was adjusted to 0.18% thiourea and 3% HCl. The final volumes for MeHg and iHg-containing solutions were 11.7 mL and 5.3 mL, respectively.

7.5 Instrumentation

All Hg analyses were performed using PerkinElmer NexION 350D ICP-MS. The parameters used for all measurements are presented in **Table 1**. An Elemental Scientific (ESI) prepFAST autosampler with a dual-flowing rinse station was used for sample introduction. The first rinse station contained 0.75% HNO_3 and 2.25% HCl, and the second one contained 3% HCl. High-purity 3% HCl was used in the carrier/diluent solution. The daily instrument check was performed for sensitivity, doubly charged ions (<3%), and oxides (<2.5%) with a commercial NexION Setup Solution according to the tuning protocol of the instrument's software.

TABLE 1 Operating parameters and conditions used for NexION 350D ICP-MS (papers **I–III**).

Mode of operation	Standard
RF power	1600 W
Plasma gas flow rate	18 L min^{-1}
Auxiliary gas flow rate	1.2 L min^{-1}
Nebulizer gas flow rate	0.89–0.96 L min^{-1}
Nebulizer	PFA-ST
Spray chamber	Baffled, glass cyclonic (2 °C)
Detector mode	Dual
Scanning mode	Peak hopping
Isotopes	Hg as the sum of ^{200}Hg, ^{201}Hg, and ^{202}Hg, ^{184}W
Internal standard	^{193}Ir (80 µg L^{-1} in 3% HCl)
Dwell time	100 ms for Hg, 10 ms for ^{184}W and ^{193}Ir
Number of sweeps/reading	50
Number of readings/replicate	1
Number of replicates	5 (papers **I** and **II**) or 3 (paper **III**)
Curve type	Linear through zero

8 RESULTS AND DISCUSSION

This chapter briefly introduces the work reported in papers **I–III**. It starts with a summary of the analytical performance of the developed methods. Then, following paper **II**, which focused on developing an analytical method for the analysis of total Hg. The next chapter discusses the development of the preconcentration method for the determination of iHg (paper **I**). It concludes with examining the development of the speciation procedure for Hg (paper **III**).

8.1 Analytical performance

The obtained instrument detection limit (IDL), method detection limit (MDL), limit of detection (LOD), and limit of quantification (LOQ) from papers **I–III** are shown in **Table 2**.

TABLE 2 Instrument and method detection limits for the determination of total Hg, MeHg, and iHg (papers **I–III**).

	Total Hg determination (paper **II**)	Preconcentration method (paper **I**)	Speciation procedure (paper **III**)
Preconcentration factor	–	93	42 for MeHg, 93 for iHg
IDL (ng L^{-1})	0.4 (of Hg)	0.013 (of Hg)*	-
MDL (ng L^{-1})	2.0 (of Hg)	0.037 (of Hg)*	0.05 (for MeHg), 0.08 (for iHg)
LOD (ng L^{-1})	–	–	0.4 (for total Hg)
LOQ (ng L^{-1})	–	–	2.0 (for total Hg)

*Considering the preconcentration factors

8.2 Method development for total Hg determination (paper II)

Sensitivity for Hg is poor compared to other elements due to its isotopic abundances being distributed among its seven natural stable isotopes. Thus, the cps for ^{200}Hg, ^{201}Hg, and ^{202}Hg were summed to achieve improved precision with a higher count rate. With this approach, the count rate of the summed isotopes (^{200}Hg+^{201}Hg+^{202}Hg) doubled the count rate compared to the most abundant ^{202}Hg isotope. Furthermore, this approach even slightly improved the IDL, MDL, and relative standard deviation (RSD) values.

Polyatomic interferences from WO^+ and WOH^+ could interfere with Hg analysis. Thus, the oxide ratio (CeO/Ce) was always kept below 2.5%. The most abundant isotope of tungsten, ^{184}W, was monitored during all measurements. For the studied samples, no interferences from W were observed. Ir was used as an internal standard as its mass is close to the mass of Hg, and its ionization potential (9.1 eV) is close to that of Hg's (10.4 eV).

In the first recovery experiments of humic-rich natural waters, when only 3% HCl was used in the sample matrix, spike recoveries of 65–85% of Hg were obtained. In addition, analysis of synthetic MeHg samples in 3% HCl showed low spike recoveries of 60–70% combined with memory effect at ng L^{-1} concentrations. Thiourea forms complexes with Hg, and it has been used to reduce the memory effect of Hg.[78] Thus, it was examined whether the addition of 0–0.2% thiourea in 0.04% increments in 3% HCl at 50 ng L^{-1} of iHg or MeHg could improve the recovery and remove the memory effect (**Fig. 5**). Indeed, the thiourea addition to all samples and standards removed the challenges associated with low spike recoveries due to the formation of stable thiourea complexes.[173] For further analysis, a concentration of 0.12% thiourea in 3% HCl was high enough to avoid challenges of poor spike recovery and memory effect of Hg but low enough not to block the orifice of the sample cone.

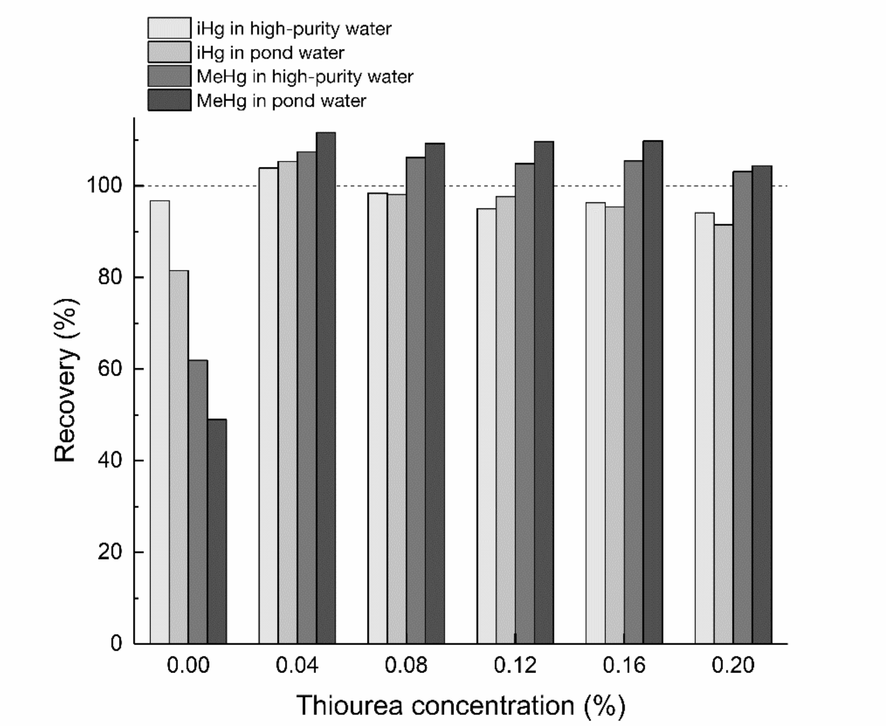

FIGURE 5 Effect of thiourea concentration (0–0.2%) on the recovery of iHg and MeHg spiked at 50 ng L^{-1} in high-purity water or pond water in 3% HCl (n=1, paper **II**). Reproduced from Kulomäki *et al.* with kind permission from Elsevier.[174]

The accuracy and precision (RSD) of the developed method were evaluated by analyzing certified reference material (ERM-CA615 natural groundwater, 37 ± 4 ng L^{-1} of Hg) and performing replicate measurements of the quality control (QC) sample and iHg standard with a concentration of 10 ng L^{-1}. The ERM-CA615 certified reference material originates from a well located in the Bree community in North-East Limburg, Belgium. It is acidified to pH ca. 2 with nitric acid and spiked with $Hg(NO_3)_2$, $Cd(NO_3)_2$, and $Pb(NO_3)_2$.[175] A mean value of 38.6 ± 0.5 ng L^{-1} of Hg (n = 10) and a precision of 1.2 % was obtained for replicate analyses of ERM-CA615 on three nonconsecutive days over a three-week period. The precision obtained from five replicate determinations of a QC sample mimicking natural water (20 mg L^{-1} of C, 5 mg L^{-1} of Na, 2 mg L^{-1} of Mg, S, and K, 5 mg L^{-1} of Ca, 1 mg L^{-1} of Fe, and 20 ng L^{-1} of iHg) within one day (n = 18) was 1.5%, and within four weeks 2.7%. The RSD for replicate determinations of the 10 ng L^{-1} iHg standard was 2.5% (n = 6) over five weeks.

8.2.1 Total Hg analysis by ICP-MS

Finally, total Hg concentrations in six natural water samples were determined using the developed method (n = 3). Samples were also spiked with 5 and 20 ng L^{-1} of iHg and then 10 ng L^{-1} of MeHg. The Hg concentrations in these six natural water samples ranged from < MDL to 10.9 ng L^{-1} (**Table 3**). The obtained spike recoveries for iHg were 96–108% and for MeHg 102–110%. For all studied samples, the RSD values were 0.6–3.6%.

TABLE 3 Determined total Hg concentrations (mean ± std. deviation, n = 3) from natural water samples (paper **II**).

Sample	Added iHg or MeHg (ng L^{-1})	Found total Hg (ng L^{-1})	Recovery (%)
Lake water 1	0	< MDL (1.07 ± 0.03)	
	5 (iHg)	6.17 ± 0.13	102.0 ± 2.6*
	20 (iHg)	21.50 ± 0.23	102.1 ± 1.1*
	10 (MeHg)	11.91 ± 0.43	108.4 ± 4.3*
Lake water 2	0	< MDL (0.94 ± 0.04)	
	5 (iHg)	5.97 ± 0.13	100.6 ± 2.6*
	20 (iHg)	20.73 ± 0.25	98.9 ± 1.2*
	10 (MeHg)	11.12 ± 0.23	101.9 ± 2.3*
Pond water 1	0	6.21 ± 0.22	
	5 (iHg)	11.10 ± 0.25	97.9 ± 5.0
	20 (iHg)	25.49 ± 0.37	96.4 ± 1.9
	10 (MeHg)	16.54 ± 0.38	103.3 ± 3.7
Pond water 2	0	7.71 ± 0.16	
	5 (iHg)	13.11 ± 0.14	108.0 ± 2.8
	20 (iHg)	28.09 ± 0.47	101.9 ± 2.3
	10 (MeHg)	18.55 ± 0.40	108.4 ± 4.0
Stream water	0	10.92 ± 0.07	
	5 (iHg)	15.80 ± 0.19	97.5 ± 3.8
	20 (iHg)	31.16 ± 0.33	101.2 ± 1.6
	10 (MeHg)	21.94 ± 0.22	110.2 ± 2.2
Ditchwater	0	7.40 ± 0.22	
	5 (iHg)	12.28 ± 0.20	97.7 ± 4.0
	20 (iHg)	26.74 ± 0.38	96.7 ± 1.9
	10 (MeHg)	17.67 ± 0.21	102.7 ± 2.1

*The Hg concentration found (without spiking) was lower than the MDL.

8.3 Preconcentration of Hg (paper I)

The suitability of several ion exchange materials and metal scavengers was investigated for the preconcentration of Hg. Of the studied materials, thiol-functionalized silica, i.e., Thiol, turned out to be the best possible scavenger for Hg and was thus used as an additive. The additive must also be added to the sample matrix. Common SPE methods to achieve this include the direct addition of an additive into the sample. It is then percolated through a solid phase or immobilized on a solid matrix by a specific synthetic procedure.[176] Commercially available additives are generally sold as powdery materials. Centrifugation or filtration is required to remove the residual particles or recover the additive from the solution. The last-mentioned immobilization method can also be very tedious. However, these challenges were avoided in this study by using SLS 3D printing. SLS 3D printing enabled the anchoring of the chemically active part to the sorbent matrix. The initially used sorbent matrix was PA12, as it is a durable, easily printable, and available polymer.

8.3.1 Adsorption experiments

The initial absorption experiments revealed that up to 97% of Hg was adsorbed to the 3D printed metal scavenging filter, i.e., PA12-Thiol from a 40 mL solution containing 100 ng L^{-1} of Hg in 3% HCl when a peristaltic pump was operated at a flow rate of ca. 8 mL min^{-1}. When using a more moderate flow rate of ca. 5 mL min^{-1}, complete adsorption was achieved. The adsorption capacities were determined for both Thiol and PA12-Thiol to demonstrate Hg adsorption efficiency. The adsorption capacities for Thiol and PA-12 Thiol were found to be 253.5 mg g^{-1} and 222.4 mg g^{-1}, respectively. These were equivalent to the manufacturer's reported loading capacity of 264.8 mg g^{-1}, indicating that 3D printing only slightly reduces the maximum capacity of the resin. In addition, PA12-Thiol was shown to be highly selective for Hg in 3% HCl as Na, Mg, K, Ca, Cr, and Mn were not retained to PA12-Thiol. Percentage retention values for Fe, Co, Ni, Cd, and Pb were <1.0%.

8.3.2 Elution experiments

After adsorption, the elution step was performed to elute the retained Hg from the PA12-Thiol. Acidic thiourea solutions have previously been used for the elution of Hg,[125,151,156,177–180] and thiourea in HCl was found to be the most potential eluent. Combinations of high thiourea and HCl concentrations resulted in the complete elution of Hg. However, it was found that thiourea affected the intensity of the internal standard during the ICP-MS analysis. Thus, thiourea and HCl matrix-matched standards were used to analyze eluent solutions in subsequent analyses. With ICP-MS, it is recommended to maintain the concentration of the acid below 4% and total dissolved solids below 0.2%.[47] If acid concentrations that exceed tolerable limits are used, samples must be diluted before measurement. In the case of dilutions, the higher preconcentration factor is lost. Considering these limitations, 0.3% thiourea in 8% HCl was chosen as the eluent.

Furthermore, the effects of eluent volume (2, 5, and 10 mL) and eluent flow rate (ca. 1, 3, and 5 mL min^{-1}) were studied with the selected eluent. The experiments were performed with a peristaltic pump, resulting in recoveries of 98–110%. Unfortunately, it could be observed that the small volumes of eluent were not entirely recovered from the peristaltic pump tubing, so the elution phase was performed manually as described in experimental section 7.4. A sample volume of 500 mL was selected to enable a high preconcentration factor while at the same time keeping the sampling practical and the duration of the preconcentration procedure reasonable, and 5 mL of this was reserved for total Hg analysis.

8.3.3 Accuracy of the preconcentration method

Recovery experiments were performed with a 20 ng L^{-1} spiking level of Hg for five natural water samples (n = 3). The results obtained with the preconcentration method (**Table 4**) were closer to the added concentration of 20 ng L^{-1} than those obtained with direct determination (containing only 3% HCl in the sample matrix). This table also demonstrates the low recovery problem resolved in paper **II**.

TABLE 4 Hg concentrations (ng L^{-1}) ± std. deviation for 20 ng L^{-1} Hg spike with different matrices determined after preconcentration method and direct determination (paper **I**).

Sample	Preconcentration method	Direct determination
Tap water[b]	23.27 ± 0.90	19.36 ± 0.45
Lake water 1[c]	20.75 ± 1.46	15.23 ± 0.40
Lake water 2	20.81 ± 1.88	13.08 ± 0.09
River water	21.24 ± 3.96	12.02 ± 1.01
Pond water 1[a,b]	18.96 ± 1.43	12.67 ± 0.44

[a]filtered with Whatman No. 41, [b]two PA12-Thiol filters, [c]n = 2

A certified reference material ERM-CA615, was used to evaluate the accuracy and precision of the ICP-MS method. A mean value of 37.0 ng L^{-1} (n = 5) and a precision of 2.5% were obtained from a replicate analysis on two different days. Short and long-term precision was estimated by measuring the QC sample that mimicked natural water. Within one day, the precision was 2.9% (n = 7), and over four weeks it was 4.0% (n = 23).

8.3.4 Analysis of Hg in water samples

The developed preconcentration method was applied for the determination of Hg concentrations from eight different water samples. As can be seen from **Table 5**, only one sample had a Hg concentration higher than the MDL of the direct measurement method, indicating that a Hg preconcentration method is needed for reliable analysis of ultra-trace concentrations of Hg in natural waters.

TABLE 5 Hg concentrations (ng L^{-1}) ± std. deviation for selected water samples with the preconcentration method and direct determination (n = 3, paper **I**).

Sample	Preconcentration method	Direct determination
Groundwater	0.34 ± 0.02	<MDL
Tap water	0.61 ± 0.06	<MDL
Pond water 1[a]	0.61 ± 0.12	<MDL
Pond water 1[a, b]	0.65 ± 0.04	<MDL
Lake water 1	0.94 ± 0.09	<MDL
River water[a,b]	1.39 ± 0.08	<MDL
Lake water 2	1.79 ± 0.13	<MDL
Pond water 2	2.94 ± 0.12	4.44 ± 0.12

[a]filtered with Whatman No. 41, [b]two PA12-Thiol filters

8.4 Speciation of Hg (paper III)

Since previously developed 3D printed metal scavenging filters were shown to be a highly effective adsorbent for Hg, their use was extended for the speciation of Hg in natural waters at ultra-trace concentration levels. First, it was investigated how both iHg and MeHg could be adsorbed into PA12-Thiol. For the studied sample matrix compositions, which contained a total of eight different thiourea and HCl combinations, 0.01% thiourea proved to be the most suitable and was used in subsequent studies.

8.4.1 Supporting matrix of the 3D printed scavenging filters

The supporting matrix of the 3D printed metal scavenging filters was changed from petroleum-derived PA12 to biobased PA11. The new PA11-Thiol filters contained the same amount of Thiol as PA12-Thiol filters. These new filters resulted in quantitative adsorption of both iHg and MeHg. Due to the same performance and with less environmental impact, these were used in further studies.

8.4.2 Adsorption and elution studies

As previously, sample solutions were pumped through the filters with a peristaltic pump. It was found that iHg was more easily adsorbed than MeHg. Due to weaker MeHg adsorption, the flow rate was set at 5 mL min^{-1}. To further improve the adsorption of MeHg, another PA11-Thiol filter was placed in the same syringe. This approach ensured that the MeHg concentrations in the effluent were below the LOD, which was 0.4 ng L^{-1} of Hg.

In the preliminary elution experiments, separate solutions of MeHg or iHg at concentrations of 50 ng L^{-1} were used (n = 2). Eluent volumes of 10 mL were used with a peristaltic pump flow rate of 0.5 mL min^{-1}, followed by delivering 2 mL of high-purity water. The eluent flow was in the same direction as the adsorption flow. Of the eluents studied, only 0.1% thiourea in 3.5% HCl allowed the elution of MeHg to be greater than 90% while retaining more than 95% of iHg in the filters. Thus, it was selected as the eluent for MeHg. As previously, 0.3% thiourea in 8% HCl was used for the elution of iHg.

When eluting MeHg (used eluent volumes 3, 5, and 10 mL) and iHg (used eluent volumes 2, 3, and 5 mL) with their elution solutions in the same direction as adsorption at a flow rate of 1 mL min^{-1} and omitting the washing phase with high-purity water, a good recovery of 93% was obtained for MeHg with an eluent volume of 10 mL. However, unexpectedly low recoveries (54, 86, and 91%) were obtained for iHg with the studied eluent volumes. The flow rate of the eluent was in the same direction as adsorption. Previously, the elution of iHg was performed in the counter-current direction without a peristaltic pump with excellent results, so this approach was used again in subsequent studies.

To enhance the elution results of MeHg, two elution cycles were used instead of one, and a washing step was included. The flow rate of the peristaltic pump was maintained at 1 mL min^{-1}. Of the eluent volumes studied (two portions of 4, 5, or 6 mL), the highest recovery of 94% was obtained with two 5 mL portions of 0.1% thiourea in 3.5% HCl for MeHg.

8.4.3 Accuracy of the speciation method

For the recovery studies, 5 ng L^{-1} of MeHg and 5 ng L^{-1} of iHg were added for a total concentration of 10 ng L^{-1} Hg. Then, the speciation procedure was performed as described in section 7.4, and samples were measured with ICP-MS. As is shown in **Table 6**, an excellent recovery of 97% was obtained for high-purity water. Recoveries of 88–97% were obtained for natural water samples. The matrix of the brown-colored and humic-rich Korkeakoski stream water may interfere with both the adsorption and elution phases, explaining the slightly lower recovery for Korkeakoski stream water. The determined total Hg concentrations from direct ICP-MS analysis are consistent with the results obtained in the recovery studies.

TABLE 6 Recovery studies in natural waters at a total concentration of 10 ng L^{-1} Hg (paper **III**).

Sample	Elution of MeHg (ng L^{-1})	Elution of iHg (ng L^{-1})	Sum of the elution of MeHg and iHg (ng L^{-1})	Initial total Hg concentration (ng L^{-1})	Recovery (%)
Korkeakoski	5.8	7.6	13.4	15.2	87.9
Päijänne	5.6	7.0	12.6	13.3	94.4
Jyväsjärvi	5.6	5.9	11.5	11.8	97.2
High-purity water	5.4	5.0	10.4	10.7	97.4

Ten replicate measurements of the QC sample within one day gave a precision of 2.5%, and during four different days, it was 3.1% (n = 24). The developed speciation procedure was evaluated with ERM-CA615-certified reference material. The speciation procedure was performed using an 11 mL sample of an ERM-CA615, and its matrix was adjusted to 0.01% thiourea (n = 3). After the preconcentration procedure, it was found that the concentration of MeHg was below MDL, while the concentration of iHg was 41.2 ± 0.5 ng L^{-1} with an RSD of 1.2% (n = 3). Good recoveries were obtained by both the direct determination (107.6 ± 3.5%) and the speciation procedure (111.5 ± 1.4%). However, the ERM-CA615 was originally acidified with nitric acid to pH ca. 2 thus having a different matrix than the other samples. The total Hg concentration for ERM-CA615 was 39.8 ± 1.3 ng L^{-1}, and the obtained RSD was 3.2% (n = 3).

8.4.4 Analysis of natural water samples

The developed speciation procedure was applied to two lake water samples. The obtained MeHg concentration for Lake Jyväsjärvi and Lake Päijänne was 0.18 ± 0.01 ng L^{-1} and 0.24 ± 0.02 ng L^{-1}, respectively. The determined iHg concentration in Lake Jyväsjärvi was 0.62 ± 0.02 ng L^{-1}; in Lake Päijänne, it was 0.50 ± 0.02 ng L^{-1}. In all examined samples, the Hg concentration of the effluent solution was below LOQ. The developed speciation method enabled Hg speciation at exceptionally low concentrations, as seen from the results above.

SUMMARY

This study presents method development for the determination of iHg, MeHg, and total Hg at ultra-trace concentrations in natural waters. ICP-MS was demonstrated to be an effective technique for the determination of total Hg concentrations in humic-rich natural waters at ultra-trace concentrations. The determination of total Hg did not require preconcentration procedures or hyphenated techniques, and a high sensitivity with a detection limit of 0.4 ng L^{-1} was obtained. The developed method overcomes challenges associated with low spike recoveries and memory effect by adding thiourea and HCl for each sample and standard. The analysis of the ERM-CA615-certified reference material demonstrated the accuracy of the method for total Hg determination with a recovery of 104 ± 2% and RSD of 1.2%. Obtained spiking recoveries were 96–108% for iHg and 102–110% for MeHg and RSDs lower than 3.6%.

SLS 3D printing was used to prepare 3D printed metal scavenging filters applied for the preconcentration of Hg. The analytical method developed for Hg determination is based on the adsorption of Hg into a 3D printed metal scavenging filter followed by elution with acidic thiourea solution prior to ICP-MS analysis. The developed method offers a straightforward and highly selective method for the speciation and determination of ultra-trace concentrations of Hg from natural waters with an extremely low MDL of 0.037 ng L^{-1} of Hg.

The use of 3D printed metal scavenging filters was extended for the speciation of Hg in natural waters. By adjusting the matrix of the sample to contain 0.01% thiourea, both iHg and MeHg were adsorbed into a 3D printed metal scavenging filter. Then, the species were eluted with their individual elution solutions. MeHg was first eluted in the same direction as the adsorption with 0.1% thiourea in 3.5% HCl, while iHg was eluted counter-currently with 0.3% thiourea in 8% HCl. The concentration of individual species and the total Hg concentration were determined by ICP-MS. Extremely low MDLs of 0.05 ng L^{-1} and 0.08 ng L^{-1} were obtained for MeHg and iHg, respectively. For ERM-CA615, the recovery obtained by the speciation procedure was 111.5 ± 1.4%, and when directly determined, it was 107.6 ± 3.5%. The developed method for Hg speciation enables accurate determination of iHg and MeHg in ng L^{-1} concentrations in natural waters.

It is important to meet the regulations regarding acceptable pollution levels to protect human health, fish, and wildlife, which require accurate analytical methods. Also, these methods are needed to assess Hg contamination and monitor the effectiveness of the measures taken under the Minamata Convention. The results of the developed methods can further contribute to understanding the fate and toxicity of Hg and its species in aqueous systems.

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ORIGINAL PAPERS

I

DETERMINATION OF MERCURY AT PICOGRAM LEVEL IN NATURAL WATERS WITH INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY BY USING 3D PRINTED METAL SCAVENGERS

by

Suvi Kulomäki, Elmeri Lahtinen, Siiri Perämäki & Ari Väisänen, 2019

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Determination of mercury at picogram level in natural waters with inductively coupled plasma mass spectrometry by using 3D printed metal scavengers

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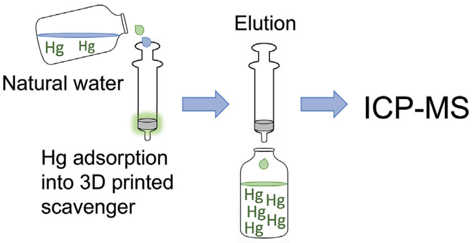
HIGHLIGHTS

- Highly selective and accurate preconcentration method for Hg.
- Hg adsorption into 3D printed metal scavenger and elution with acidic thiourea solution.
- Accurate Hg determination at picogram level in natural waters with a preconcentration factor of 92.8.
- Determination of background concentrations in natural water with ICP-MS.

GRAPHICAL ABSTRACT



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ABSTRACT

The determination of ultra-trace concentrations of Hg in natural water samples via preconcentration using 3D printed metal scavenger technique followed by inductively coupled plasma mass spectrometry (ICP-MS) was developed. The determination of Hg in certified reference material ERM-CA615 (groundwater) was performed with high accuracy and precision resulting in recovery of 100 ± 3% and RSD <2.5%, respectively. Selective laser sintering (SLS) 3D printing was used to fabricate the scavengers using a mixture of polyamide-12 powder with thiol-functionalized silica. The preconcentration procedure is based on the adsorption of Hg on the scavenger and followed by elution of the preconcentrated Hg from the filter with 0.3% thiourea in 8% HCl prior to its determination by ICP-MS. A preconcentration factor of 92.8 can be achieved by filtering 495 mL of water followed with the elution step. Very low instrumental detection limit and method detection limit were obtained resulting in 0.013 and 0.037 ng L^{-1}, respectively. The method was applied successfully for the determination of Hg in different lake and river water samples. The developed method is the first preconcentration method enabling simple and accurate determination of Hg in pg L^{-1} concentrations in natural waters with ICP-MS.

1. Introduction

Mercury is one of the most toxic elements due to its accumulative and persistent nature in the environment and living organisms. It occurs naturally in the environment in a variety of chemical and physical forms at very low concentrations [1]. In pristine natural waters, Hg concentrations are typically between pg and ng L^{-1} range. Recent reports estimate a total mercury concentration in natural waters ranging from 0.03 to 90 ng L^{-1}, while

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contaminated waters may contain up to several μg L^{-1} of Hg [1,2]. Although mercury is not an abundant element in nature, its presence in polluted waters and the uptake of Hg by aquatic media has become an area of great environmental concern and a potential risk to human health [3]. All forms of mercury are poisonous and its toxicity is generally increased by transformation into organic forms via biomethylation [1]. The monitoring of mercury traces in the hydrosphere has been regulated in many countries, e.g., in Europe by the European Water Framework Directive [2,4]. Hg is included in Annex I of the Directive 2013/39/EU as a priority hazardous substance and a maximum allowable concentration of Hg and its compounds of 0.07 μg L^{-1} in inland and other surface waters has been established [5,6]. In Finland, heavy metal concentrations in natural waters are usually at a very low level. For this reason, the method detection limits for Ni, Cd, Pb, and Hg are stricter than those presented in the Water Framework Directive. For Hg, the recommendation for the method detection limit is 5 ng L^{-1} [7].

Accurate quantification of Hg traces in natural waters is challenging due to very low Hg concentrations. Highly sensitive detection techniques are available for mercury e.g. cold vapor atomic fluorescence spectrometry (CV-AFS) and inductively coupled plasma mass spectrometry (ICP-MS) but most modern analytical techniques do not succeed in direct determination of low Hg concentrations [1,2,8]. Thus, a preconcentration step is needed to reach the detection limits required for the environmental monitoring of Hg [9]. The analytical performance of non-chromatographic methods using different types of preconcentration methods has been reviewed previously [1,10,11]. Most of these methods are using a tedious cold vapor generation coupled to atomic spectroscopic techniques with detection limits between 0.05 and 500 ng L^{-1} for Hg^{2+}. Still, many of these approaches have inadequate detection limit for ultra-trace Hg analysis in environmental samples and the development of simple and sensitive methods are highly desirable.

During the preconcentration step, the analyte is usually also separated from most of the matrix elements, in order to eliminate possible interferences and lowering the detection limit [12]. Various approaches have been applied for separation and preconcentration of Hg based mainly on liquid-liquid extraction [13–15], co-precipitation [16,17] and solid-phase extraction (SPE) [3,6,18–20]. SPE is the most commonly used preconcentration method for trace metals due to its simplicity, flexibility, and ability to achieve high enrichment factors. Extraction efficiency and selectivity are greatly dependent on the sorbent material used and therefore the choice of the appropriate adsorbent with high affinity and selectivity for Hg is the most critical step in SPE method development [12]. Numerous materials, such as chelating resins [21], ion exchangers [22–24], modified carbon nanotubes [25,26] and nanoparticles [2,3], modified silica gel [27], and polyurethane foam [28,29] have been suggested as SPE sorbents. There are also some commercially available metal scavengers for Hg but their disadvantage is that they are usually sold as powdery materials and hence centrifugation or filtration is needed to recover the used adsorbent or to purify the sample solutions from remaining particles. These difficulties make the use of the adsorbent laborious and challenging. Moreover, the use of solid samplers instead of small particle material would enable in situ sampling which would be almost impossible with a material consisting of small particles. These challenges can be avoided by anchoring the chemically active component to a sorbent matrix by using 3D printing.

Over the past few years, 3D printing has received increasing attention as a way to fabricate macroscopic objects with actual functionality instead of just mechanical or aesthetical properties [30–35]. 3D printing has found many potential applications in the fields of separation sciences [36] and analytical chemistry [37]. However, many of these reports have been focusing on either extrusion-based methods or stereolithography whereas methods such as Selective Laser Sintering (SLS) have received less attention. This is even though the SLS 3D printing possesses some obvious advantages if targeting, for example, any kind of flow-related process. The challenge with the aforementioned extrusion-based and stereolithography methods is that they produce objects, where the surfaces of the printed objects are completely solid and the reactive surface area inside the object is not accessible.

The SLS 3D printing doesn't suffer from similar challenges as it uses a laser to partially sinter small polymer beads together, leaving accessible voids between the polymer particles. These voids are crucial for analytical chemistry purposes, as they allow the fluid to flow through the pores of the objects and therefore allow for controlled chemical interaction between the fluid and the object. This level of sintering, and therefore the porosity, can be controlled by adjusting the printing parameters such as laser power, laser speed and temperature [38–41]. Even different areas of the same object can be made to possess different porosities. For example, the outer walls of the object can be made impermeable for fluids while leaving the inside of the object highly macroporous [42]. The chemical functionality can be embedded into the printed objects either by simply mixing the additive into the starting material or by post-processing the printed objects. Almost anything can be used as the additive since as long as the additive can withstand the temperature of the printing process, it can be printed to form chemically functionalized macroscopic objects without losing the activity of the additive during the process [42,43]. Turning powdery materials into easily handleable objects with customizable shape, size and porosity often make their use in actual applications much more feasible if compared to using them in their powdery form.

This paper presents a 3D printed Hg scavenger (3D-Thiol) method for the preconcentration and determination of ultra-trace mercury concentrations in natural waters. A series of sorption experiments were conducted using flow-through procedures to study the effect of sorption, elution, and selectivity for mercury and some other metals. In addition, adsorption isotherms for thiol functionalized silica (Thiol) and 3D-Thiol were determined. Finally, an analytical method for Hg determination in ultra-trace concentrations was developed, based on the sorption of Hg on 3D-Thiol and elution using HCl and thiourea solution followed by ICP-MS detection. To our knowledge, this is the very first study enabling Hg detection with ICP-MS for pg L^{-1} concentrations without the utilization of cold vapor technique. In addition, this is the first instance where thiol functionalized material, use-case and functionality have been combined with SLS 3D printing.

2. Experimental section

2.1. Reagents

All reagents were of analytical grade and all aqueous solutions were prepared in high purity water (18.2 MΩ cm) produced by Elga Purelab Ultra water purification system (Buckinghamshire, U.K.). Single-element standard of Hg as Hg^{2+} (10 mg L^{-1}, Pure Plus), Cd, Co, Cr, Fe, Ir, K, Mg, Mn, Na, Ni, and Pb (1000 mg L^{-1}, Pure) were supplied by PerkinElmer. 1000 mg L^{-1} stock solution of Ca was prepared from $CaCO_3$ (Merck, ≥99.0%). The ICP-MS performance was checked daily with a NexION Setup Solution (1 μg L^{-1} Be, Ce, Fe, In, Li, Mg, Pb, and U in 1% HNO_3, Pure Plus, PerkinElmer). Thiourea (99.8%) was obtained from VWR Chemicals and SiliaMetS Thiol was purchased from SiliCycle (Quebec City, Canada). Polyamide-12 (PA12), thermoplastic polyurethane and polypropylene printing powders were purchased from BASF (Ludwigshafen am Rhein, Germany). Certified reference material (ERM-CA615, groundwater) was produced and certified by the Joint Research Centre, Institute for Reference Materials and Measurements (JRC-IRMM). Ultrapure hydrochloric acid (34–37%) was purchased from ANALYTIKA, spol. s r.o. (Prague, Czech Republic). Puriss. p.a. hydrochloric acid ($\geq$37%) and nitric acid ($\geq$65%) were purchased from Merck and were used for the preparation of washing solutions for ICP-MS. All glass containers that could come into contact with samples and standards were filled with 10% (v/v) hydrochloric acid ($\geq$37%) for at least 24 h and tested for Hg followed by ICP-MS analysis. Sample bottles were first rinsed thoroughly with ultrapure water and then completely filled with it. A blank sample was prepared by using this water and was then analyzed with ICP-MS. Sample bottles were used if the measured Hg concentration was below the instrument detection limit (IDL). After testing, glass containers were filled with dilute HCl (~0.5%) and were thoroughly rinsed with high purity water before usage. Calibration standards and smaller volume samples were prepared into 50 mL polypropylene centrifuge tubes (VWR International).

2.2. *Sampling and sample pretreatment*

A total of seven different water samples were collected in 2L glass bottles from Central Finland. Tap water was collected from the research laboratory of the Department of Chemistry (Jyväskylä), groundwater from Heinäahonlähde (Laukaa), lake water 1 from lake Päijänne (Korpilahti), lake water 2 from lake Jyväsjärvi (Jyväskylä), river water from river Tourujoki (Jyväskylä), pond water 1 from Myllylampi (Tikkakoski), and pond water 2 from Mustalampi (Jyväskylä). Samples were filtered (Whatman No. 41) only if they contained solid material. Then they were acidified with ultrapure HCl to obtain 3% (v/v) acid concentration and stored at 4 °C in a refrigerator overnight if not analyzed immediately after the sample collection.

2.3. *Instrumentation*

All mercury analyses were performed on a PerkinElmer (Massachusetts, U.S.A.) NexION 350D ICP-MS in standard mode. The ICP-MS was interfaced with an Elemental Scientific (Nebraska, U.S.A.) prepFAST 4DX sample/standard autodilution system. A NexION Setup Solution was used for the daily optimization procedures for the ICP-MS according to the manufacturer's instructions. Data were acquired in counts per second (cps) for Hg (the sum of the counts at *m/z* 200, 201 and 202), and Ir (*m/z* 193) was used as the internal standard. The most abundant W isotope ^{184}W was monitored during all measurements, and it was concluded that WO did not interfere with Hg in the studied water samples when the 3D-Thiol method was used. Calibration standards were prepared in the range of 0–500 ng L^{-1} (blank, 50, 250 and 500 ng L^{-1}) Hg and the standards and samples were adjusted to contain 3% (v/v) HCl. For analysis of elution solutions containing thiourea and HCl, the standards were matrix-matched with 0.1125% (m/v) thiourea and 3% (v/v) HCl. The system was first rinsed with a mixture of 0.75% (v/v) HNO$_3$ and 2.25% (v/v) HCl and then with 3% (v/v) hydrochloric acid between samples. The optimum operating conditions and measurement parameters for ICP-MS are listed in Table 1.

Elements present in the selectivity study were analyzed on Avio 500 inductively coupled plasma optical emission spectrometer (ICP-OES) (PerkinElmer, Massachusetts, U.S.A.) or with ICP-MS. More detailed information about the analytical procedure is presented in Tables S1 and S2. Black/white 3.18 mm i.d. PVC peristaltic pump tubing (PerkinElmer, Massachusetts, U.S.A.) was used for the preconcentration process and the flow rate of the samples was adjusted using an ISCO (Nebraska, U.S.A.) WIZ peristaltic pump. Helium imaging was performed with Carl Zeiss (Oberkochen, Germany) ORION NanoFab helium ion microscope (HIM). The beam energy was set to around 30 kV while the beam current varied between 0.211 and 0.234 pA. Scan dwell time of 0.2 µs with a viewing distance of 5.101–7.833 mm was utilized for the imaging. Flood gun was used throughout the imaging to counteract the charging event present. Samples did not receive any additional pretreatment before the imaging process.

Table 1
ICP-MS instrumental operating parameters and conditions.

System/Operating parameter	Type/Value/Mode
Sample introduction	ESI prepFAST
Nebulizer	PFA-ST nebulizer for prepFAST
Spray chamber	Baffled cyclonic, glass, cooled to 2 °C
RF power	1600 W
Plasma gas flow rate	18 L min^{-1}
Auxiliary gas flow rate	1.2 L min^{-1}
Nebulizer gas flow rate	0.89–0.93 L min^{-1}
Isotope	The sum of ^{200}Hg, ^{201}Hg, and ^{202}Hg
Internal standard	^{193}Ir at 100 µg L^{-1} with 10 ms dwell time
Detector mode	Dual
Sweeps/reading	50
Replicates	5
Dwell time	100 ms
Scan mode	Peak hopping
Curve type	Linear through zero

2.4. *Designing and preparation of the 3D printed scavengers*

The objects were designed using FreeCad v. 0.16 and then sliced with Slic3r v. 1.2.9 into 100 µm layers to obtain filters with desired shape and size (5 mm tall disc with 16.5 mm diameter). The starting material for the SLS 3D printing was made by mixing polyamide-12 powder (~50 µm diameter) with either 5 or 10 wt% of thiol-functionalized silica (40–63 µm). The material was then mechanically mixed and placed into the 3D printer. The actual printing process was done using Sharebot SnowWhite SLS 3D printer with a laser power of 30–40% (of a maximum of 14 W), a laser speed of 1800–2400 mm s^{-1} and printing temperature of 170 °C. No post-processing, aside for cleaning the objects of any unsintered powder, was done for the 3D printed objects.

2.5. *Adsorption experiments*

The developed 3D-Thiol method was tested with model solutions prior to the separation and preconcentration of Hg from real samples. For this purpose, 3D printed filters were placed into a 10 mL syringe (Fig. 1) and washed from any unsintered powder with ultrapure water. The extraction of Hg from aqueous solutions was investigated in a flow-through process. The sorption was performed in centrifuge tubes containing 100 ng L^{-1} of Hg in 40 mL of 3% (v/v) HCl. Initial tests were carried out using a peristaltic pump with a maximum flow rate (~8.3 mL min^{-1}). The adsorbed concentration of Hg was determined using ICP-MS. Adsorption percentage was calculated using the following equation:

Fig. 1. SLS 3D printed 5 mm (diam. 16.5 mm) 3D-Thiol filter (left) and filter placed in a syringe (right).

$$\text{Adsorption\%} = \frac{C_A - C_B}{C_A} \cdot 100\% \tag{1}$$

where C_A and C_B are initial and final concentration (ng L^{-1}) in the solution, respectively [44]. In the elution step, 2–10 mL of 0.3–2% (w/v) thiourea and 8–40% (v/v) hydrochloric acid solution was passed through 3D-Thiol at a flow rate of ~1–8.3 mL min^{-1}.

2.6. Determination of adsorption capacity

Equilibrium isotherm studies were carried out with different initial concentrations of Hg in flow-through experiments. The adsorption capacity was determined for both Thiol and 3D-Thiol. The adsorption efficiency of Thiol was performed by adding 10 mg of beads into 40 mL of different solutions containing different concentrations of Hg (1, 25, 50, 75, and 100 mg L^{-1}) in 3% (v/v) HCl. The tube was sealed and then agitated using Stuart SF1 at 500 osc min^{-1} for 2 h at room temperature. The thiol-functionalized silica was removed from the Hg solution by filtration (Whatman No. 42) and samples were analyzed by using ICP-MS after appropriate dilution.

The adsorption capacity for 3D-Thiol was performed with the same sample volume and Hg concentrations as above. To obtain approximately 10 mg of Thiol in the printed scavenger, the weight fraction of the additive was dropped to 5 wt% in the printed scavenger and 3D-Thiol filters were placed into a 5 mL syringe. 40 mL solutions were passed through one filter five times with a peristaltic pump at a maximum flow rate (~8.3 mL min^{-1}). After adsorption, the residual concentration of Hg was analyzed by ICP-MS after appropriate dilution. The concentration of Hg adsorbed per unit mass of the adsorbents was calculated from the following equation:

$$q_e = \frac{(C_0 - C_e)V}{m} \tag{2}$$

where q_e is the adsorption capacity (mg g^{-1}) of the adsorbent at equilibrium; C_0 and C_e are the initial and final concentration of Hg (mg L^{-1}), respectively; V is the volume of the solution (L), and m is the mass of the adsorbent (g).

2.7. Selectivity experiments

A variety of 40 mL samples containing spiked lake water or synthetic sample were passed through a 10 mL syringe containing one 3D-Thiol filter with a ~8.3 mL min^{-1} flow rate. Spiking levels were 5 ng L^{-1} of Hg, 10 mg L^{-1} of Ca, 6 mg L^{-1} of Na, 2 mg L^{-1} of Mg, 1 mg L^{-1} of K and Fe, and 50 μg L^{-1} of Cd, Co, Cr, Mn, Ni, and Pb. Ca, Na, Mg, K, Fe, Cr, and Mn were measured with ICP-OES and Co, Ni, Cd, Pb, and Hg were analyzed with ICP-MS. More detailed information about the analytical procedure is presented in Table S3.

2.8. Spiking studies and the treatment of the real sample

Samples were spiked with 20 ng L^{-1} of Hg and prepared in 45 mL volume of which 5 mL was set aside for direct ICP-MS analysis. The real samples were prepared into 500 mL volume and 5 mL of the sample was saved for direct measurement with ICP-MS. The sample solution was pushed through the 3D-Thiol with ~5 mL min^{-1} flow rate using a peristaltic pump resulting in the time needed for preconcentration to be about 1 h 40 min. The elution step was done without a peristaltic pump in backflush mode. The retained Hg was eluted with 2 mL of 0.3% (w/v) thiourea in 8% (v/v) HCl solution. The syringe plunger was used to gently push the first drop of the eluent through 3D-Thiol. Then the rest of the eluent was allowed to drain through the filter. Finally, the 3D-Thiol was washed with 3.335 mL of ultrapure water to obtain 3% (v/v) HCl concentration in the final solution, which was analyzed by ICP-MS. The elution and washing took approximately 5 min.

3. Results and discussion

3.1. Analytical performance of ICP-MS analysis

The method showed good linearity over the calibration range of 0–500 ng L^{-1} (Fig. S1) and the correlation coefficient of linear regression (r) was higher than 0.9999. IDL achieved for Hg determination was 1.2 ng L^{-1} (calculated from the average of 10 replicate measurements of the calibration blank plus three times the standard deviation). The method detection limit (MDL) was determined according to the U.S. Environmental Protection Agency method detection limit procedure [45] and it was calculated to be 3.5 ng L^{-1}. The short and long term precision were estimated by measuring a quality control sample containing 20 ng L^{-1} of Hg, 20 mg L^{-1} of C, 5 mg L^{-1} of Ca and Na, 1 mg L^{-1} of Fe, and 2 mg L^{-1} of K, Mg, and S. The precision (relative standard deviation, RSD) within one day was 2.9% ($n = 7$) and during four weeks 4.0% ($n = 23$). In addition, the precision and accuracy of the ICP-MS method were estimated using a certified groundwater reference material (ERM-CA615, certified value 37 ± 4 ng L^{-1}; expanded uncertainty at 95% confidence level). Replicate analyses on two different days gave a mean value of 37.0 ng L^{-1} ($n = 5$) with a precision of 2.5%.

3.2. Selection of the chemically active component and supporting matrix

Several commercially available metal scavengers and ion exchange materials were examined for the preconcentration of Hg. Thiol-functionalized silica proved to be the most suitable scavenger for Hg so it was chosen for a chemically active component for the 3D printed scavenger. Resins containing the thiol functional groups have been found to be able to adsorb Hg [46,47]. Unfortunately, many of these are no longer commercially available (Spheron-Thiol, Duolite GT73, Ambersep GT74). Polyamide-12, thermoplastic polyurethane, and polypropylene printing powders were tested for 3D printing supporting matrix. PA12 proved to be a suitable material since it is a readily available, durable and easily printable polymer. In addition, Hg was not retained by the PA12 after elution with acidic thiourea solution.

3.3. Macroscopic structure of the 3D printed filter

The HIM imaging was used to get information about the macroscopic structure of the printed object as well as the distribution and the attachment of the additive. HIM imaging, instead of the more traditional SEM imaging, was conducted for the printed filters to combat the charging effect often present when imaging nonconductive samples [48]. It is also evident, that the silica particles have not been capsulated by the polymer and therefore they remain accessible for chemical reactions (Fig. 2 left). The overall structure of the printed filter can be seen to be highly macroporous, consisting of polymeric beads that have been partially sintered together by the laser during the printing process. Additionally, the distribution of the silica particles throughout the printed object seems to be rather even (Fig. 2 right). However, this was expected as the distribution and the attachment for a similar system has been previously reported [42,43] and thus was not studied more in depth here.

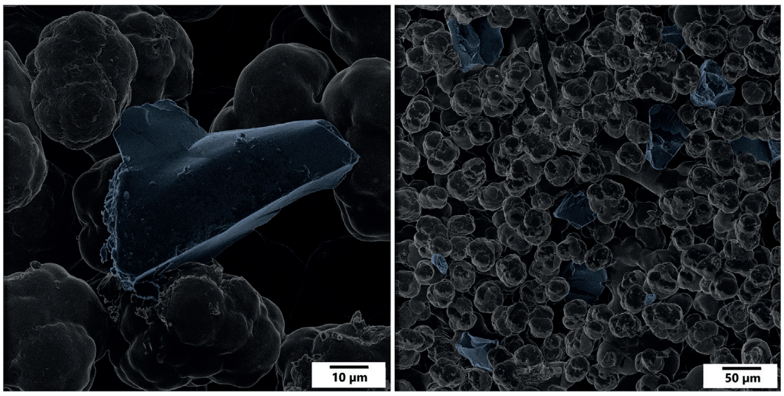

Fig. 2. HIM image showing the attachment of the thiol-functionalized silica particle onto the 3D printed PA12 framework (left). HIM image of the overall macroporous structure of the 3D printed filter containing 10 wt% of functionalized silica on the PA12 framework. Thiol-functionalized silica particles are highlighted for clarity.

3.4. *Adsorption and adsorption isotherm studies*

Initial adsorption experiments were conducted using a peristaltic pump. During the experiment, up to 97% of the Hg was adsorbed, which shows the rather satisfactory retention kinetics. Complete adsorption could be achieved with a more moderate flow rate (~5 mL min^{-1}). In this case, the Hg concentration in the remaining solution is very close or below the instrument detection limit (1.2 ng L^{-1}) indicating complete adsorption. Since the Hg concentration in pristine natural waters can be only a few ng L^{-1}, a high preconcentration factor is desired. The preconcentration factor can be maximized by increasing the sample volume and decreasing the eluent volume. However, due to practical reasons such as ease of sampling and duration of pretreatment, the volume of the real natural water sample should be kept reasonable. Taking these considerations into account, a sample volume of 500 mL was chosen, of which 5 mL was set aside for direct ICP-MS analysis.

The equilibrium conditions of the sorption process can be described using sorption isotherms. The most frequently used adsorption isotherm equations [23,49,50] of the Langmuir (3) and Freundlich (4) isotherms were applied to utilize the linearized equations indicated below:

$$\frac{C_e}{q_e} = \frac{1}{K_L \cdot q_{max}} + \frac{C_e}{q_{max}} \tag{3}$$

$$\ln q_e = \ln K_F + \frac{1}{n} \ln C_e \tag{4}$$

where q_e (mg g^{-1}) is the equilibrium adsorption capacity, C_e (mg L^{-1}) is the Hg concentration at equilibrium, q_{max} (mg g^{-1}) is the monolayer maximum adsorption capacity, K_L (L mg^{-1}) is the Langmuir equilibrium constant, K_F (L g^{-1}) is a coefficient related to the amount of adsorbed metal ions, and n is the Freundlich adsorption constant related to surface heterogeneity. The Langmuir equation assumes the same energy for all adsorption sites and that monolayer is formed when the solid surface reaches saturation [51]. The plot of C_e/q_e against C_e gives a straight line and the values of q_{max} and K_L are obtained from the slope and intercept of the plot [23]. The Freundlich isotherm, in turn, is an empirical equation and assumes that adsorption is multilayer and is based on heterogeneous surface adsorption suggesting that binding sites are not equivalent [23,51].

Linear plots of $\ln q_e$ versus $\ln C_e$ revealed that the adsorption obeys well the Freundlich isotherm (Fig. S2). The results showed that the Hg adsorption onto Thiol can be considered to be a multilayer adsorption system (Table 2). The loading capacity for Thiol is 264.8 mg g^{-1} according to the manufacturer. The experimental value obtained from the Langmuir isotherm model for Thiol was 253.5 mg g^{-1} and for 3D-Thiol 222.4 mg g^{-1}. These results indicate that 3D printing decreases the maximum capacity of the resin only slightly. Hg concentrations in natural waters are so low that the maximum loading capacity is not easily reached. For example, Hg concentration of 46 mg L^{-1} with a sample volume of 500 mL is required to saturate all absorption sites of 3D-Thiol.

3.5. *Selectivity*

In order to study the selectivity of 3D-Thiol towards Hg, competitive adsorption of Ca, Cd, Co, Cr, Fe, K, Mg, Mn, Na, Ni, and Pb from spiked lake water and synthetic sample were investigated. In spite of the high concentrations of other metal ions (0.05–10 mg L^{-1}), Hg was retained to 3D-Thiol completely as the Hg concentration of the sample that passed through the filter was below the limit of detection. Moreover, Ca, Na, Mg, K, Cr, and Mn were not retained at all, Fe, Co, Ni, Cd, and Pb showed very low percentage retention values to be $\leq$ 1.0% (Table S4). 3D-Thiol proved to be extremely selective for Hg in 3% (v/v) HCl. These results provide further evidence of the great selectivity of 3D-Thiol for Hg.

3.6. *The effect of eluent composition and its volume*

According to initial testing with different eluents and literature review [1,52,53], thiourea in hydrochloric acid solution was found

Table 2
Constants of examined isotherms for Hg adsorption.

Isotherm model	Constants	Value for 3D-Thiol (n = 1)	The average value for Thiol (n = 3)
Langmuir	q_{max} (mg g^{-1})	222.4	253.5
	K_L (L mg^{-1})	0.160	0.337
	R^2	0.975	0.940
Freundlich	K_F (L g^{-1})	23.5	54.2
	n	1.53	2.07
	R^2	0.982	0.980

Table 3
Recovery (%) ± std. deviation for Hg from 3D-Thiol using different eluent compositions.

Eluent	Recovery (%)	Required dilution factor for analysis
0.3% thiourea in 8% HCl	90.1 ± 3.8	2.67
1% thiourea in 8% HCl	99.7 ± 4.9	5
2% thiourea in 10% HCl	98.8 ± 2.9	10
2% thiourea in 20% HCl	104.0 ± 3.7	13.33
0.3% thiourea in 40% HCl	105.1 ± 2.7	13.33
2% thiourea in 40% HCl	101.3 ± 4.2	13.33

to be the most promising eluent. HCl has also been reported [54] to have an enhanced performance due to its complexing abilities with mercury and therefore it was of interest to investigate the influence of thiourea and HCl concentration on the elution efficiency for 3D-Thiol. Thiourea was found to affect the intensity level of the internal standard in ICP-MS analysis, hence, the samples with different eluent compositions were analyzed without internal standard correction (Table 3). To enable the use of internal standard, matrix matching was employed in subsequent analyses.

The effect of thiourea and HCl concentration on elution efficiency was studied in duplicates with 10 mL eluent volumes and ~8.3 mL min^{-1} flow rate without internal standard correction (Table 3). Higher thiourea and acid concentration results in complete elution of Hg from the 3D-Thiol, however, it is desirable to keep the acid concentration and total dissolved solids below 4% and 0.2%, respectively for ICP-MS [55]. Samples have to be diluted before measurement if the acid concentration is above the tolerable limits and hence the higher preconcentration factor would be lost. In addition, at least 4 mL sample volume is needed for the measurement. Because of these reasons, 0.3% thiourea in 8% HCl was chosen for further studies.

The effect of eluent volume (2, 5, and 10 mL) and eluent flow rate (~1, 3, and 5 mL min^{-1}) were evaluated using a peristaltic pump with the selected eluent. It was observed, that the recovery was satisfactory (98–110%) with all the tested eluent volumes and flow rates. However, these small eluent volumes were not completely recovered when using a peristaltic pump. To obtain quantitative recoveries, the elution step was performed manually without a peristaltic pump as described in section 2.8. Considering the sample volume of 495 mL, the final elution volume of 2 mL, and a dilution factor of 2.67, the preconcentration factor becomes 92.8. The method detection limit for the 3D-Thiol method becomes 0.037 ng L^{-1} when the preconcentration factor is taken into account.

3.7. Reusability of the sorbent

The reusability of 3D-Thiol as a sorbent was examined by assessing the change in the recoveries of the analyte through several sorption-elution cycles at a maximum flow rate of the peristaltic pump following the procedure described in section 2.5. The results revealed that one 3D-Thiol filter could be reused up to 10 times without a loss of adsorption efficiency (Fig. S3). The recovery and relative standard deviation throughout the reusability test for adsorption was 95.2% and 0.7%, respectively (n = 2). The adsorption was satisfactory even when the highest flow rate was used. As stated previously, the extraction efficiency is easily increased with a more moderate flow rate. Nonetheless, in order to avoid any contamination, new 3D-Thiol filters were employed throughout the study.

3.8. Spiking tests

A total of five spiked real samples were analyzed with the 3D-Thiol method as well as directly without any preconcentration. The results obtained using the 3D-Thiol method were significantly closer to the spiked concentration of 20 ng L^{-1} compared with the direct measurement as can be seen in Table 4. This can be seen more clearly in the case of yellow to brown color waters (lake water 2, river water, and pond water 1) which contain more humic substances than clearer waters (tap water and lake water 1). Some spiking tests were also performed at 10 ng L^{-1} level, for which direct measurement from tap water gave 10.2 ng L^{-1} (RSD 7.2%) and 3D-Thiol method 10.2 ng L^{-1} (RSD 1.7%). It is likely that the challenging matrix of the colored waters suppresses the signal of mercury in direct ICP-MS determination and might result in biased low concentrations (Table 4). With the 3D-Thiol method, Hg can be separated from most of the matrix and interferences are reduced when compared to direct determination. It was observed that RSD values could slightly be improved by using two 3D-filters in the same syringe (Tables 4 and 5). As the amount of the functional group is increased it is more likely that Hg interacts with the thiol group and different kinds of samples behave in the same manner. Although the RSD values were improved with the use of two 3D-Thiol filters, RSD values were considered to be sufficient even when only one filter was used. Obtained results further confirm the usefulness of the proposed method.

Table 4
Average Hg concentration (ng L^{-1}) ± std. deviation for 20 ng L^{-1} spike to different matrices (n = 3).

Sample	3D-Thiol method	Direct determination
Tap water[b]	23.27 ± 0.90	19.36 ± 0.45
Lake water 1[c]	20.75 ± 1.46	15.23 ± 0.40
Lake water 2	20.81 ± 1.88	13.08 ± 0.09
River water	21.24 ± 3.96	12.02 ± 1.01
Pond water 1[a,b]	18.96 ± 1.43	12.67 ± 0.44

[a] Filtered with Whatman No. 41.
[b] Two 3D-Thiol filters.
[c] n = 2.

3.9. Analysis of mercury concentration in real samples

The proposed method was applied for the determination of Hg in different water samples. The samples were analyzed both with the direct determination and with the preconcentration method. With direct determination, Hg concentration of the water samples was below the MDL except for one sample (Table 5). This represents the need for a 3D-Thiol method to enable a reliable analysis of extremely low concentrations of Hg in natural waters. The reproducibility of the method is very good considering the low ng L^{-1} concentrations. In Central Finland, Hg concentrations of natural waters are at a very low level and with the 3D-Thiol method, the recommended 5 ng L^{-1} method detection limit was easily reached.

Table 5
Average Hg concentration (ng L^{-1}) ± std. deviation for different water samples with the 3D-Thiol method and direct determination (n = 3).

Sample	3D-Thiol method	Direct determination
Tap water	0.61 ± 0.06	<MDL
Groundwater	0.34 ± 0.02	<MDL
Lake water 1	0.94 ± 0.09	<MDL
Lake water 2	1.79 ± 0.13	<MDL
River water[a,b]	1.39 ± 0.08	<MDL
Pond water 1[a]	0.61 ± 0.12	<MDL
Pond water 1[a,b]	0.65 ± 0.04	<MDL
Pond water 2	2.94 ± 0.12	4.44 ± 0.12

[a] Filtered with Whatman No. 41.
[b] Two 3D-Thiol filters.

4. Conclusions

A SLS 3D printed 3D-Thiol scavenger for Hg preconcentration was prepared and applied for the determination of Hg from water samples with ICP-MS. The proposed method offers a simple and highly selective method for the extraction and determination of ultra-trace Hg. 3D-Thiol has an excellent selectivity towards Hg over Ca, Cd, Co, Cr, Fe, K, Mg, Mn, Na, Ni, and Pb. The interfering effects of the matrix are reduced when the 3D-Thiol method is applied. In addition, the loading capacity of 222.4 $\mathrm{mg\,g^{-1}}$ for 3D-Thiol is significantly higher than adequate for this application. The captured Hg can be quantitatively eluted from the 3D-Thiol filter with a mixture of 0.3% (w/v) thiourea and 8% (v/v) HCl solution. If desired, the 3D-Thiol scavenger can be reused at least 10 times with no decrease in its extraction efficiency. The results indicate that the new 3D-Thiol is an exceptional candidate for Hg sorption from natural waters. Due to the high preconcentration factor of 92.8 and extremely low detection limit of 0.037 $\mathrm{ng\,L^{-1}}$, ultra-trace amounts of Hg at pg $\mathrm{L^{-1}}$ levels in water samples can be quantified by the method developed.

Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. S.K. did the Hg adsorption and elution experiments and carried out the ICP-MS and ICP-OES analyses. E.L. prepared the 3D model of the scavenger and performed 3D printing. S.P. assisted in the designing of the experiments and A.V. supervised the project.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aca.2019.09.075.

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II

ADDITION OF THIOUREA AND HYDROCHLORIC ACID: ACCURATE NANOGRAM LEVEL ANALYSIS OF MERCURY IN HUMIC-RICH NATURAL WATERS BY INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY

by

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Addition of thiourea and hydrochloric acid: Accurate nanogram level analysis of mercury in humic-rich natural waters by inductively coupled plasma mass spectrometry

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ABSTRACT

An analytical method was developed for the direct determination of total mercury in natural waters at low ng L^{-1} level by inductively coupled plasma mass spectrometry (ICP-MS). The presented method overcomes previously observed problems relating to poor spike recoveries by adding 0.12% thiourea in addition to 3% HCl to all samples and standards. The sample preparation is fast and easy to perform by the developed method since it requires only the addition of HCl and thiourea to the water samples. A very low instrument detection limit (0.4 ng L^{-1}) was obtained without time-consuming preconcentration procedures. The accuracy and precision of the developed method were found excellent by the analysis of a certified groundwater reference material (ERM-CA615). The determined Hg concentration of 38.6 ± 0.5 ng L^{-1} was within the 95% confidence interval of the certified concentration of 37 ± 4 ng L^{-1}. The analysis of natural water samples showed that total mercury levels ranged from concentrations lower than the method detection limit (2.0 ng L^{-1}) to 10.9 ng L^{-1}. Excellent recoveries of 96–108% for inorganic mercury (iHg) and 102–110% for methylmercury (MeHg) were obtained for spiked humic-rich natural water samples. To our knowledge, the developed method is the first ICP-MS method for the analysis of humic-rich natural water samples at ng L^{-1} concentrations without the need for hyphenated techniques or preconcentration procedures.

1. Introduction

Mercury (Hg) is a highly toxic and persistent pollutant due to its bioaccumulation capabilities [1,2]. Dissolved total Hg concentration in pristine coastal waters and freshwaters is up to 10 ng L^{-1} and humic-rich lake waters and particle rich river waters can contain some tens of ng L^{-1} of Hg [3–5]. The total Hg concentration is the sum of the various Hg species occurring naturally due to oxidation, reduction, and biomethylation processes [1–3,5]. In natural waters, the dissolved total Hg contains 3–50% of elemental mercury and 30–90% of inorganic mercury (iHg). The methylated species consist mainly of monomethylmercury (MeHg), which content ranges from less than 1% up to 40%, and dimethylmercury to a much lower extent (< 2%) [3].

Simple and accurate determination of Hg in ultra-trace concentrations is a challenge at the low ng L^{-1} range. Typically, lowest detection limits are achieved by coupling cold vapor (CV) generation to atomic absorption spectrometry (AAS), atomic fluorescence spectrometry (AFS), or inductively coupled plasma mass spectrometry (ICP-MS) [1,6,7]. United States Environmental Protection Agency (US EPA) methods for Hg analysis are mainly intended for CVAAS or CVAFS techniques [8]. EPA Method 245.7 (Mercury in Water by Cold Vapor Atomic Fluorescence Spectrometry) can achieve a method detection limit (MDL) of 0.04 ng L^{-1} for Hg [9] while the highest determined MDL in reagent water in an interlaboratory study was 1.8 ng L^{-1} [10]. ICP-MS is used for Hg determination in EPA Methods 200.8 and 6020B [11,12]. According to Method 200.8, the estimated instrument detection limit (IDL) for ^{202}Hg is 0.2 μg L^{-1} and due to the memory effect, the concentration range should be limited to ≤5 μg L^{-1}. These methods are using gold to preserve the Hg and to prevent it from adhering to the sample introduction system [11,12]. However, the excess addition of gold may increase the maintenance frequency of the ICP-MS, and gold may buildup in the interface, on cones, and in the sample introduction system [13]. Additionally, the use of relatively high concentrations of gold as a preservative is not an option in many laboratories analyzing gold at trace concentration levels. Application notes [14,15] have reported method detection limits between 2 and 11 ng L^{-1} for ^{202}Hg using EPA Method 200.8. These are noticeably higher than obtained with CVAAS and CVAFS techniques. The preferred analytical technique

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often depends on available instruments and the regulations that various laboratories are required to follow.

The measurement of Hg in aqueous solutions is challenging with ICP-MS. Sensitivity for Hg is low relative to other elements since isotopic abundances of Hg are distributed among its stable isotopes, all less than 30% abundant. Additionally, a very high ionization potential (10.4 eV) leads to low ionization (4%), and due to its volatility, Hg can be lost during sample digestion [16,17]. Another problem observed during ICP-MS analysis is the memory effect [6,18–20]. Even relatively low Hg concentrations can adhere to the sample introduction system and persistence of Hg remains after a reasonable flushing time. As a result, a gradual increase of the Hg signal strength with time, non-linear calibration curves, poor accuracy, and low reliability are observed [6,18,21]. This makes accurate and precise determinations of Hg very challenging using ICP-MS [6,19]. Several reagents in addition to gold (III) chloride, such as L-cysteine, 2-mercaptoethanol, thiourea, potassium ferricyanide, and ammonium pyrolidinedithiocarbamate (APDC) have been used to eliminate the memory effect of Hg [6,13,18,19,22–24]. In general, previous publications focus on comparing different reagents to eliminate the memory effect of Hg in relatively high concentrations of a few μg L^{-1} to up to 10 mg L^{-1}.

Despite aforementioned challenges, ICP-MS has been used for the determination of Hg concentration in blood samples [13,25,26], biological samples [16,27–30], sediments [21,30,31], food [23,32,33], and drinking water [34,35]. The measured Hg concentrations are typically at μg L^{-1} level. For the investigation of pristine natural waters, which contain only a few ng L^{-1} of Hg, the achieved detection limits are often not sufficient. New publications on the measurement of total Hg in water by ICP-MS without hyphenated techniques or time-consuming preconcentration procedures seem to be scarce [1,36]. A review [36] published in 2016 included 13 references for total Hg analysis in water and only one of them used ICP-MS. Detection and quantification limits for this developed CV-ICP-MS method were 0.7 ng L^{-1} (3σ) and 2.2 ng L^{-1} (10σ), respectively [4]. It was stated in the review, that many of the developed analysis methods were applied to natural waters after spiking the water with Hg. In many cases, the spiking level used was probably higher than that needed for natural waters, which does not reflect the natural state of the environment. There are very few, if any, publications using ICP-MS for total Hg determination without the utilization of cold vapor generation or preconcentration techniques at low ng L^{-1} concentrations.

In our previous study [37], quantitative Hg recoveries could be obtained by a developed preconcentration procedure at the picogram level. However, a direct ICP-MS method for total Hg determination in humic-rich natural waters resulted in biased low recoveries in the approximate range of 65–85% when only 3% HCl was added to the samples. Therefore, the aim of this study was to develop a simple, fast, and reliable method for the determination of low total Hg concentrations in natural waters using ICP-MS without preconcentration procedure.

2. Material and methods

2.1. Reagents

All reagents were of analytical grade and all acidified solutions were made by dilution on a volume/volume basis. High-purity water with a resistivity of 18.2 MΩ cm obtained from an ELGA PURELAB Ultra water purification system (High Wycombe, Buckinghamshire, UK) was used throughout the experiment. High-purity hydrochloric acid (ANALPURE, 34–37%) and nitric acid (ANALPURE, 67–69%) were obtained from ANALYTIKA, spol. s r.o. (Prague, Czech Republic). Washing solutions for ICP-MS were prepared from puriss. p.a. hydrochloric acid (≥37%) and nitric acid (≥65%) purchased from Merck. Standard stock solutions of C, Fe, Ir, K, Mg, Na, S (1000 mg L^{-1}, Pure), and NexION Setup Solution (1 μg L^{-1} Be, Ce, Fe, In, Li, Mg, Pb, and U in 1% HNO_3, Pure Plus) were obtained from PerkinElmer. 1000 mg L^{-1} stock solution of Ca was prepared from $CaCO_3$ (≥99.0%) which was obtained from Merck. A stock standard solution of iHg (10 mg L^{-1} in 5% HNO_3, Pure Plus) was obtained from PerkinElmer and stored at 4 °C. A stock solution of MeHg (310.8 mg L^{-1} as Hg) was prepared from methylmercury chloride (≥98%, Merck) by dissolving the reagent in a small volume (~1.5 mL) of methanol (BAKER ANALYZED, (Ultra) Gradient HPLC Grade, J.T. Baker) and further dilution in 0.5% HCl. MeHg stock solution was stored in an amber-colored glass bottle at 4 °C.

For the preparation of calibrations standards (blank, 10, 20, and 100 ng L^{-1} of iHg) and spiking solutions of iHg or MeHg, adequate volumes of stock standards were diluted in 3% HCl and 0.12% (m/v) thiourea (ReagentPlus ≥99.0%, Merck) was added. The standards and samples were made up to 40 or 20 mL (by weight) in a new 50 mL polypropylene centrifuge tubes (VWR International) and mixed by vortex mixer (IKA Lab dancer, Germany). These centrifuge tubes are compatible with the used autosampler, so samples can be prepared and measured from the same tube, thus reducing the risk of contamination. To prepare Hg samples, HCl was added before iHg followed by dilution with sample water and addition of thiourea. Finally, the samples were made up to the volume with sample water. Due to the high toxicity of MeHg, it was last added to the samples in a fume hood to prepare samples containing MeHg. To assess the accuracy, the certified reference material of groundwater (ERM-CA615) from the Joint Research Centre, Institute for Reference Materials and Measurements (JRC-IRMM) was analyzed. It was matrix-matched with the used HCl and thiourea concentrations and made up to a volume of 5 mL in a 15 mL polypropylene centrifuge tube (VWR International). Plastic and glass containers that could come into contact with samples and standards, expect new polypropylene centrifuge tubes, were stored in 10% HCl (puriss. p.a.) over 24h and rinsed thoroughly with high-purity water before use. Sample bottles were filled with 10% HCl (puriss. p.a.) over 24h, rinsed with high-purity water, and filled with 0.5% HCl (high-purity) until sampling.

2.2. Sampling and sample pretreatment procedure

Natural water samples were collected from Central Finland, Southern Savonia, and Tampere Region in 500 mL amber colored glass bottles. Lake water 1 was obtained from lake Jyväsjärvi (Jyväskylä), lake water 2 from lake Ahvenisjärvi (Tampere, Hervanta), pond water 1 from a small pond (Toivakka, Ruuhimäki), pond water 2 from Mustalampi (Jyväskylä, Keltinmäki), stream water from a small stream flowing into Mustalampi (Jyväskylä, Keltinmäki), and ditchwater from a ditch (Pieksämäki, Pieksäjärvi). Samples were collected from late October to mid-November in 2019. Some days the outdoor temperature dropped below 0 °C and the sample had to be taken under ice (pond water 1 and ditchwater). Unfiltered samples were prepared in 20 mL volume and acidified to 3% with high-purity HCl and 250 μL of 9.6% thiourea was added to obtain 0.12% thiourea concentration. Samples were stored overnight at 4 °C if not analyzed immediately after sample collection.

2.3. Instrumentation

Analyses were performed with a PerkinElmer (Waltham, MA, USA) NexION 350D ICP-MS fitted with a prepFAST 4DX sample/standard autodilution system (Elemental Scientific Inc. (ESI), Omaha, NE, USA). The first rinse station of prepFAST contained a mixture of 0.75% HNO_3 and 2.25% HCl, and 3% HCl was used in the second rinse station. Daily instrument performance check was conducted using the NexION Setup Solution to ensure that sensitivities, oxides, and background count were all within the necessary ranges. Table 1 shows the instrumental parameters for ICP-MS.

Table 1
Instrumental parameters and conditions for ICP-MS.

System/Operating parameter	Type/Value/Mode
ICP-MS	PerkinElmer NexION 350D
Sample introduction	ESI prepFAST 4DX
Mode of operation	Standard
RF power	1600 W
Plasma gas flow	18 L min^{-1}
Auxiliary gas flow	1.2 L min^{-1}
Nebulizer gas flow	0.91–0.93 L min^{-1}
Nebulizer	PFA-ST
Spray chamber	Baffled, glass cyclonic
Spray chamber temperature	Peltier-cooled to 2 °C
Detector mode	Dual
Triple cone interface material	Nickel/Aluminum
Scanning mode	Peak hopping
Isotopes	Hg as the sum of ^{200}Hg, ^{201}Hg, and ^{202}Hg, ^{184}W
Internal standard	^{193}Ir at 80 µg L^{-1}
Dwell time	100 ms for Hg, 10 ms for ^{184}W and ^{193}Ir
Number of sweeps/reading	50
Number of readings/replicate	1
Number of replicates	5
Curve type	Linear through zero

2.4. Effect of thiourea concentration

The influence of thiourea concentration on Hg recovery and suspected memory effect was studied separately in the initial experiments. Solutions of 50 ng L^{-1} of iHg or MeHg were prepared in 3% HCl or 2% HNO_3 and 0–0.2% thiourea was added with 0.04% increments (n = 1). The experiments were conducted using both high-purity water and pond water 1. The calibration solutions contained 0.12% thiourea and 3% HCl or 2% HNO_3. Total Hg concentration was analyzed by ICP-MS. Blank samples were analyzed after real samples to monitor the memory effect.

2.5. Stability and precision studies

The stability of the sample introduction system, as well as short- and long-term precision, were evaluated by periodically measuring a quality control (QC) sample. QC stock solution mimicking natural water contained 200 ng L^{-1} of iHg, 200 mg L^{-1} of C, 50 mg L^{-1} of Ca and Na, 10 mg L^{-1} of Fe, and 20 mg L^{-1} of K, Mg, and S in 30% HCl. This stock solution was diluted 10-fold and thiourea concentration was adjusted to 0.12% before ICP-MS measurements. The precision and stability at lower Hg concentrations were estimated by measuring the same standard once a day on each measurement day containing 10 ng L^{-1} of iHg, 3% HCl and 0.12% thiourea over five weeks. The standard was stored in a polypropylene centrifuge tube at room temperature.

2.6. Spiking studies

To apply the developed method to natural water samples and evaluate the effectiveness of the addition of thiourea, samples were analyzed without the addition of Hg, and by adding low and high concentrations of 5 and 20 ng L^{-1} of iHg. Furthermore, 10 ng L^{-1} of MeHg was added to samples to better assess the performance of the method. All samples were prepared in triplicates and adjusted to contain 3% HCl and 0.12% thiourea. Total Hg concentration was measured with ICP-MS.

3. Results and discussion

3.1. Method development

In the analysis of synthetic MeHg samples by the direct ICP-MS method utilizing a 3% HCl matrix, low spike recovery combined with memory effect was observed even though the studied concentrations were at the low ng L^{-1} level. Analysis of 100 ng L^{-1} of MeHg in 3% HCl resulted in 60–70% recoveries. After analyzing solutions containing 100 ng L^{-1} of MeHg, the residue could be several ng L^{-1} of Hg which was difficult to remove. The memory effect was not evident when iHg samples were analyzed without the addition of MeHg. However, natural waters can contain up to 40% MeHg, making a separate investigation of MeHg necessary for reliable total Hg analysis [3]. To improve the washout of Hg, the used prepFAST method was modified to increase the rinsing of the nebulizer and spray chamber. The washout of Hg improved to some extent, although the memory effect could not be eliminated with this approach.

Different experiments were conducted to investigate the performance characteristics of the method in terms of selectivity, instrument detection limit (IDL), method detection limit (MDL), linearity, accuracy, stability, and robustness. Additionally, the influence of thiourea concentration on Hg recovery and memory effect was studied. Method performance was assessed using spike recovery tests using iHg or MeHg and analyzing certified groundwater reference material ERM-CA615.

3.1.1. Selectivity and spectral interferences

To obtain a higher count rate and improved precision, ^{200}Hg was summed with the signals from ^{201}Hg and ^{202}Hg, which doubled the count rate compared to ^{202}Hg alone. Additionally, ^{202}Hg was separately monitored for further assessment. The concentrations obtained by the isotope summing method and ^{202}Hg were remarkably close to each other, but the former had slightly better IDL, MDL, and RSD values. The concentration difference between these two was not more than 0.8 ng L^{-1} in the analysis of natural waters. Internal standards including Bi, In, Ir, Pt, Rh, Te, and Tl have been used for the determination of Hg by ICP-MS [6,13,25,29,38–40]. Iridium was chosen as the internal standard since it has a mass similar to Hg and the ionization potential of Ir (9.1 eV) is close to that of Hg (10.4 eV). Tungsten (W) could interfere with Hg analysis as oxides or hydroxides ($^{183}W^{16}OH^+$, $^{184}W^{16}O^+$, $^{184}W^{17}O^+$, and $^{186}W^{16}O^+$), which would lead to a significant positive bias [41]. For this reason, the most abundant W isotope ^{184}W was monitored during all measurements. The ratio of CeO to Ce was always kept below 2.5% to minimize the possible interference. The studied samples had 2–35 times higher cps for ^{184}W than for Hg but no W interference on Hg analysis was observed.

3.1.2. Effect of thiourea on recovery

Initial experiments showed that when only 3% HCl was added to all samples and standards, the Hg spike recoveries for humic-rich natural waters ranged approximately from 65 to 85%, which was not considered acceptable. In Finland, natural waters are abundant in natural organic matter and the matrix of these humic-rich waters can also cause problems in Hg analysis. Dissolved organic matter is known to interact very strongly with Hg, affecting its speciation, solubility, mobility, and toxicity in the aquatic environment [42]. The addition of thiourea to the samples seems highly feasible since it can complex with iHg and MeHg in aqueous solutions and thus obviate the memory effect [18,43]. According to the theory of hard and soft acids and bases (HSAB theory), thiourea can be used in the stabilization of Hg as soft base anion ligands readily coordinate with soft acid cation metals [20,44]. Generally, too high Hg recoveries (approximately 110%) are reported [29,34] rather than too low recoveries, which were found in this study. Zhu and Alexandratos [18] examined 0.1% thiourea to eliminate the memory effect from 2 mg L^{-1} Hg solutions using ICP-OES. However, they did not use only thiourea but also added 0.1% Triton X-100 to the thiourea solution as a wetting agent. In a column published in Spectroscopy, Pappas [20] discussed sample preparation problem solving for ICP-MS in terms of solubility, chelation, and memory effects and presented that even 0.01% thiourea worked as effectively as 100 µg L^{-1} gold (III) chloride + 0.01% EDTA to rinse out several µg L^{-1} of Hg spiked into blood.

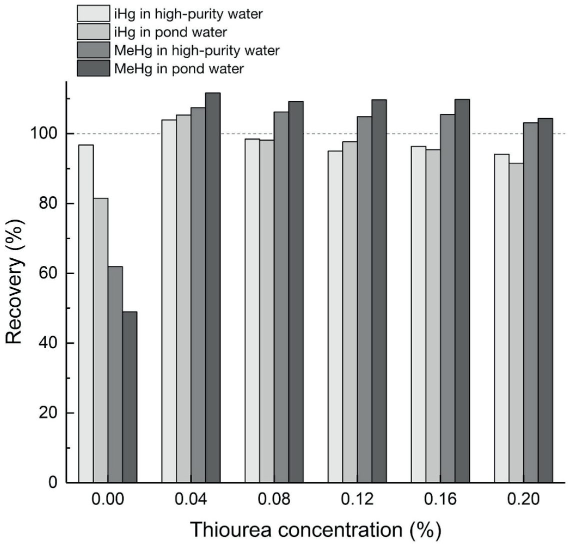

Fig. 1. Recovery (%) of 50 ng L^{-1} iHg and MeHg spike in high-purity water or pond water in 3% HCl using 0–0.2% thiourea (n = 1).

Thiourea forms complexes with Hg, hence, it was studied whether adding thiourea to all samples and standards could solve both the problem of biased low recoveries and memory effect. The effect of various concentrations of thiourea was studied with a concentration of 50 ng L^{-1} iHg or MeHg prepared in high-purity water and in pond water containing 3% HCl as described in section 2.4. Both iHg and MeHg were studied as these are the most commonly found Hg species in natural waters. At concentrations of 0.04%–0.2% thiourea, sufficient recoveries were obtained, and recoveries were relatively poor without the addition of thiourea except for iHg in high-purity water, resulting in a recovery of 97% (Fig. 1). This can be explained by iHg forming stable $[HgCl_4]^{2-}$ complexes with HCl in high-purity water, which can be interfered in natural waters containing e.g. humic substances. The results show that the addition of thiourea to all samples and standards eliminated the problem of low spike recoveries for both iHg and MeHg as stable thiourea complexes are formed [45]. The findings also suggest that the method is robust as the recoveries were close to 100%, even if the thiourea concentration was not matched to that of the calibration standards, which contained 0.12% thiourea.

3.1.3. *Effect of thiourea on memory effect*

Blank samples were measured after 50 ng L^{-1} of iHg and MeHg samples (section 2.4) to monitor the carry-over from Hg. After analyzing the MeHg samples without thiourea in high-purity water and pond water, approximately 10 and 3 ng L^{-1} Hg residue were measured, respectively. The analysis of MeHg in 0.04% thiourea resulted in residual Hg, which concentration was only just lower than the MDL (2.0 ng L^{-1}), and at concentrations of 0.08–0.16% thiourea, the measured Hg concentration in the blank sample was at the highest ~1 ng L^{-1}. The memory effect was not observed at 0.2% thiourea concentration. In the analysis of iHg samples, the residual concentration was lower than the IDL (0.4 ng L^{-1}). Although no memory effect was observed after analysis of iHg samples, the recovery was only 82% when pond water was used (Fig. 1), which is consistent with the above-mentioned initial experiments where only 3% HCl was used. No memory effect was observed for iHg when 0.12% thiourea was utilized in ICP-MS measurement, even after measuring 1 μg L^{-1} iHg sample solution followed by a blank sample. Within this concentration range and especially in natural waters, the low spike recovery was found to be more problematic than the memory effect. The selected concentrations are discussed in detail in section 3.1.5.

3.1.4. *Influence of nitric acid*

The same thiourea addition experiments (section 2.4) were carried out using 2% HNO_3 instead of 3% HCl to study whether the HNO_3 matrix could be utilized with thiourea since it is mostly preferred in multi-element ICP-MS analysis. However, μg L^{-1} level dilutions of iHg in HCl are more resistant towards adsorption to the container walls than the HNO_3 based dilutions. It is also recommended that HNO_3 matrices should be used for Hg determinations by ICP-MS at low concentrations only if Au is used as a stabilizing agent [46]. Paniz *et al.* [23] analyzed several elements by ICP-MS, including Hg, from plants and foodstuffs utilizing 0.2% L-cysteine and 2.5% (v/v) HNO_3, which improved the Hg stability for calibration and reduced the memory effect. However, the Hg concentration in the final solution analyzed was approximately 1–3 μg L^{-1}, which is again much higher than the concentrations examined in this study. Our tests show that without the addition of thiourea in the HNO_3 matrix, the spike recoveries were low. However, analysis of iHg in high-purity water resulted in 93% recovery which is similar to results obtained in the HCl matrix in high-purity water. The addition of thiourea from 0.04% to 0.2% using 2% HNO_3 resulted in varying recoveries from 51 to 141%, hence, it was concluded that the HNO_3 matrix was not suitable with or without thiourea and 3% HCl was used in further studies.

3.1.5. *Selected concentrations*

Quantitative recoveries were obtained with all used thiourea concentrations when thiourea was added to all samples and standards. With thiourea concentration of 0.2%, no memory effect was observed. However, the total dissolved solids are desirable to keep below 0.2% with ICP-MS because of possible decomposition of the matrix components around the sampler cone orifice [47]. Vermillion *et al.* [45] used 10–60 mM (≈0.08–0.45%) thiourea to release MeHg from the dissolved matrix of HCl-acidified freshwater samples to form a more labile MeHg-thiourea complex prior to the MeHg preconcentration procedure and detection with CVAFS. The used concentration of thiourea needs to be high enough in order to avoid problems relating to low spike recovery and memory effect but low enough not to block the sample cone orifice. Thus, 0.12% thiourea concentration with 3% HCl was selected for the analysis. In addition, the selected thiourea concentration is compatible with our previously developed preconcentration method and the same calibration standards can be used with both methods.

3.2. *Validation and analytical figures of merit*

3.2.1. *Range of the linearity of the calibration graph*

The calibration was found to be linear up to 1 μg L^{-1}. However, Hg concentrations in natural water samples are usually at a very low level, so the highest calibration standard was set at 100 ng L^{-1}. All calibration curves obtained during the study were found to be linear over the analytical range and the correlation coefficient was higher than 0.9999. After linear regression, the lack of fit was tested by examining the residuals (Fig. A.1). At first, the calibration was performed with six levels (blank, 1, 5, 10, 20, 50, 100 ng L^{-1}). Since the RSD of the two lowest calibration standards was higher than 5%, calibration was performed using a blank, 10, 20, and 100 ng L^{-1} of iHg in subsequent measurements.

3.2.2. *Instrument and method detection limits*

The IDL was calculated to be the concentration equal to the average of 10 replicate measurements of the calibration blank plus three times the standard deviation. The MDL was calculated according to the recommended protocol outlined in the U.S. Environmental Protection Agency method detection limit procedure [48]. Seven replicate measurements of a calibration blank spiked with iHg at a concentration of

1.5 ng L^{-1} were analyzed on three nonconsecutive days. The calculated IDL and MDL were 0.4 and 2.0 ng L^{-1} of Hg, respectively.

3.2.3. Accuracy and precision

The European Union established a maximum allowable concentration for Hg and its compounds to be 0.07 μg L^{-1} in inland and other surface waters [49]. There are only two freshwater reference materials for Hg which have a certified value below this concentration [50]. ERM-CA615 (natural groundwater, 0.037 ± 0.004 μg L^{-1}) from JRC-IRMM was chosen to be used in this study due to its availability during the experiments. The precision of the method was evaluated using RSD values from the analysis of certified reference material as well as from the quality control sample and low-level iHg standard (section 2.5). The concentration determined from ERM-CA615 was 38.6 ± 0.5 ng L^{-1} ($n = 10$) and the concentration was within the 95% confidence interval of the certified concentration of 37 ± 4 ng L^{-1}. The replicate sample analyses were performed on three nonconsecutive days over three weeks and the precision was found to be 1.2%. The RSD for five replicate determinations of the QC sample within one day was 1.5%, and over four weeks the precision was 2.7% ($n = 18$). Precision for replicate assays of 10 ng L^{-1} iHg standard was calculated to be 2.5% ($n = 6$) and the average recovery was 102% over five weeks (Fig. A.2). It can be concluded that the lowest standard was stable throughout this period.

3.3. Analysis of natural waters

The proposed method was applied to six natural water samples from different parts of Finland. The concentrations determined and the recoveries of the spiked samples are given in Table 2. The analyzed total Hg concentrations ranged from < MDL to 10.9 ng L^{-1} with lowest Hg concentrations in lake waters and highest in stream water. The recoveries of iHg spiked samples varied between 96 and 108% and MeHg spiked samples between 102 and 110%. RSD values for three sample replicates were 0.6–3.6% for all studied samples. Although the Hg concentration in lake waters resulted in lower concentration than the MDL, the obtained concentration was used in the calculation of the spike recovery so that the recovery would not be overestimated. Considering that the analyzed water samples had different matrices, the standard deviations obtained were suitable and the successful recovery of Hg in all samples indicates the high robustness of the developed method. Moreover, the method was demonstrated to be robust for the measurement of samples with thiourea concentration in the range of 0.04–0.2% (Fig. 1). In the presented method, one sample can be prepared in about 1 min, while the estimated sample preparation time using our previously developed preconcentration method is close to 2 h. In addition, the required sample volume is reduced from 500 mL to 20 mL, which is more than adequate for ICP-MS measurement. Even without the use of any preconcentration procedures, the method still has a lower method detection limit (2 ng L^{-1}) than that recommended in Finland (5 ng L^{-1}) [51].

Table 2
Analytical results (mean ± std. deviation, $n = 3$) for total Hg in water samples.

Sample	Added iHg or MeHg (ng L^{-1})	Found total Hg (ng L^{-1})	Recovery (%)
Lake water 1	0	< MDL (1.07 ± 0.03)	
	5 (iHg)	6.17 ± 0.13	102 ± 3[a]
	20 (iHg)	21.5 ± 0.3	102 ± 2[a]
	10 (MeHg)	11.9 ± 0.5	108 ± 5[a]
Lake water 2	0	< MDL (0.938 ± 0.035)	
	5 (iHg)	5.97 ± 0.13	101 ± 3[a]
	20 (iHg)	20.7 ± 0.3	99 ± 2[a]
	10 (MeHg)	11.1 ± 0.3	102 ± 3[a]
Pond water 1	0	6.21 ± 0.22	
	5 (iHg)	11.1 ± 0.3	98 ± 5
	20 (iHg)	25.5 ± 0.4	96 ± 2
	10 (MeHg)	16.5 ± 0.4	103 ± 4
Pond water 2	0	7.71 ± 0.16	
	5 (iHg)	13.1 ± 0.2	108 ± 3
	20 (iHg)	28.1 ± 0.5	102 ± 3
	10 (MeHg)	18.6 ± 0.4	108 ± 4
Stream water	0	10.9 ± 0.1	
	5 (iHg)	15.8 ± 0.2	98 ± 4
	20 (iHg)	31.2 ± 0.4	101 ± 2
	10 (MeHg)	21.9 ± 0.3	110 ± 3
Ditchwater	0	7.40 ± 0.22	
	5 (iHg)	12.3 ± 0.3	98 ± 5
	20 (iHg)	26.7 ± 0.4	97 ± 2
	10 (MeHg)	17.7 ± 0.3	103 ± 3

[a] Recovery was calculated even though the concentration without spiking resulted in a lower concentration than the MDL.

4. Conclusions

ICP-MS was shown as a powerful technique for the determination of ng L^{-1} Hg concentrations in natural waters only when HCl and thiourea were utilized. The developed method is based on the addition of 3% HCl and 0.12% thiourea in natural water, whereby Hg is released from the matrix and stabilized in the solution. By using at least 0.04% thiourea and 3% HCl in all samples and standards, the low spike recovery problem was overcome. However, the concentration of thiourea was further increased to 0.12% to reduce the memory effect from MeHg. Using 0.12% thiourea, the developed method is also compatible with our previously developed preconcentration method. It was found that the HNO_3 matrix was not suitable for Hg analysis at ng L^{-1} concentrations by ICP-MS and compared to other studies, the concentration range has been reduced to ng L^{-1}. High sensitivity with a detection limit of 0.4 ng L^{-1} was achieved, which is even lower than obtained with a CV-ICP-MS technique [4,24]. Successful analysis of the certified groundwater reference material ERM-CA615 demonstrated the accuracy of the method resulting in the recovery of 104 ± 2% and RSD 1.2%. Moreover, the spike recoveries varied between 96 and 108% for iHg and 102–110% for MeHg with RSDs lower than 3.6%. The use of thiourea and HCl alone substantially clarifies and speeds up the analytical procedure compared to methods with preconcentration steps. The developed method proved to be accurate and reliable for the detection of low ng L^{-1} concentrations of Hg in humic-rich natural waters.

CRediT authorship contribution statement

Suvi Kulomäki: Conceptualization, Formal analysis, Investigation, Resources, Validation, Visualization, Writing - original draft, Writing - review & editing. **Siiri Perämäki:** Conceptualization, Funding acquisition, Resources, Supervision, Writing - review & editing. **Ari Väisänen:** Funding acquisition, Project administration, Resources, Supervision, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.talanta.2020.121125.

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III

PRECONCENTRATION AND SPECIATION ANALYSIS OF MERCURY: 3D PRINTED METAL SCAVENGER-BASED SOLID-PHASE EXTRACTION FOLLOWED BY ANALYSIS WITH INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY

by

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Preconcentration and speciation analysis of mercury: 3D printed metal scavenger-based solid-phase extraction followed by analysis with inductively coupled plasma mass spectrometry

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ABSTRACT

A selective method for preconcentration and determination of methylmercury (MeHg) and inorganic mercury (iHg) in natural water samples at the ng L^{-1} level has been developed. The method involves adsorption of Hg species into a 3D printed metal scavenger and sequential elution with acidic thiourea solutions before ICP-MS determination. Experimental parameters affecting the preconcentration of MeHg and iHg such as the sample matrix, effect of the flow rate on adsorption, eluent composition, and elution mode have been studied in detail. The obtained method detection limits, considering the preconcentration factors of 42 and 93, were found to be 0.05 ng L^{-1} and 0.08 ng L^{-1} for MeHg and iHg, respectively. The accuracy of the method was assessed with a certified groundwater reference material ERM-CA615 (certified total iHg concentration 37 ± 4 ng L^{-1}). The determined MeHg concentration was below MDL while iHg concentration was determined to be 41.2 ± 0.5 ng L^{-1}. Both MeHg and iHg were also spiked to natural water samples at 5 ng L^{-1} concentration and favorable spiking recoveries of 88–97% were obtained. The speciation procedure was successfully applied to two lake water samples where MeHg and iHg concentrations ranged from 0.18 to 0.24 ng L^{-1} and 0.50–0.62 ng L^{-1}, respectively. The results obtained demonstrate that the developed 3D printed metal scavenger-based method for preconcentration and speciation of Hg is simple and sensitive for the determination of Hg species at an ultra-trace level in water samples.

1. Introduction

Mercury (Hg) and its compounds are highly hazardous pollutants, with organic Hg compounds generally more toxic than their inorganic counterparts (iHg). Methylmercury (MeHg) is the species of highest concern due to its bioaccumulation ability, affinity to macromolecules, and slow metabolism [1]. According to the United Nations Environment Programme's (UNEP) Global Mercury Assessment 2018 report [2], human activities have increased total atmospheric Hg concentrations by about 450% above natural levels, thus leading to Hg loads in some food webs reaching levels of concern for ecological and human health. Typical concentrations of total Hg in natural water are in the low ng L^{-1} range, with MeHg content ranging from only a few percentages up to 30–40% of the total Hg [3–5]. The speciation of Hg is essential, especially to understand the bioavailability and toxicity of MeHg to assess the health risks of Hg and to better understand its biogeochemical cycle [6]. Unfortunately, speciation analysis is often challenging due to extremely low concentrations of Hg in non-polluted waters.

Various Hg speciation analysis methods have been developed and reviewed in recent years [1,5,7–15]. The most common methods used for Hg speciation are chromatographic methods including gas chromatography (GC) and high-performance liquid chromatography (HPLC) hyphenated to highly sensitive elemental specific detection techniques such as cold vapor atomic fluorescence spectrometry (CV-AFS) and inductively coupled plasma mass spectrometry (ICP-MS). In addition to chromatographic methods, non-chromatographic methods can be used to separate Hg species based on the different chemical and/or physical properties [5].

Non-chromatographic methods use simple instrumentation and are more available than chromatographic ones. Additionally, they can offer shorter analysis time, lower cost, and sometimes even better sensitivity than a chromatographic approach [16]. Unfortunately, these methods have more limited separation power i.e., they are not applicable for the separation of many species [17]. Nevertheless, non-chromatographic

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speciation methods are a well-accepted way of speciation analysis for many elements with competitive limits of detection (LOD) [16,17]. Different non-chromatographic methods for the determination of Hg species in natural waters have been reviewed [1,5,11,18] and many of these techniques are utilizing solid-phase extraction for Hg preconcentration. As mentioned by Amde *et al.* [1], most of the non-chromatographic techniques are for iHg species alone and often have an insufficient limit of detection, exceeding the low ng L^{-1} range required for natural waters. Hence, the development of sensitive, simple, and multi-Hg species-oriented methods is highly desirable.

In our previous work [19], a 3D printed metal scavenger (3D-Thiol i.e., PA12-Thiol) for Hg preconcentration was prepared and applied for the determination of iHg from water samples with ICP-MS. The PA12-Thiol proved to be a very efficient adsorbent for Hg, so the focus of this study was to expand the method for speciation of Hg in natural water samples at ng L^{-1} concentration level. Experimental parameters including the sample matrix, effect of the flow rate on adsorption, eluent composition, and elution mode were optimized to achieve preconcentration and speciation analysis of Hg. The developed speciation procedure was tested on a certified reference material (ERM-CA615) and finally applied to natural water samples.

2. Material and methods

2.1. Reagents

High-purity water (18.2 MΩ cm) used throughout the study was prepared from an ELGA PURELAB Ultra water purification system (High Wycombe, Buckinghamshire, UK) and all solutions were made by dilution on a volume/volume basis. High-purity hydrochloric acid (ANALPURE, 34–37%) was obtained from ANALYTIKA, spol. s r.o. (Prague, Czech Republic) and p.a. hydrochloric acid (≥ 37%) from Merck (Darmstadt, Germany). Nitric acid (≥ 65%) and thiourea (ReagentPlus ≥ 99.0%) were purchased from Merck. A stock solution of iHg (10 mg L^{-1} in 5% HNO_3) was obtained from PerkinElmer (Waltham, MA, USA) and stored at 4 °C. The stock solution of MeHgCl (PESTANAL, analytical standard ≥ 98%, Merck, 310.8 mg L^{-1} as Hg) was prepared by dissolving the reagent in a small volume of methanol (BAKER ANALYZED, (Ultra) Gradient HPLC Grade, J.T. Baker, Deventer, Netherlands) and further diluted in 0.5% HCl and stored at 4 °C in an amber-colored glass bottle. Standard stock solutions of C, Fe, Ir, K, Mg, Na, and S (1000 mg L^{-1}, Pure) were obtained from PerkinElmer. Ca (1000 mg L^{-1}) was prepared from $CaCO_3$ (≥ 99.0%, Merck). NexION Setup Solution for ICP-MS instrument set-up (1 μg L^{-1} Be, Ce, Fe, In, Li, Mg, Pb, and U in 1% HNO_3, Pure Plus) was purchased from PerkinElmer. Certified reference material of groundwater (ERM-CA615) was from the Joint Research Centre, Institute for Reference Materials and Measurements (JRC-IRMM). SiliaMetS Thiol (i.e., Thiol) was used as a chemically active component in the 3D printed scavengers and was obtained from SiliCycle (Quebec City, Canada). Polyamide-12 (PA12) and polyamide-11 (PA11) printing powders were purchased from BASF (Ludwigshafen am Rhein, Germany). Only new 15 or 50 mL polypropylene centrifuge tubes or 10% HCl acid-washed volumetric flasks and glass bottles were used during the study.

2.2. Instrumentation

Total Hg determination was performed with a PerkinElmer NexION 350D ICP-MS (Waltham, MA, USA) in standard mode similarly as in our previous study [20] and the operational conditions are listed in Table 1. A four-channeled peristaltic pump (LabV1, Baoding Shenchen Precision Pump Co., Ltd., Baoding, China) with black/white 3.18 mm i.d. PVC peristaltic pump tubing (PerkinElmer) was used for the delivery of solutions through 3D printed metal scavengers.

Table 1
Operational conditions used for Hg determination by ICP-MS.

System/Operating parameter	Type/Value/Mode
ICP-MS	PerkinElmer NexION 350D
Sample introduction	ESI prepFAST 4DX
Washing solutions for ESI prepFAST	Rinse 1: A mixture of 0.75% HNO_3 and 2.25% HCl (p.a.), Rinse 2: 3% HCl (p.a.), Carrier/diluent: 3% HCl (high-purity)
Mode of operation	Standard
RF power	1600 W
Plasma gas flow rate	18 L min^{-1}
Auxiliary gas flow rate	1.2 L min^{-1}
Nebulizer gas flow rate	0.92–0.96 L min^{-1}
Nebulizer	PFA-ST
Spray chamber	Baffled, glass cyclonic (2 °C)
Detector mode	Dual
Scanning mode	Peak hopping
Isotopes	Hg as the sum of ^{200}Hg, ^{201}Hg, and ^{202}Hg, ^{184}W
Internal standard	^{193}Ir at 80 μg L^{-1} in 3% HCl
Dwell time	100 ms for Hg, 10 ms for ^{184}W and ^{193}Ir
Number of sweeps/ reading	50
Number of readings/ replicate	1
Number of replicates	3
Curve type	Linear through zero

2.3. Preparation of 3D printed metal scavengers

The 3D printed metal scavengers (PA12-Thiol or PA11-Thiol) were prepared by mixing PA12 or PA11 powder (~50 μm diameter) with 10 wt% of Thiol (3-mercaptopropyl-functionalized silica gel, 40–63 μm), respectively. The printing process was done using a Sharebot SnowWhite SLS 3D printer as described in our previous work [19]. The 3D printing parameters used for PA12-Thiol are also described in our previous work [19]. For PA11-Thiol, the used laser power was 40–50% (of a maximum of 14 W), a laser speed of 2160–2560 mm s^{-1}, and a printing temperature of 175–177 °C. A layer thickness of 0.1 mm was used for all 3D printed filters. The filters were thoroughly cleaned from any unsintered powder. One or two filters were placed in a 10 mL syringe and washed with high-purity water.

2.4. Adsorption experiments

In all experiments, sample solutions were prepared in an appropriate volume (e.g., 45 or 500 mL), of which 5 mL was reserved for ICP-MS analysis to obtain the initial total Hg concentration. The remaining solution was then used for adsorption experiments. All 3D printed filters were conditioned with ~10 mL of high-purity water to prepare the filter, solvate the functional groups, and remove any unsintered printing powder [21].

2.4.1. Effect of the sample matrix on adsorption

Initial adsorption experiments were conducted using PA12-Thiol filters. To investigate the effect of different sample matrix compositions on both MeHg and iHg adsorption, 10 mL synthetic solutions ($n = 2$) containing 20 ng L^{-1} of MeHg or iHg with different concentrations of thiourea (0, 0.01, 0.05, and 0.12%) and HCl (0, 0.5, 3, 4, and 6%) were slowly passed through the PA12-Thiol filter without using a peristaltic pump (**A**: high-purity water, **B**: 0.01% thiourea, **C**: 0.12% thiourea, **D**: 3% HCl, **E**: 0.12% thiourea in 0.5% HCl, **F**: 0.12% thiourea in 3% HCl, **G**: 0.12% thiourea in 4% HCl, **H**: 0.12% thiourea in 6% HCl). For **A**–**F** and **H**, calibration was performed using 0.12% thiourea and 3% HCl in the sample matrix, and for **G** using 0.12% thiourea and 4% HCl in the sample matrix. The sample matrices were also adjusted to these concentrations before ICP-MS analysis. The Hg concentrations of the initial sample solutions and effluent solutions were analyzed by ICP-MS and adsorption percentages were calculated.

2.4.2. Support matrix of the 3D printed metal scavenger

The use of PA12 or PA11 in the supporting matrix of the 3D printed metal scavenger was investigated. The chemically active component (Thiol) and its amount were kept constant in all experiments. Filters were prepared as described in section 2.3. Adsorption tests were performed in the same manner as previously described in 2.4.1 and 0.01% thiourea was used in the sample matrix.

2.4.3. Improving the adsorption

The effect of different flow rates (5, 6, and 7 mL min $^{-1}$) on the adsorption of MeHg and iHg was investigated. Sample solutions of 40 mL (n = 4) containing either 20 ng L^{-1} of MeHg or iHg in 0.01% thiourea were passed through a PA11-Thiol filter with a peristaltic pump. It was also studied whether the adsorption of MeHg could be improved by placing two PA11-Thiol filters in the same syringe instead of one filter. A sample solution of 40 mL containing 50 ng L^{-1} of MeHg was passed through either one or two PA11-Thiol filters (n = 6).

2.5. Elution experiments

Preliminary elution experiments were performed with a PA11-Thiol filter using a peristaltic pump with the eluent volume of 10 mL at a flow rate of 0.5 mL min-1, followed by delivery of 2 mL of high-purity water. Experiments were performed in individual MeHg or iHg sample solutions at 50 ng L^{-1} concentrations (n = 2). The flow of the elution solution was in the same direction as adsorption in the preliminary experiments. In the optimized procedure (section **2.6**), the flow of the elution solution (flow rate of 1 mL min^{-1}) was in the same direction as adsorption for MeHg, and counter-current flow was used for iHg without a peristaltic pump.

2.6. Optimized procedure

In the optimized procedure, the initial sample solution was adjusted to contain 0.01% thiourea, prepared in suitable sample volume, and a 5 mL of the solution was reserved for total Hg analysis. PA11-Thiol filters were conditioned with ~10 mL of high-purity water. The adsorption step was performed using a peristaltic pump and the sample solution was pumped through two filters at a flow rate of 5 mL min^{-1}. The retained MeHg species were eluted from the PA11-Thiol with two 5 mL aliquots of elution solution consisting of 0.1% thiourea in 3.5% HCl, followed by 1 mL of high-purity water at a flow rate of 1 mL min^{-1}. At the end of each elution step, the peristaltic pump was set at maximum speed for a brief time to recover the sample solutions from the filters.

Elution of iHg species was performed by countercurrent flow without a peristaltic pump. 2 mL of the elution solution consisting of 0.3% thiourea in 8% HCl was placed in a syringe holding the PA11-filters. The syringe plunger was used to push the first drop of elution solution through the PA11-Thiol filters and the rest of the elution solution was allowed to flow freely through the filters. Next, 2 mL of high-purity water was passed through the PA11-Thiol filters in the same manner. Before the washing step and at the end of the procedure, air was pumped through the filters with a syringe plunger to recover the sample solution. Individual sample solutions containing either MeHg or iHg species were adjusted to contain 0.18% thiourea and 3% HCl before ICP-MS analysis. Thus, the final volumes of the solutions containing either MeHg or iHg were 11.7 and 5.3 mL, respectively. The PVC tubes of the peristaltic pump used for sample delivery were washed with 5 mL of a solution containing 0.5% thiourea in 10% HCl at a flow rate of 5 mL min^{-1}, followed by 50 mL of high-purity water at a flow rate of ~50 mL min^{-1}.

2.7. Accuracy and precision

The analytical performance of the method was evaluated with replicate determinations of quality control (QC) sample (total Hg determination), and certified groundwater reference material of ERM-CA615 (n = 3, with and without speciation procedure). A natural water-mimicking QC sample was prepared by 10-fold dilution of QC stock solution (200 ng L^{-1} of iHg, 200 mg L^{-1} of C, 50 mg L^{-1} of Ca and Na, 10 mg L^{-1} of Fe, and 20 mg L^{-1} of K, Mg, and S in 30% HCl) and was adjusted to contain 0.18% thiourea and 3% HCl. For the analysis of certified reference material, 11,5 μL of 9.6% thiourea was added to 11 mL of ERM-CA615. As before, 5 mL of this solution was saved for total Hg analysis, and the rest of the solution was used for speciation. Speciation was performed as described in the optimized procedure (section 2.6).

2.8. Spiking studies and the treatment of the natural water samples

Water samples were collected either in 500 mL (spiking studies) or 2 L amber-colored glass bottles from Central Finland in April 2021. Sample bottles were cleaned as previously described [20]. Lake water 1 was collected from lake Jyväsjärvi (Jyväskylä) and lake water 2 from lake Päijänne (Korpilahti). Stream water was collected from Kor-ke-a-kos-ki (Jyväskylä). For spiking studies (n = 2), samples were spiked with 5 ng L^{-1} of MeHg and 5 ng L^{-1} of iHg, adjusted to contain 0.01% thiourea, and prepared to a volume of 45 mL. Natural water samples were prepared in triplicate to a sample volume of 500 mL. Samples were treated as described in the optimized procedure (section 2.6). Elution solutions were stored at room temperature overnight and analyzed by ICP-MS the following day. Considering the sample volume of 495 mL, the final volumes of 11.7 mL for MeHg and 5.3 mL for iHg, preconcentration factors of 42 and 93 are obtained for MeHg and iHg, respectively.

3. Results and discussion

3.1. Effect of the sample matrix on adsorption

The effect of eight different combinations of thiourea and HCl in the sample matrix was assessed on the adsorption of MeHg and iHg at 20 ng L^{-1} concentrations, and the results are shown in Fig. 1. Excellent adsorption (98–102%) was obtained for both MeHg and iHg with **A**, **B**, and **C**. However, when **A** was used in the sample matrix, the recovery of iHg in the initial sample solution was reduced to only 80%, indicating either Hg volatilization or possible loss of Hg by adsorption on the

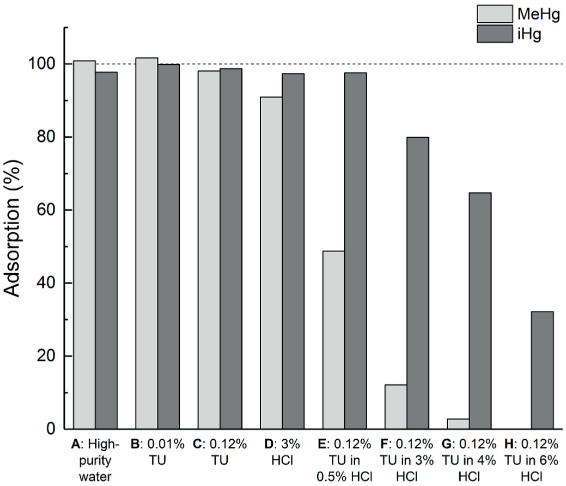

Fig. 1. The adsorption (%) of MeHg and iHg into PA12-Thiol using different combinations of thiourea (TU) and HCl in the sample matrix. The dotted line represents the target value of 100%.

container walls or the sample introduction system [22]. This was not observed with other sample matrices. When using matrix **D**, the adsorption of iHg was very efficient, but only about 90% of MeHg was adsorbed. It appears that iHg is more readily adsorbed than MeHg. Strong retention of iHg at lower pH could be explained as a higher affinity of Hg(II) towards –SH groups and the possibility for complex formation [23,24]. The retention of MeHg is often highly pH-dependent and the highest retention is usually achieved in the pH range of 4–8 [23,25,26]. Outside the optimum pH range, quantitative sorption of MeHg would be inhibited due to either its larger size, lower charge, and/or lower stability constant compared to iHg [23]. This also explains why low adsorption of MeHg was observed in matrices **G** and **H**, where higher HCl concentrations were used.

Indeed, other studies have aimed either to retain both forms of Hg [25] or retain iHg alone while MeHg is passed through the adsorbent [27–29]. For example, with an anion-exchanger material [29], iHg species are retained under selected conditions by the anion-exchanger as $HgCl_4^{2-}$, and they do not pass through the material. MeHg species, in turn, are passed through the ion-exchanger as MeHgCl [29]. It has been shown [30] that with thiourea-based coordinating resins, as the concentration of HCl in the feed solutions increases, the adsorption capacity of iHg is decreased. A similar effect was found in this study when PA12-Thiol was employed with a higher HCl concentration. It seems that the use of thiourea alone (**B** or **C**) in the sample matrix is the most appropriate as both Hg species are retained into PA12-Thiol. Therefore, **B** was used in further studies as this concentration of thiourea in the sample matrix is sufficient for the adsorption of both Hg species. Additionally, it is desirable to keep the total dissolved solids below 0.2% with ICP-MS [31], which also supports the choice of lower concentrations of thiourea. It should be noted that the effluent concentrations were in some cases below the limit of quantification (LOQ) and the obtained Hg concentrations have been used to calculate the adsorption percentages. Otherwise, significantly higher concentrations should have been used in all experiments, which does not reflect the natural state of the environment.

3.2. *Support matrix of the 3D printed metal scavenger*

The possibility of using PA11 in the supporting matrix instead of PA12 was investigated. PA12 is petroleum-derived while PA11 is a bio-based polyamide polymer made from castor oil, making PA11 a more sustainable alternative. The same amount of Thiol was used as a chemically active component in both filters. PA11-Thiol filters proved to be as efficient as PA12-Thiol filters, resulting in quantitative adsorption of both MeHg and iHg. The two polymers differ only by one carbon in the polymer backbone, but PA11 has been shown to have a better fatigue life than PA12 [32] thus being more durable. Due to the similar performance and lower environmental impact, PA11-Thiol filters were used in subsequent studies. Since the preparation of the metal scavengers was performed in the same manner as previously described and the macroscopic structure of PA12-Thiol has already been studied [19], this matter was not further investigated.

3.3. *Optimization of the adsorption procedure*

3.3.1. *Flow rate*

After finding a more sustainable supporting matrix for 3D printed metal scavengers, the effect of different flow rates on MeHg and iHg adsorption was investigated as described in section 2.4.3. It can be seen from Table 2 that iHg was more readily adsorbed than MeHg and the weaker adsorption of MeHg restricts the use of higher flow rates. Therefore, an adsorption flow rate was set at 5 mL min^{-1}.

Table 2
Adsorption (%) of MeHg and iHg at different flow rates using a PA11-Thiol filter.

Flow rate (mL min^{-1})	Adsorption of MeHg (%)	Adsorption of iHg (%)
5	96.1 ± 2.0	100.9 ± 0.7
6	93.7 ± 1.8	99.8 ± 0.7
7	89.4 ± 1.9	101.2 ± 0.4

Generally, SPE methods for preconcentration or speciation of Hg employ a sample flow rate of 1.5–5 mL min^{-1} [23,24,31–41], and the selected sample flow rate of 5 mL min^{-1} is at the higher end of this range. When using one PA11-Thiol filter, the adsorption of MeHg and iHg can be considered near quantitative.

3.3.2. *Improvement of the adsorption of MeHg*

It was examined whether the adsorption of MeHg could be improved by using two PA11-Thiol filters in the same syringe instead of one filter (section 2.4.3). When only one PA11-Thiol filter was used, in two of the six cases, the MeHg concentration of the effluent was above LOD (0.4 ng L^{-1}) but below LOQ (2 ng L^{-1}). By using two PA11-Thiol filters in the same syringe, the effluent concentrations of MeHg were below LOD. As two filters are placed in the same syringe, they are more tightly packed compared to just one filter. In our previous study [19], it was observed that relative standard deviation (RSD) values could also be improved if two filters were used instead of one, so two filters were used in subsequent experiments.

The PA12-Thiol has been shown [19] to be effective in adsorbing iHg with a maximum adsorption capacity of ~220 mg g^{-1} obtained from the Langmuir isotherm model. Because the adsorption capacity was found to be relatively large and far more than adequate for this purpose, and the chemically active component was not changed, the adsorption capacity was not further investigated in this study. In addition, if the adsorption capacity was to be determined, relatively high concentrations (several mg L^{-1} of MeHg) should be treated which is quite hazardous due to the high toxicity of MeHg.

3.4. *Elution experiments*

Acidic thiourea solutions have previously been used to elute iHg and/or MeHg from different adsorbents [19,24–26,35,44]. Krishna *et al.* [25] were able to elute MeHg alone from polyaniline using 10 mL of 0.3% HCl, followed by elution of iHg with 10 mL of 0.02% thiourea in 0.3% HCl. Mladenova *et al.* [23], in turn, used 2 mL of 4 mol L^{-1} HCl for MeHg elution from L-cysteine grafted silica gel and iHg was then eluted with 2 mL of 0.1 mol L^{-1} thiourea in 0.1 mol L^{-1} HCl. The concentration of thiourea and HCl used should be as low as possible considering the restrictions of total dissolved solids and acid concentrations with ICP-MS, while the volumes of sample solution and eluent, as well as the dilution of the sample solution, determine the preconcentration factor to be achieved. Since other studies have been successful in the sequential elution of MeHg and iHg, this approach was further investigated in this study.

3.4.1. *Elution experiments focusing on MeHg*

After optimizing the sample matrix and adsorption flow rate, elution tests were performed as described in preliminary experiments in section 2.5. When using only one PA11-Thiol filter, almost all MeHg could be eluted from the filter with the acidic thiourea solutions used. Unfortunately, also 9–38% of iHg was eluted from the filter (Table S1). To retain iHg more tightly in the 3D printed metal scavenger, two PA11-Thiol filters were used instead of one. This modification had obvious advantages for elution, as can be seen in Table 3. The retention of iHg was significantly increased, while good recoveries were obtained for MeHg. Of the eluents studied, 0.1% thiourea in 3.5% HCl was the only eluent that allowed elution of MeHg to be higher than 90% while retaining over 95% of iHg in the filter, so it was selected as the eluent for MeHg.

3.4.2. *Improvement of the elution procedure*

After MeHg is eluted from the filters, the next step is to elute iHg. In our previous study [19], 0.3% thiourea in 8% HCl was shown to be

Table 3
Recovery (%) of MeHg and iHg in preliminary experiments using two PA11-Thiol filters.

TU (%)	HCl (%)	Recovery of MeHg (%)	Recovery of iHg (%)
0.1	3	88.9	0.6
0.1	3.5	90.3	2.8
0.1	4	92.1	11.1
0.15	2	88.4	1.3
0.2	2	92.1	5.5
0.25	2	94.8	11.8
0.3	2	92.3	18.4

TU = Thiourea.

effective in eluting iHg, so it was also used as an eluent for iHg in this study. Elution of both MeHg and iHg was performed with their optimized eluents. The eluent volumes assessed for MeHg were 3, 5, and 10 mL and for iHg, these were 2, 3, and 5 mL. Elution was performed with a peristaltic pump in the same direction as adsorption. The flow rate of the eluent was increased from 0.5 mL min^{-1} to 1 mL min^{-1} and the washing step was omitted in this experiment. Good recoveries were obtained for MeHg with 10 mL eluent volume even if the flow rate of the eluent was increased as shown in Table 4.

However, unexpectedly low recoveries were obtained for iHg in this experiment. The low recoveries can be explained by the fact that the flow rate of the eluent was in the same direction as adsorption, so the elution was not as efficient if the flow was in the counter-current direction. To enable the separation of MeHg and iHg, the elution of MeHg was performed with a peristaltic pump in the same direction as adsorption, while the elution of iHg was performed counter currently in the following experiments as in our previous study [19].

Since two elution cycles are preferred to a single step [21], elution of MeHg was performed with two 4, 5, or 6 mL aliquots using the selected eluent at a flow rate of 1 mL min^{-1} followed by a washing step with high-purity water. As can be seen from Table 5, the highest recovery of MeHg was obtained with two 5 mL aliquots followed by a washing step with 1 mL of high-purity water.

These results suggest that selective and sequential elution could be performed if MeHg was first eluted from the filter in the same direction as adsorption, whereas the recovery of iHg could be significantly increased if eluted counter-currently [21].

3.5. *Spiking studies*

As the elution procedure described in the previous chapter seemed very promising, spiking studies were performed in different matrices as described in section 2.8. A total concentration of 10 ng L^{-1} of Hg was chosen for the experiments to have a similar concentration with natural water samples, but also to have a high enough concentration to make it possible to analyze Hg directly from the samples for comparison with the preconcentration method. The results are shown in Table 6.

When using high-purity water, an excellent recovery of 97% was obtained. Suitable recoveries (88–97%) were also obtained with natural water samples. The color of the water from both Lake Jyväsjärvi and Päijänne was slightly yellowish while Korkeakoski's water was brown-colored. The matrix of this brown-colored, humic-rich stream water might disturb both adsorption and elution steps, explaining slightly lower recovery. The results are consistent with the obtained total Hg concentrations from the direct ICP-MS analysis, confirming the applicability of the developed method for accurate and precise Hg speciation in natural waters at ultra-trace concentrations.

Table 4
Recovery (%) of MeHg and iHg from individual sample solutions using 0.1% TU in 3.5% HCl for MeHg and 0.3% TU in 8% HCl for iHg.

Eluent volume (mL)	Recovery (%) of MeHg with 0.1% TU in 3.5% HCl	Recovery (%) of iHg with 0.3% TU in 8% HCl
2	–	54.4
3	72.8	86.0
5	82.0	91.3
10	92.8	–

Table 5
Recovery (%) of MeHg using two aliquots of 0.1% TU in 3.5% HCl followed by washing step with high-purity water.

A total volume of the eluent + volume of high-purity water in the washing step (mL)	Recovery of MeHg (%)
8 + 2	74.6
10 + 2	87.3
10 + 1	94.0
12 + 2	90.6

Table 6
Results of the MeHg and iHg spiking studies in different matrices at a total concentration of 10 ng L^{-1}.

Sample	Elution of MeHg (ng L^{-1})	Elution of iHg (ng L^{-1})	Sum of the elution of MeHg and iHg (ng L^{-1})	Initial total Hg concentration (ng L^{-1})	Recovery (%)
High-purity water	5.4	5.0	10.4	10.7	97.4
Jyväsjärvi	5.6	5.9	11.5	11.8	97.2
Päijänne	5.6	7.0	12.6	13.3	94.4
Korkeakoski	5.8	7.6	13.4	15.2	87.9

3.6. *Analytical performance and the analysis of natural water samples*

The analytical performance of the proposed method was evaluated under the optimal experimental conditions. Linear calibration was performed using a blank, 10, 50, 250, and 500 ng L^{-1} of iHg in 0.18% thiourea and 3% HCl with a coefficient of determination higher than 0.999. LOD and LOQ for total Hg determination were estimated as the total Hg concentration corresponding to the average of 10 replicate measurements of the calibration blank plus three or ten times the standard deviation, respectively. The LOD and LOQ values were determined to be 0.4 and 2.0 ng L^{-1}, respectively.

To determine the method detection limits (MDLs) for MeHg and iHg using the speciation procedure, seven calibration blank solutions (500 mL) were taken through the speciation procedure as described in the optimized procedure (section 2.6) [45]. The elution solutions were prepared and analyzed over three non-consecutive days. To calculate the MDL for MeHg and iHg, the standard deviation of the blanks was multiplied by 3.14 (Student's *t*-test value for six degrees of freedom) and the preconcentration factors for MeHg and iHg were considered. The MDLs calculated for MeHg and iHg were 0.02 ng L^{-1} and 0.08 ng L^{-1}, respectively. However, considering the detection limits for total Hg determination, the MDL for MeHg was set at 0.05 ng L^{-1}, obtained by dividing the LOQ for total Hg determination by the preconcentration factor used for MeHg.

The RSD for ten replicate determinations of the QC sample within one day was 2.5%, and within four different days, the precision was 3.1% (n = 24). The obtained total Hg concentration for ERM-CA615 was 39.8 ± 1.3 ng L^{-1} with an RSD of 3.2% (n = 3). When the speciation procedure was utilized, MeHg was determined to be lower than MDL, while the iHg concentration was 41.2 ± 0.5 ng L^{-1} and an RSD of 1.2% (n = 3). The ERM-CA615 is originally acidified with HNO_3 to pH ~2 and spiked with inorganic $Hg(NO_3)_2$ to increase its Hg concentration to about 40 ng L^{-1} with a certified total Hg concentration of 37 ± 4 ng L^{-1}. Good recoveries for ERM-CA615 were obtained with (111.5 ± 1.4%) and without speciation procedure (107.6 ± 3.5%). According to the *t*-

test, no significant differences were found between the Hg concentrations using the speciation procedure and direct ICP-MS determination at the 95% level of confidence.

The proposed preconcentration and speciation procedure was applied to determine the concentrations of MeHg and iHg in lake Jyväsjärvi and Päijänne, as described in section 2.8. The determined MeHg and iHg concentration for lake Jyväsjärvi were 0.18 ± 0.01 ng L^{-1} and 0.62 ± 0.02 ng L^{-1}, respectively. Correspondingly, for lake Päijänne these concentrations were 0.24 ± 0.02 ng L^{-1} and 0.50 ± 0.02 ng L^{-1}. The results presented above demonstrate that the developed method enables Hg speciation at extremely low concentrations. The Hg concentration of the effluent solution was below LOQ in all studied samples.

The detection limits obtained in this study are considerably lower than in other similar solid-phase extraction studies for Hg speciation [23,26,33–35,37,39–43,46], with the exception of Krishna *et al.* [25], who achieved a LOD of 0.05 ng L^{-1} for iHg (Table S2). However, they used a more tedious method involving selective elution of MeHg and iHg from a homemade polyaniline column, UV irradiation for degradation of MeHg to iHg, CV generation, and trapping onto gold collector [25]. In the above-mentioned studies, the off-line preconcentration has also been performed by the flow-through process and the detection limits varied between 0.05 and 4300 ng L^{-1} for MeHg and 0.05–800 ng L^{-1} for iHg. Even lower detection limits have been obtained when online preconcentration has been utilized [3,38]. In addition, HPLC [33,34,37,39,41,42,46] or ion chromatography (IC) [38] have been commonly used to separate Hg species after preconcentration, which is not required in this study, simplifying the process significantly.

In our study, a 495 mL sample volume was chosen due to practical reasons such as ease of sampling and duration of the preconcentration procedure, which only takes about an hour and 40 min. The reusability of PA12-Thiol filters was studied previously [19] and the filters could be reused up to 10 times without a loss of adsorption efficiency. However, to avoid any contamination, only new filters were employed throughout the study. In our previous work [19], the preconcentration procedure is only applicable to iHg species, thus the method developed in this study is a significant improvement over the previous one. The method could further be improved by performing the preconcentration procedure in the field [11]. In this case, the loss of Hg species by evaporation and the risk of contamination from vessels would be minimized as Hg species are bound to the solid phase before transportation.

4. Conclusions

In the present study, a method has been developed and applied for the preconcentration and speciation of Hg in natural water samples at ng L^{-1} level offering an alternative to hyphenated techniques. The sample matrix is adjusted to contain 0.01% thiourea, which allows both iHg and MeHg to be adsorbed into a 3D printed metal scavenger. Selective and sequential elution is achieved by using 0.1% thiourea in 3.5% HCl for MeHg and 0.3% thiourea in 8% HCl for iHg. MeHg and iHg species, as well as the total Hg concentration, can be determined by the same ICP-MS method, which further improves the reliability of the developed method. Relatively high preconcentration factors of 42 for MeHg and 93 for iHg enabled extremely low MDLs of 0.05 ng L^{-1} and 0.08 ng L^{-1}, respectively. A comparison of the developed method with the previously reported solid-phase extraction methods for Hg speciation indicates that the proposed method is simpler than the existing methods and has an extremely low detection limit. The developed method can further help to understand the fate and toxicity of Hg species in aqueous systems.

Credit author statement

Suvi Kulomäki: Conceptualization, Formal analysis, Investigation, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. Elmeri Lahtinen: Conceptualization, Writing – review & editing. Siiri Perämäki: Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing. Ari Väisänen: Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.talanta.2021.123163.

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