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INNOVATIVE ANALYTICAL METHODOLOGIES BASED ON IONIC LIQUIDS TO FACILITATE MERCURY DETERMINATION IN NATURAL WATERS

Gemma Elias Estañol

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Universitat de Girona

Doctoral Thesis

Innovative analytical methodologies based on ionic liquids to facilitate mercury determination in natural waters

Gemma Elias Estañol

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Doctoral program in water science and technology

Supervised by:

Dr. Clàudia Fontàs Dr. Sergi Díez

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Universitat
de Girona

Dr. Clàudia Fontàs of the University of Girona and Dr. Sergi Díez, of the IDAEA-CSIC from Barcelona:

WE DECLARE:

That the thesis titled “Innovative analytical methodologies based on ionic liquids to facilitate mercury determination in natural waters”, presented by Gemma Elias Estañol to obtain a doctoral degree, has been completed under our supervision and meets the requirements to opt for an International Doctorate.

For all intents and purposes, we hereby sign this document.

Dr. Clàudia Fontàs

Dr. Sergi Díez

Girona, May 2nd of 2019

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i al millor company de camí que es pot tenir.

“You can climb a ladder up to the sun,

or write a song nobody has sung,

or do something that’s never been done.”

Talk - *Coldplay*

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LIST OF PUBLICATIONS

The research described in this thesis has result in the publication of two scientific papers:

Elias, G., Marguí, E., Díez, S., Fontàs, C., 2018. Polymer inclusion membranes as an effective sorbent to facilitate mercury storage and detection by X-ray fluorescence in natural waters. Analytical Chemistry 90, 4756 – 4763
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DOI: 10.1016/j.jhazmat.2019.03.017
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LIST OF ABBREVIATIONS

A336-MTBA	Trioctylmethylammoium 2-(methylthio) benzoate
AAS	Atomic absoption spectrometry
AES	Atomic emission spectrometry
AFS	Atomic fluorescence spectrometry
Aliquat 336	Trioctylmethylammonium chloride
AMA	Advanced mercury analyzer
AMP	Ammonium molybdophosphate
ASGM	Artisanal and small-scale gold minig
BA	Benzoate
BBPA	bis (1-butylpentyl) adipate
BEHP	bis (2-ethylhexyl) phthalate
BTS	butyl stearate
CD_3CL_3	Deuterated chloroform
Cel(p)	Cellulose particles
Cel(p)-TOMATS	Cellulose particles impregnated with TOMATS
Chypos IL 101	Tetraalkylphosphonium chloride
CPIPA	Chloro phenyl imino propenyl aniline
CTA	Cellulose triacetate
CVAFS	Cold vapour fluorescence spectrometry
D	Difussion coeficient
D2EHPA	Di-(2-ethylhexyl)phosphoric acid
DBL	Diffusive boundary layer
DBP	Dibutylphtalate
DBS	Dibutylsebacate
DBS	dibutyl sebacate
DC18C6	Dicyclohexano-18-crown-6
DEHA	bis(2-ethylhexyl) adipate
DGT	Diffusive gradient in thin films
DMA	Direct mercury analyzer
DNNS	Dinonylnaphtalene sulfonic acid
DOS	di (2-ethylhexyl) sebacate
EDTA	Ethylendiamine tetraacetic acid

EDXRF	Energy dispersive X-ray fluorescence
EE	Extraction efficieny
ENMF	Electrospun nanofibrus mercury filter
EPA	Environmental protection agency
EQA	Environmental quality assessment
EQS	Environmental quality standards
EtHg	Ethyl mercuri
EU	European Union
F	Flux
FFAT	5-(4-phenoxyphenyl)-6H-1,3,4-thiadizin-2-amine
FPNPE	2-fluorophenyl 2-nitrophenyl ether
GEOS	Goddard earth observing system
GMA	Global Mercury Assessment
GMOS	Global mercury observation system
GW	Groundwater
HEEX	Hexanoate
Hg-P	Hg associated with particulate matter
HSAB	Hard and soft acid and base
IC	Ion chromatgraph
IC	Ion chromatograph
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma optic spectrometry
IES	Ion-selective elèctrodes
IL	Ionic líquid
LLE	Liquid-liquid extraction
LM	Liquid membrane
LOD	Limit of detection
LOQ	Limit of quantification
LPME	Liquid phase micro extraction
MCL	Maximum contaminant level
MeHg	Methyl mercuri
MNPs	Magnetic nanoparticles
MP	Mercaptopropyl

MPTMS	(3-mercaptopropuy) trimethoxycilane
MTBA	2-(methylthio) benzoate
NPPE	Nitrophenyl phenyl ether
NPOE	nitrophenyl octyl ether
NPs	Nanoparticles
PIM	Polymer inclusion membranes
PVA	Polyvinyl alcohol
PVC	Polyvinyl chloride
RSD	Reltive standard deviation
S/N	Signal to noise ratio
SCN	Thiocyanate
SD	Standard deviation
SDD	Silicon drift detector
SDWA	Safe drinking water act
SEM	Scanning electron microscopy
Si(np)	Silicone dioxid nanoparticles
Si(np)-TOMATS	Silicone dioxide nanoparticles impregnated with TOMATS
SNW	Simulated naturall wàter
SOM	Soil organic matter
SPE	Solid phase extraction
TBP	Tributylphosphate
TE	Transport efficiency
TEHP	Tris (2-ethylhexyl) phosphate
T_g	Glass transition temperature
THF	tetrahydrofuran
THg	Total mercuri
T_m	Melting temperature
TOA	Tri-n-octylamine
TOC	Total organic carbon
TOMAS	Triotylmethylammonium salicylate
TOMATS	Trioctylmethylammonium thiosalicylate
TPPS	Triphenylphosphine sulphide
TS	Thiosalicylate

TSIL	Task-specific ionic liquid
TSIL	Task-specific ionil liquid
TWA	Time-weighted average
TXRF	Total reflection X-ray fluorescence
UNEP	United nations environment programme
USA	United States of America
USA DMSPE	Ultrasound-assisted dispersive micro solid-phase extraction
USEPA	United States environmental protection agency
VCM	Vinyl chloride monomer
WDXRF	Wavelenght dispersive X-ray fluorescence
WFD	Water framework directive
WHO	World health organization
XRF	X-ray fluorescence

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ABSTRACT

Water is an indispensable resource for living bodies, not only for consumption but also as a habitat. Since it is a scarce resource, it is important to control its quality. One of the main parameters of concern is the contamination of water by heavy metals such as mercury (Hg). This metal found in water may come from natural sources as well as from anthropogenic sources, such as fossil fuel combustion or urban waste incineration. Although Hg concentration in aquatic systems is usually low, the problem related to this metal is its biomagnification and bioaccumulation along the trophic web. Due to its high toxicity, analytical methodologies are needed to facilitate Hg determination and monitoring in natural waters at low concentration levels.

The investigation presented in this thesis is focused on the design of new analytical methodologies to allow Hg preconcentration using ionic liquids (IL) incorporated in functionalized membranes or impregnated onto solid sorbents.

To carry out this investigation, two ILs, trioctylmethylammonium salicylate (TOMAS) and trioctylmethylammonium tiosalicylate (TOMATS), have been prepared and characterized, and effectively incorporated into polymeric inclusion membranes (PIMs) using cellulose triacetate (CTA) as the polymer. Their extraction capacity has been evaluated. Membranes prepared with TOMATS, which contains a thiol group in its chemical structure, have proven to be highly effective for Hg extraction in different natural waters, without showing matrix effect, and at low concentration levels. This has allowed the development of an analytical methodology using a PIM with TOMATS as a medium for Hg extraction and the subsequent analysis of the membrane by means of Energy Dispersive X-ray Fluorescence (EDXRF). It has been demonstrated that this methodology enables the analysis of water samples at levels required by Council Directive 98/83/EC. It is also worth noting that this investigation has demonstrated that Hg extracted in a PIM is conserved for a long time period. Thus, these membranes are presented as a suitable medium for the preservation of the metal, enabling it to be detected not on immediate analysis without affecting its quality.

In addition, a new methodology has been designed with the PIM containing these ILs, consisting of the preconcentration of the metal present in water samples in a receiving phase of cysteine. This methodology has allowed the analysis of the sample by means of Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and Inductively Coupled

Plasma Optical Emission Spectrometry (ICP-OES), which require aqueous samples. It has been observed that the incorporation of a plasticizer in the PIM composition is necessary in order to allow the transport of Hg to the receiving phase, and different studies on the effect of the plasticizer's characteristics on transport efficiency have been carried out. These studies have demonstrated that plasticizers with characteristics as different as nitrophenyl octyl ether (NPOE) and dibutyl sebacate (DBS), lead to very efficient membranes.

For the first time, a study on the possible biofilm growth onto a PIM surface made of CTA, TOMATS and NPOE has been performed. It has been proven that after seven days being soaked in a natural aquatic environment, the presence of biofilm was extremely scarce and the transport efficiency of the membrane was not affected. This fact is of paramount importance when designing Hg passive sampling systems.

Finally, the possibility of preparing impregnated sorbents with TOMATS for their use as a binding phase in samplers based on the Diffusive Gradient in Thin Films (DGT) technique has been studied in this thesis. Thus, two kinds of sorbents have been evaluated; one of an organic nature such as cellulose, and another inorganic one such as silica dioxide nanoparticles. Both materials have allowed impregnation and the obtaining of materials easy to manipulate and highly efficient for Hg extraction. Also, it has been proven that their inclusion in an agarose gel matrix did not affect their extraction efficiency and Hg elution was achieved using a cysteine solution. Last but not least, new DGT devices have been prepared using agarose as a diffusive phase and a new binding phase consisting of silica dioxide nanoparticles impregnated with TOMATS, which can be employed for Hg monitoring in natural aquatic systems.

RESUM

L'aigua és un recurs fonamental pels éssers vius, no només pel seu consum sinó també perquè és l'hàbitat de molts d'ells. Atès a que és un bé escàs, és important controlar la seva qualitat. Un dels paràmetres més preocupants és la contaminació d'aigües per metalls pesants tals com el mercuri (Hg). Aquest metall és pot trobar a l'aigua tant de manera natural com degut a activitats humanes resultat de la combustió de combustibles fòssils o la incineració de residus urbans, entre d'altres. Malgrat les concentracions de Hg en sistemes aquàtics són molt baixes, aquest metall presenta la problemàtica que es bioacomula i biomagnifica a la xarxa tròfica. Degut a la seva alta toxicitat, és necessari disposar de metodologies analítiques que facilitin la seva determinació i monitoratge en mostres d'aigua natural i a baixos nivells de concentració.

La investigació que es presenta en aquesta tesi es centra en el disseny de noves metodologies analítiques per permetre la preconcentració de Hg utilitzant líquids iònics (LI) incorporats a membranes funcionalitzades o impregnats en sorbents sòlids.

Per dur a terme aquesta investigació s'han preparat i caracteritzat dos LI, el salicilat de trioctilmetil amoni (TOMAS) i el tiosalicilat de trioctilmetil amoni(TOMATS), els quals s'han incorporat de manera efectiva en membranes d'inclusió polimèrica (PIMs) utilitzant el polímer triacetat de cel•lulosa (CTA) i s'ha avaluat la seva capacitat d'extracció per Hg. Les membranes preparades amb TOMATS, que conté un grup tiol en la seva formulació, han mostrat ser molt efectives per l'extracció de Hg en diverses aigües naturals, sense efecte de la matriu, i a nivells baixos de concentració. Això ha permès desenvolupar una metodologia analítica utilitzant les PIM amb TOMATS com a medi d'extracció de Hg i realitzar el posterior anàlisi de la membrana per espectroscòpia d' Energia Dispersiva de Fluorescència de Raigs-X (EDFXR). S'ha demostrat que aquesta metodologia permet realitzar anàlisi de mostres d'aigua als nivells requerits per la Directiva 98/83/EC. És de destacar també que fruit d'aquesta investigació s'ha demostrat que el Hg extret en una PIM es manté inalterat amb el pas dels temps. Per això, es presenten aquestes membranes com un medi adequat per a la preservació del metall, fet que pot permetre realitzar la seva detecció no de forma immediata sense que això afecti a la qualitat de l'anàlisi.

Utilitzant també PIMs amb els LI s'ha dissenyat una metodologia que consisteix en una preconcentració del metall present en mostres d'aigua a una fase receptora de cisteïna.

Aquesta metodologia permet l'anàlisi de la mostra amb tècniques d'Espectroscòpia de Masses per Plasma d'Acoblament Inductiu (ICP-MS) o d'espectroscòpia Òptica per Plasma d'Acoblament Inductiu (ICP-OES), les quals requereixes mostres aquoses. S'ha observat que la incorporació de plastificant a la PIM és necessària per tal de permetre el transport a la fase receptora, i s'han fet estudis sobre les característiques del plastificant incorporat a la PIM en l'eficiència de transport de Hg. Els estudis han demostrat que plastificants amb característiques ben diferents com són nitrofenil octil éter (NPOE) i dibutil sebacat (DBS) , donen lloc a membranes molt eficients.

Per primera vegada s'ha realitzat un estudi sobre el possible creixement de biofilm a la superfície de les PIM preparades amb CTA, TOMATS i NPOE, i s'ha comprovat que en set dies d'estar submergida en un ambient aquàtic natural la presència de biofilm era molt escàs i que no afectava a l'eficiència de transport de la membrana. Aquest fet és de rellevància a l'hora de dissenyar sistemes de mostreig passiu per a Hg.

Finalment, en aquesta tesi s'ha estudiat la possibilitat de preparar sorbents impregnats amb TOMATS per a ser utilitzats com a base extractant en dispositius de mostreig basats en la tècnica de Diffusive Gradient in Thin films (DGT). Per això s'han avaluat dos tipus de sorbents, un de naturalesa orgànica, com és la cel•lulosa, i un d'inorgànic, nanopartícules de diòxid de silici. Ambdós materials han permès la seva impregnació i l'obtenció de materials de bon manipular i altament eficients per a l'extracció de Hg. També s'ha comprovat que la seva inclusió en una matriu de gel d'agarosa no afectava a l'eficiència d'extracció i que l'elució del Hg extret s'aconseguia utilitzant una dissolució de cisteïna. Finalment, s'han preparat de forma efectiva nous dispositius DGT utilitzant agarosa com a fase de difusió i la nova fase extractant formada per nanopartícules de diòxid de silici impregnades amb TOMATS que poden ser emprats pel monitoratge de Hg en sistemes aquàtics naturals.

RESUMEN

El agua es un recurso fundamental para los seres vivos, no solo por su uso sino también porque es el hábitat de muchos de ellos. Debido a que es un bien escaso, es de vital importancia controlar su calidad. Uno de los parámetros más preocupantes es la contaminación de aguas por metales pesados tales como el mercurio (Hg). Este metal se puede encontrar en el agua de manera natural o como resultada de actividades humanas tales como la combustión de combustibles fósiles o la incineración de residuos urbanos, entre otros. Aunque las concentraciones de Hg en sistemas acuáticos don muy bajas, este metal presenta asociada la problemática que se bioacomula y biomagnifica en la red trófica. Debido a su alta toxicidad, es necesario disponer de metodologías analíticas que faciliten su determinación y monitoreo en aguas naturales y a bajos niveles de concentración.

La investigación que se presenta en esta tesis se centra en el diseño de nuevas metodologías analíticas para permitir la preconcentración de Hg utilizando líquidos iónicos (LI) incorporados en membranas funcionalizadas o impregnados en sorbentes sólidos.

Para llevar a cabo esta investigación se han preparado y caracterizado dos LI, el salicitato de trioctilmetil amonio (TOMAS) y el tiosalicilato de trioctilmetil amonio (TOMATS), los cuales se han incorporado de manera efectiva en membranas de inclusión polimérica (PIMs) utilizando polímero triacetato de celulosa (CTA) y se ha evaluado su capacidad de extracción de Hg. Las membranas preparadas con TOMATS, que contiene un grupo tiol en su formulación química, han demostrado ser muy efectivas para la extracción de Hg en diversas aguas naturales, sin ser afectados por la matriz, y a niveles bajos de concentración. Esto ha permitido desarrollar una metodología analítica utilizando PIMs con TOMATS como medio de extracción de Hg y realizar su posterior análisis con espectroscopía de Energía Dispersiva de Fluorescencia de Rayos-X (EDXRF). Se ha demostrado que esta metodología permite realizar análisis de muestras de agua a niveles requeridos por la Directiva 98/83/EC. Es también de destacar que fruto de esta investigación se ha demostrado que el Hg extraído en una PIM se mantiene inalterado con el paso del tiempo. Por tanto, se presentan estas membranas como un medio adecuado para la preservación de este metal, hecho que puede permitir realizar su detección no de forma inmediata sin que este hecho afecte a la calidad del análisis.

Utilizando también PIMs con los LI, se ha diseñado una metodología que consiste en una preconcentración del metal presente en muestras de agua en una fase receptora de cisteína. Esta metodología permite el análisis de la muestra con técnicas de Espectroscopía de Masas por Plasma de Acoplamiento Inductivo (ICP-MS) o de Espectroscopía Óptica por Plasma de Acoplamiento Inductivo (ICP-OES), las cuales requieren muestras acuosas. Se ha observado que la incorporación de plastificante en la composición de la PIM es necesaria para permitir el transporte a la fase receptora, i se han realizado estudios sobre las características del plastificante incorporado a la PIM en la eficiencia de transporte de Hg. Los estudios has demostrado que plastificantes con características tan diferentes como son el nitrofenil octil éter (NPOE) y el dibutil sebacato (DBS), dan lugar a membranas muy eficientes.

Por primera vez se ha realizado un estudio sobre el posible crecimiento de biofilm en la superficie de PIMs preparadas con CTA, TOMATS y NPOE, y se ha comprobado que en siete días de inmersión en un ambiente acuático natural la presencia de biofilm era muy escasa y que no afectaba a la eficiencia de transporte de la membrana. Este hecho es de relevancia a la hora de diseñar sistemas de muestreo para Hg.

Finalmente, en esta tesis se ha estudiado la posibilidad de preparar sorbentes impregnados con TOMATS para ser utilizados como base extractante en dispositivos de muestreo basados en la técnica de *Diffusive Gradien in Thin films* (DGT). Para ello se han evaluado dos tipos de sorbentes, uno de naturaleza orgánica como es la celulosa, y uno de inorgánico como las nanopartículas de dióxido de silicio. Ambos materiales han permitido su impregnación y la obtención de materiales fáciles de manipular y altamente eficientes para la extracción de Hg. También se ha comprobado que su inclusión en una mtriz de gel de agarosa no afectaba a la eficiencia de extracción y que la elución de Hg se conseguía utilizado una disolución de cisteína. Finalmente, se han preparado de forma efectiva nuevos dispositivos DGT utilizando agarosa como fase de difusión y la nueva fase extractante formada por nanopartículas de dióxido de silicio impregnadas con TOMATS que pueden ser usados para el monitoreo de Hg en sistemas acuáticos naturales.

Chapter 1

General introduction

1.1. Mercury

1.1.1 Occurrence and physicochemical properties

Mercury (Hg) is a heavy metal naturally occurring in the Earth's crust that can be found at concentrations of about 0.05 µg g^{-1} in the upper continental crust [1]. It has been known and used since remote times and its name and abbreviation proceed from the Latin name for the Greek *hydragyros* (liquid silver) [2,3]. Its principal ore mineral, cinnabar (HgS), was used as a red pigment (vermillion) by different antique civilisations, such as the Egyptians and Romans, to decorate tombs or villas respectively, and even in China to prepare red ink. Hg has been used historically not only as a pigment but also in art, alchemy, cosmetics, in gold and silver amalgamation, as a medicine and even as a poison. It has also been widely used in recent centuries in scientific, agricultural, industrial and medical applications. Probably the most widely-known application of Hg was in the development of the barometer in 1643 by Torricelli and the Hg thermometer, innovated by Fahrenheit in 1714. Although currently banned, Hg was also used in agricultural chemicals.

Among the physicochemical properties of Hg, its atomic number is 80 and its atomic weight, 200.59. Hg is shiny and a silver-white colour, it belongs to group 12 in the periodic table and is characterized by being the only metal in liquid state at ambient temperature and pressure. It is a highly dense metal (density at 20ºC is 13.55 g mL^{-1}), with the lowest melting point among metals (-38.83 ºC) while its boiling point is 356.61 ºC [2].

Hg presents seven stable isotopes (^{196}Hg, ^{198}Hg, ^{199}Hg, ^{200}Hg, ^{201}Hg, ^{202}Hg, ^{204}Hg), the most abundant being ^{202}Hg (29.86%), and 35 unstable isotopes [2]. Its properties and behaviour depend on its oxidation state which mainly include: elemental or metallic mercury (Hg(0)), monovalent mercury (Hg(I)) and divalent mercury (Hg(II)) [3]. In general, elemental mercury is the dominant form of Hg in the atmosphere, whereas the inorganic Hg(II) species is the most common in water, soil and sediments and the organic form of methylmercury (MeHg) is dominant in biota [4].

Hg is the most hydrolyzed among the alkaline earth metal ions. Its high polarizability as well as its high electronegativity, lead to an increase in the covalence of the M-O bonds and, consequently, almost every Hg compound goes through covalent bonds [5,6]. According to the theory of hard and soft acids and bases (HSAB theory), Hg is classified as a soft Lewis acid and therefore forms strong bonds with soft base ligands, such as N-, P- and S-donor ligands, with which Hg forms complexes the stabilities of which are rarely exceeded by those of any other divalent cation [6,7]. The highest affinity is for S, presenting high insoluble and inert compounds such as HgS [7]. It also forms conventional ammine and amine complexes but it also directs bonds covalently to nitrogen by the displacement of hydrogen [7]. The solubility in water of Hg halides complexes decreases when the molecular weight increases, and the stability of the complexes follows the order $Cl^- < Br^- < I^-$, with little or no tendency to form fluoride complexes [6,7].

1.1.2 Emission sources

It is estimated that the Hg global annual input to the atmosphere is around 5000-6000 t per year^{-1} [4]. Hg has different pathways to enter into the ecosystem and can be emitted from natural or anthropogenic sources [8]. An overview of the current global mercury emissions sources and the associated uncertainties are represented in Figure 1.1. It is important to remark that estimations of Hg emissions are expected to be reassessed during 2019, since the Global Mercury Assessment (GMA) 2013 is due to be updated for this year.

Figure 1.1. Sources of mercury emissions to the ecosystem (UNEP 2008) [8]**.**

Natural sources

The different natural sources that release Hg to the environment are basically geological activities (coming from volcanic and geothermic activity), volatilization of Hg from marine environments and Hg emission from terrestrial environments, such as natural erosion of Hg enriched soils [4]. An estimation of a global mercury emission by natural sources in a year is represented in Table 1.1. It is complicated to determine precisely natural Hg emissions due to the large uncertainties associated with these reported sources, and because it is difficult to distinguish primary emissions from re-emissions, which can proceed from natural and anthropogenic sources [4,9]. The Global Mercury Observation System (GMOS) has estimated that natural emissions (including re-emissions) amount to about 5207 t year^{-1} [10,11], but this value has been studied over years ranging from 3600 to 6200 t year^{-1} [12]. Of this total, 80-600 t year^{-1} correspond to primary natural sources [13].

Table 1.1. An estimation of global mercury emissions from natural sources for 2008 [11].

Source	Hg (tonnes yr^{-1})	Contribution (%)
Oceans	2682	52
Lakes	96	2
Forests	342	7
Tundra/Grassland/Savannah/Prairie/Chaparral	448	9
Desert/Metalliferous/Non-vegetated zones	546	10
Agricultural areas	128	2
Evasion after mercury depletion events	200	4
Biomass burning	675	13
Volcanoes and geothermal areas	90	2
TOTAL	5207	100

Anthropogenic sources

It is estimated that 1540 Gg of Hg have been released into the environment from human activities, 73% of which was emitted after 1850 [14], associated with the industrial revolution [15]. Nowadays, Hg emission from anthropogenic sources is estimated to be approximately 2000 tonnes/year [16]. Anthropogenic emissions can be divided into primary and secondary sources.

Primary sources refer to those with geological origin, where Hg is directly mobilized from deep mineral reservoirs to the environment [17]. The principle sources of this group are those in which mercury is emitted as a `by-product', such as mining, extraction and fossil fuel combustion, cement production and waste incineration [10,15,18].

Since coal is relatively rich in Hg compared to other fossil fuels, its combustion directly implies important emissions of geologically sequestered Hg to the atmosphere [18]. A high degree of uncertainty is associated with the estimation of Hg emission from coal burning due to the wide variation of Hg contained in coal [15].

Secondary anthropogenic sources are those in which mercury is used intentionally, including mercury use in industrial processes, waste from consumer products, dental applications and artisanal and small-scale gold mining (ASGM) [10,15]. Although the industrial use of Hg has been largely reduced in developed countries, it is still significantly contributing as a secondary source in developing areas [4].

ASGM practices consist of using mercury to create an amalgam to separate gold from other minerals or alluvial deposits, and then heating it to recover the gold. This process is also associated with a large number of uncertainties because it is commonly carried out in unregulated or even illegal conditions [15].

Concerning industrial processes, its major use is in the chlor-alkali industry, where mercury-cell technology is used in the electrolytic production of chlorine and caustic soda. Hg is also used as a catalyst in the industrial production of vinyl chloride monomer (VCM) from acetylene, especially in China [15].

It is considered that stationary fossil fuel combustion, mainly coal combustion, is the most important source of Hg emissions to the environment (800-900 Mg y^{-1}) followed by ASGM (400 Mg y^{-1}), but UNEP's global Hg assessment estimated ASGM emissions higher than 700 Mg y^{-1} due to unregulated and illegal ASGM in some countries [16].

The global distribution of anthropogenic emissions to the air is represented in Figure 1.2, whereas its influence by industrial sector is depicted in Table 1.2.

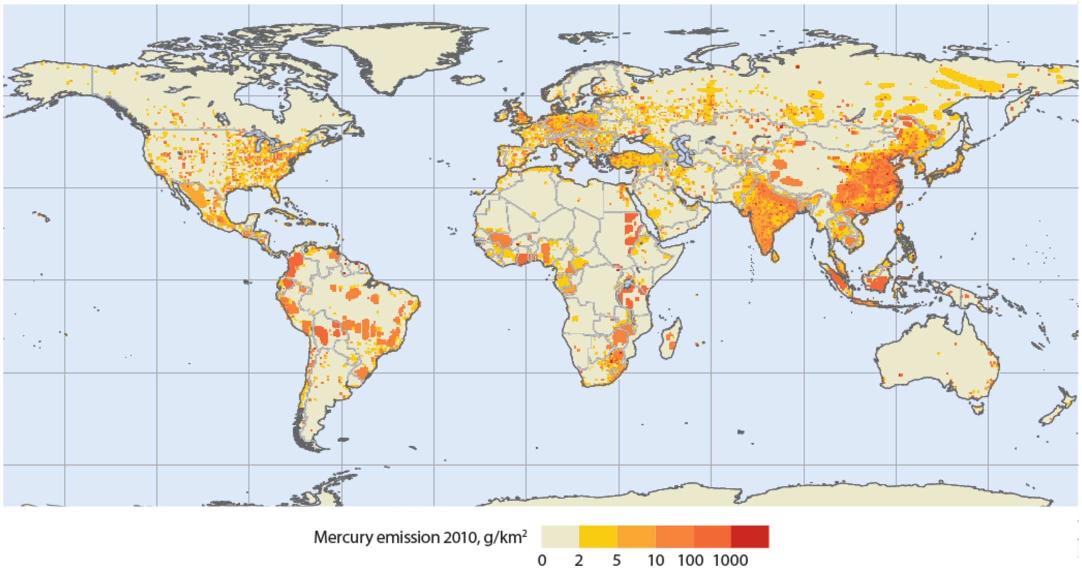


Figure 1.2. Global distribution of anthropogenic emissions to air in 2010 (UNEP, 2013) [15].

Table 1.2. Emissions from various sectors in tonnes per year (mean value and range), and as a percentage of total anthropogenic emissions [15].

Sector	Emission (range) (Mg yr^{-1})	Contribution (%)
By-product or unintentional emissions		
Fossil fuel burning		
Coal burning (all uses)	474 (304 - 678)	24
Oil and natural gas burning	9.9 (4.5 - 16.3)	1
Mining, smelting & production of metals		
Primary production of ferrous metals	45.5 (20.5 - 241)	2
Primary production of non-ferrous metals	193 (82 - 660)	10
Large-scale gold production	97.3 (0.7 - 247)	5
Mine production of Hg	11.7 (6.9 - 17.8)	<1
Cement production	173 (65.5 - 646)	9
Oil refining	16 (7.6 - 26.4)	1
Contaminated sites	82.5 (70 - 95)	4
Intentional uses		
Artisanal and small-scale gold mining	727 (416 - 1040)	37
Chlor-alkali industry	28.6 (10.2 - 54.7)	1
Consumer product waste	95.6 (23.7 - 330)	5
Cremation (dental amalgam)	3.6 (0.9 - 11.9)	<1
Grand Total	**1960 (1010 -4070)**	**100**

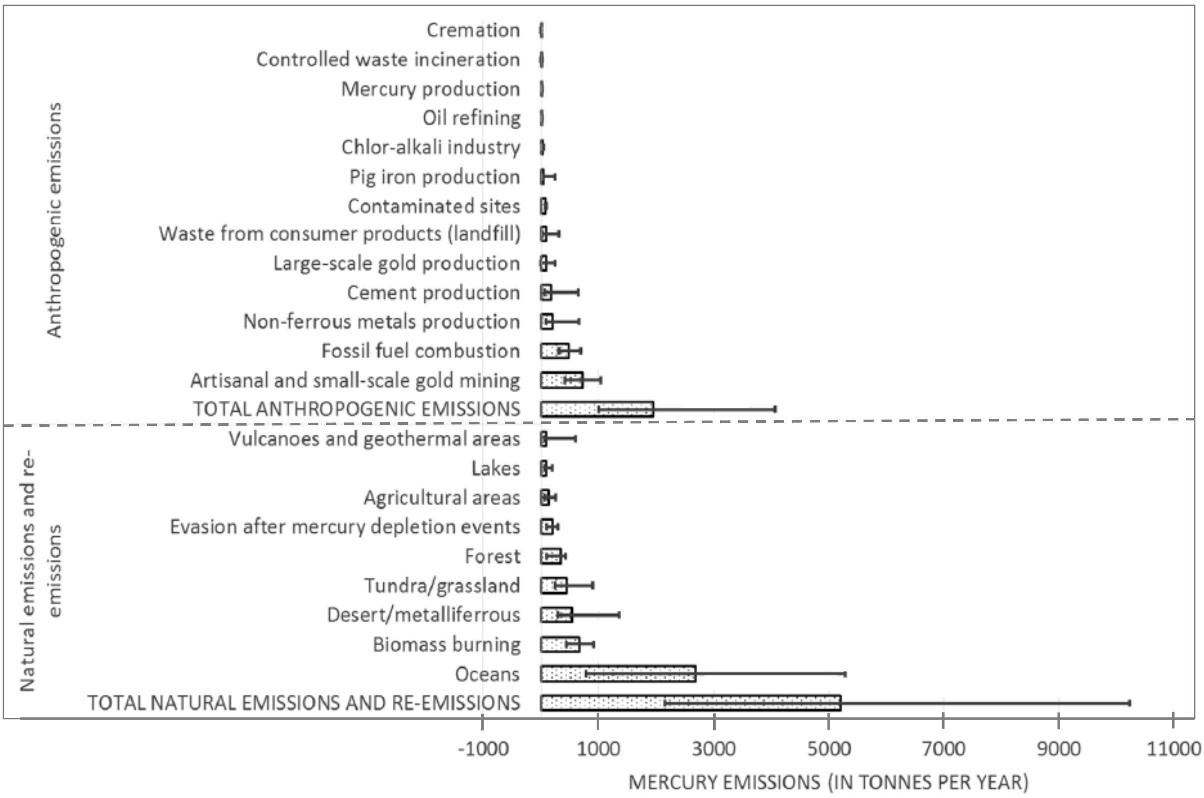
Re-emission and remobilization

Mercury remobilization occurs when mercury accumulated or deposited on soils, which has previously been taken out of circulation, is released again. For example, rain or floods can remobilize the Hg accumulated in soils into the aquatic system [15]. Re-emission is a result of a natural process that converts inorganic and organic forms of mercury to elemental mercury which is emitted back to the air, such as mercury taken up by vegetation that can be re-emitted during a forest fire or biomass burning.

Although it is difficult to distinguish if re-emitted mercury has a natural or anthropogenic origin [15], it is estimated that legacy anthropogenic Hg, previously deposited and subsequently re-emitted, accounts for 60% of present-day atmospheric deposition [17].

It is likely that all the kinds of emission sources (anthropogenic, natural and re-emitted) have large associated uncertainties, which make these amounts difficult to accept for decision-making purposes. The uncertainties associated with each emission source are represented in Figure 1.3. Although the most recent global emission inventories are complete and accurate regarding some anthropogenic sources, the need still exists to improve inventories for waste incineration and ASGM, as well as for total natural and re-emitted emissions. In addition, the chemical and physical behaviour of mercury in the atmosphere needs to be enhanced since it affects its scale and range of transport.

Figure 1.3. Global sources of Hg with associated uncertainties [19].



1.1.3 Mercury in the environment: Hg cycle

Although Hg is a naturally occurring element in the Earth’s crust, it has been directly mobilized by humans into atmospheric, aquatic and terrestrial systems [13]. The continuous flux of this element between the different environmental compartments is called the mercury cycle, and is represented in Figure 1.4. This cycle is based on Hg behaviour in the different media, the implied chemical reactions, and also on transport parameters and final destination [20,21]. The biogeochemical cycle of mercury begins with its emission to the air, then passing to the aquatic or terrestrial ecosystem by means of wet and dry deposition [13]. Once in the aquatic ecosystem, the metal is taken up by living organisms and introduced into the food chain [22]. The Hg cycle is likely to be profoundly altered by global changes, such as climate, land use and Hg emissions, which are dependent on choices made now (and in the future) in policies on Hg emission mitigation and climate change [16].

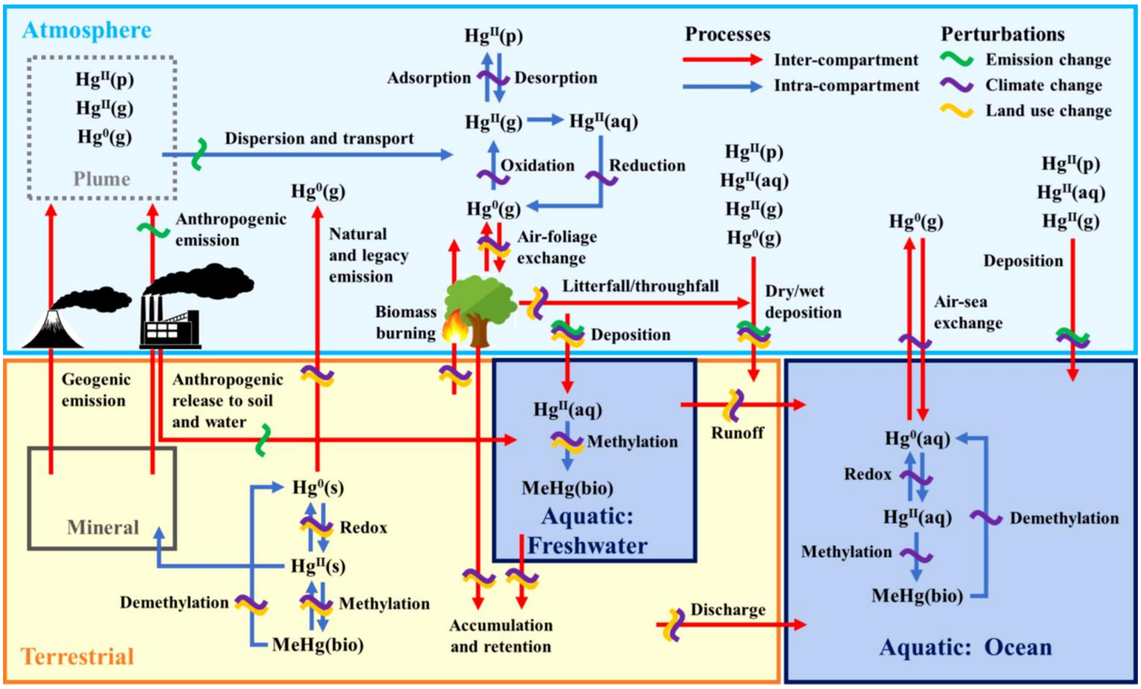


Figure 1.4. The Hg cycle and its critical processes of global importance, including perturbations and processes between environmental compartments, and within the same one. Figure obtained from [16].

Atmospheric cycle

The atmosphere is the foremost transport pathway of Hg emissions and there are four basic processes involved in this atmospheric cycle: emissions, transport, chemistry reaction and deposition [13]. Emissions may originate in natural or anthropogenic sources. The first include Hg volatilization from land and ocean surfaces and emissions from geological sources, and are considered to be mainly in the form of Hg(0) [20]. However, anthropogenic sources, including industrial processes, such as fuel combustion or mining, can emit mercury in the form of a gaseous ionic divalent (Hg(II)), or associated with particulate matter (Hg(P)) [23].

The temporal and spatial scales of Hg transport in the atmosphere and its transfer to the other ecosystems depend on its chemical and physical form [13]. The atmospheric residence time of Hg(0) is from several months to years, while Hg(II) and Hg(P) is from hours to days [24]. This behaviour associates emitted Hg(II) with local Hg deposition while Hg(0) is related to global scale pollution [13]. Although Hg is well mixed in the atmosphere, concentrations are commonly higher in the Northern Hemisphere because the majority of emission sources are located there [25] and also because Hg(0) transport between hemispheres is not really efficient in contributing to the deposition of the pollutant in the same hemisphere [26].

Hg(II) and Hg(P) can be removed from the atmosphere and deposited in ecosystems through dry and wet depositions because they are water-soluble [20,27]. In contrast, elemental mercury presents lower solubility and is removed upon oxidation to ionic Hg and subsequent deposition [13,20], but there is considerable uncertainty about the chemical species involved [13]. It was commonly considered that the most important Hg(0) oxidant was hydroxide (OH) or ozone (O_3) [28–30] , but in recent years it has been shown that these reactions are unlikely to play a significant role in mercury oxidation in the atmosphere [31–33]. Bromine (Br) has also been considered as one of the main oxidants for Hg (0) in the free troposphere [27] and in the marine boundary layer [34], but more recent studies suggest that this assumption requires further evaluation [35]. The proposed chemical reduction pathways in the aqueous phase of the atmosphere are the sulphate-mediated reduction of Hg and photo reduction of mercury(II) hydroxide ($Hg(OH)_2$), but recent observations and model studies also support that the reduction of Hg-organic complexes may play an important role in the atmospheric cycle of Hg [9]. Also, Hg oxidation chemistry has been updated in recent years in the Goddard Earth Observing System(GEOS)-Chem model, describing in-cloud Hg(II) photoreduction as potentially important in Hg redox chemistry [36]. A general scheme of the Hg atmospheric cycle, including gaps in knowledge, is represented in Figure 1.5.

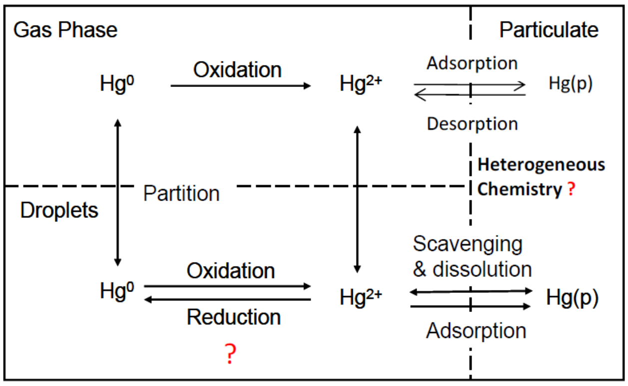


Figure 1.5. Atmospheric Hg chemistry. The question mark indicates some major knowledge gaps in Hg cycling in the environment. Scheme obtained from [9].

So, nowadays, a discrepancy still exists between measured and theoretical atmospheric mercury concentrations due to the combination of different factors including (but not limited to) uncertainties in Hg measurement, knowledge gaps in theoretical and modelling studies and the requirement of more precise emission inventories [9,37].

Terrestrial cycle

As stated before, mercury occurs naturally in the Earth's crust in low concentrations, around 0.5 $\mu g\ g^{-1}$ [38]. However, this concentration can be higher in certain compartments when Hg is released from natural processes, such as hot springs or volcanic explosions [39]. Commonly, the average ranges from 0.001 – 1.5 mg/kg depending on the soil's properties and proximity to an emission site, but several studies have determined higher Hg concentrations in soils adjacent to contaminated sources [40]. It is remarkable that anthropogenic activities have doubled Hg storage in soils [17].

Hg is mainly transported to the land surface from wet and dry deposition of atmospheric mercury [39] but it has been shown recently that a dominant pathway is also through litterfall [16]. After deposition, a considerable portion of the Hg deposited on land is evaded back to the atmosphere in its elemental form, which can be subjected again to processes of transport, oxidation or deposition [13]. The remaining fraction is incorporated into the soil, and undergoes different chemical reactions which will depend on soil properties and characteristics [39]. Since Hg presents high affinity to soil organic matter (SOM), sulphide anions and minerals, it rarely occurs as free Hg(II) in soils [39]. This fact implies that soils with low concentration of SOM, such as sandy soils, leach more Hg into groundwater [41].

The small portion of free Hg(II) in soils is available for soil microorganisms, which are mainly responsible for its reduction to Hg(0) and its subsequent volatilization and return to the atmosphere [39]. Soil microorganisms can also transform Hg(II) to organo-mercury compounds such as MeHg which can subsequently be concentrated and bioaccumulated in the food chain [13,39].

Hg can be readily uptaken by plants and introduced in the food chain [42]. It is basically taken up from the atmosphere through above-ground parts, while the Hg uptaken from roots is lower [43]. Hg present in vegetation returns to the soil via throughfall [16].

Aquatic cycle

Atmospheric deposition, direct release from Hg-containing effluents and runoff from watershed containing atmospheric Hg, are the main sources of Hg in freshwater ecosystems [16], whereas atmospheric deposition is the main route of Hg input to oceanic waters [13]. The predominant Hg species in aquatic environments are Hg(0), Hg(II) (which is also found bound with chloride, sulphides and organic acids), organic Hg (MeHg, or ethylmercury (EtHg)), Hg(P) and colloidal Hg [21]. Figure 1.6 represents the general mercury cycle in aquatic systems whereas the typical total Hg concentration ranges of dissolved mercury species in uncontaminated waters are depicted in Table 1.3. Hg(II) reduction to Hg(0), and its subsequent evasion back to the atmosphere, is one of the most important routes of Hg loss in aquatic ecosystems [20,21,44]. Indeed, around 75% of deposited Hg in freshwater and oceans is lost from these ecosystems by this pathway [44]. Inorganic forms of Hg can also be converted to MeHg, which is of a particular concern due to its efficient trophic transfer through the aquatic food web [45].

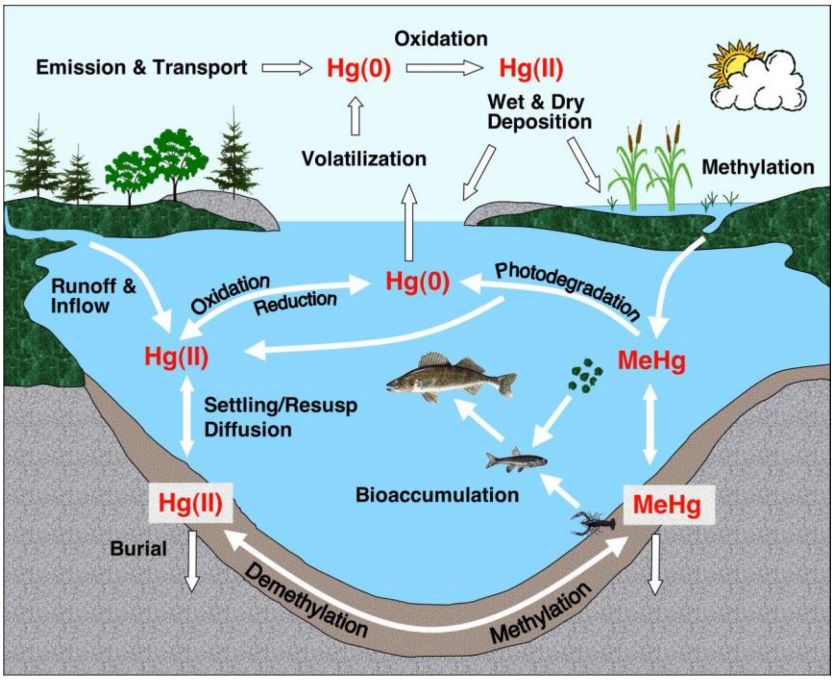


Figure 1.6. Mercury cycle in a lake and its watershed [22].

Table 1.3. Typical total mercury concentration ranges of different dissolved mercury species in uncontaminated natural waters [46].

Water	Total [Hg] (ng L^{-1})
Open ocean	0.03 - 3.24
Mediterranean Sea	0.05 - 1.8
Black Sea	0.36 - 2.37
Estuarine and coastal waters	0.1 35
River	1.0 - 5.0
Lake	0.2 – 80
Rain	5.0 – 90
Groundwater	0.1 – 16

Microbial production is the main origin of MeHg in wetlands and lakes, generally producing more amounts in surface sediments than in the water column [16]. However, biological methylation of mercury in the water column is also an important source [47]. In marine environments, MeHg is mainly produced in sediments [48] and the upper ocean [13] but there is also evidence of its production in the water column [49]. The rate of MeHg production is controlled by different environmental parameters, such as temperature, pH, hydrology, redox conditions, the presence of labile organic carbon and the supply of Hg(II) in an available form [13,16]. This microbial process, in anoxic sediments, is largely regulated by sulphate-reducing bacteria, which are also the most studied inorganic Hg methylators [21,50]. Iron-reducing bacteria [51] and methanogens [52] are also involved in MeHg production.

MeHg can be demethylated by an inverse process, demethylation. This transformation can be biotic, facilitated by microorganisms, or abiotic, including photodegradation [46]. Biological demethylation can be oxidative, involving methanogenic and sulphate-reducing bacteria presumably degradating MeHg to carbon dioxide (CO_2), methane (CH_4) and Hg(II), or reductive , carried out by a system of enzymes (organomercury lyase and mercury reductase) that catalyse demethylation producing Hg(II) and $CH_{4,}$ and its subsequent Hg reduction to Hg(0) [48]. The oxidative degradation pathway seems to predominate in less contaminated ecosystems, whereas reductive degradation seems to be more common in polluted environments [53]. In contrast, abiotic demethylation is caused by the photodegradation of MeHg in superficial oxic waters in lakes and seas [21,54].

Although Hg(II) and MeHg can be efficiently uptaken by living organisms [21], only the organic form has the capacity to be biomagnified through the food web, which means that Hg concentration in a consumer is higher than in its prey [21,55]. Indeed, bioaccumulation and biomagnification can lead to Hg concentrations 10^6 times higher in predator species than in species at the bottom of the food chain[45], and transfer efficiency from the bottom to top predator is determined by the structure of the food web involved [21]. It has been observed that biomagnification increases with latitude, a phenomenon related to the combination of interdependent parameters associated with temperature [45]. Although Hg(II) presents high cell toxicity [56], MeHg can also bound thiol (-SH) groups in subcellular constituents due to its high affinity to this group, attenuating or even blocking their correct function [57] . Recent research has demonstrated that the abiotic photochemical formation of HgS in surface water diminishes Hg bioavailability in the food web [58].

1.1.4 Legal regulation in water

Water policy and regulation exist as major tools in environment protection. Although the atmosphere is mainly responsible for transporting Hg emissions, aquatic ecosystems are the drivers of the main human exposure to MeHg via the trophic chain [13]. Thus, Hg levels in aquatic systems need to be regulated.

In this context, on 23rd October 2000, Directive 200/60/EC of the European Parliament and the Council was approved establishing a framework of community actions regarding water policies under the name of Water Framework Directive (WFD). The main objective of this European Union Directive is to ensure a quality and ecological improvement and also the protection of the different water bodies (including ecosystems linked to them) by the year 2021. In 2008, this Directive was amended by Directive 2008/105/EC of the European Parliament and the Council on environmental quality standards (EQS) in the field of water policy, where environmental quality standards for "33 priority substances" were established, including Hg and its compounds. However, both Directives were amended in 2013 by Directive 2013/39/EC, in which the list of priority substances was replaced and limits on concentrations in surface waters were established for 45 substances. Concerning Hg, the Member States should ensure a maximum allowable concentration of 0.07 µg L^{-1} in inland surface waters and other surface waters. The EQA of Hg in biota was also established at 20 µg Kg^{-1} of weight.

The WFD allows all the aspects contained therin to be transposed to each Member State's legislation. Accordingly, in 2011, the Spanish Royal Decree 60/2011 on EQS was approved, maintaining the maximum allowable concentration of Hg in surface water and in biota (0.07 µg L^{-1} and 20 µg Kg^{-1} respectively). In 2015, it was updated by Royal Decree 817/2015 in which the EQS of Hg concentration in surface waters and biota were maintained.

Hg was not mentioned by the International Standards for Drinking Water in its first version in 1958. It was not taken into account until 1971, when the upper concentration limit of total Hg was established at 1 µg L^{-1}. In 1993, the World Health Organization (WHO) proposed some European standards for different chemical compounds in drinking water, retaining 1 µg L^{-1} for total Hg. Five years later, in 1998, Directive

98/83/EC on the quality of water intended for human consumption, established a maximum allowable total Hg concentration in drinking water of 1 µg L^{-1} in all Member States. In 2008, the WHO proposed another guideline level of 6 µg L^{-1} of inorganic Hg instead of total Hg, which it is still the currently accepted guideline value [59]. It was claimed that direct risk of the intake of organic mercury compounds as a result of the ingestion of drinking water was unlikely since the form found in drinking water is inorganic mercury.

In the case of the United States of America (USA), the federal legislation controlling drinking water quality is the Safe Drinking Water Act (SDWA), which is implemented by the United States Environmental Protection Agency (USEPA). The maximum contaminant level (MCL) allowed for inorganic Hg in drinking water is 2 µg L^{-1}.

1.1.5 Adverse health effects

Mercury is a global threat to human health for its toxicity. Indeed, it symptoms largely differ depending on the exposure pathway and also on its chemical form: elemental, inorganic or organic mercury [60,61].

Elemental mercury

Elemental mercury generally enters into the atmosphere in a vaporized state and can be quickly absorbed by inhalation [57]. Although inhalation is the most common pathway for vapour mercury to enter the body, it can also occur through ingestion and dermal adsorption [3]. Approximately 80% of inhaled mercury vapour is retained in the body, and 7-14% is exhaled within a week after exposure [62]. Exposures can be originated in workplaces, such as mines or chlor-alkali production plants, as well as in devices like thermostats or thermometers, or in dental amalgam [3,57].

Once inside the body, mercury vapour bonds to sulphur-containing amino acids throughout the body, easily passing the blood-brain barrier and thereby, damaging the central nervous system [57,61]. It can also be deposited in the thyroid, muscles, myocardium, kidneys, lungs and other organs causing their dysfunction [61]. Its biological half-life is approximately two to four days and 90% is excreted through urine and faeces [63]. Vapour mercury in the body is also oxidized to Hg(II) [63].

Acute exposure to mercury vapour can lead to pneumonitis or bronchitis and it may be accompanied by tremors or erethism, while chronic exposure produces neurological dysfunction. In cases of low-level exposure, symptoms are more non-specific, such as weakness, fatigue or weight loss, whereas high exposure levels lead to tremors, personality changes, emotional excitability, insomnia, fatigue, depression or loss of memory [61].

Inorganic mercury compounds

Inorganic mercury salts have been found in laxatives, cosmetic products, teething powders, diuretics, skin-lightning creams and antiseptics, but some have also been used historically as a preservative for vaccines and for the development of photographic film or as a means of suicide [57,61]. Inorganic mercury is mostly excreted through urine and faeces [61,62], but also through sweat, tears, breast milk and saliva [61].

The toxicity of mercury salts varies with their solubility; the less soluble species being the less toxic, and the most soluble, such as mercury chloride salts, the most toxic [64]. When in the blood-stream, mercury salts adhere to sulfhydryl groups on erythrocytes, metalothionein, or glutathione or in suspended plasma accumulating either in the liver or kidneys [61,64]. Inorganic mercury salts are not lipid soluble and consequently do not cross the blood-brain barrier as efficiently as mercury vapour [64]. Although its absorption through the digestive tract is comparatively low, a large intake of Hg causes digestive tract and kidney disorders, which can result in death [63]. Concerning gestation, it accumulates quantitatively in the placenta, fetal tissues and amniotic fluid [61].

Acute poisoning of inorganic mercury salts presents symptoms, such as abdominal pain, vomiting, bloody diarrhoea, and potential necrosis of the gut mucosa which may produce death [61]. Otherwise, chronic poisoning affects the central nervous system and also leads to immune dysfunctions like asthma and dermatitis or the suppression of natural killer cells [61,64].

Organic mercury

Organomercurial compounds are very lipophilic so they are efficiently absorbed and can easily cross the biological membranes [61,65]. Organic forms of mercury can be found in fungicides, for example, and were also used as a preservative in vaccines in the form of thimerosal [62]. They can enter the body via inhalation, skin contact or consumption [61]. Even though the main route of human exposure to organic mercury is through the consumption of marine and freshwater fish, recent literature points out that in some regions of Asia the consumption of rice can also be an important source for prenatal exposure [19].

Organic forms of mercury can range from simple molecules to larger and more complex structures, the most common and toxic form being MeHg [8]. About 95% of MeHg ingested in fish is absorbed in the gastrointestinal tract [60]. It reacts with sulfhydryl groups throughout the body, potentially interfering with the function of any cellular or subcellular structure [61]. MeHg is distributed through the body by blood flow, and easily enters the central nervous systems after passing the blood-brain barrier [60,63]. Since the brain has a strong affinity to MeHg, the concentration in this organ is about 5 times higher than in the blood [60]. It is also transported through the placenta and, consequently, MeHg concentration in fetal blood is about 1.6 times higher than in the mother [63]. Its half-life in the body is about 45 to 70 days, but it differs from one tissue to another [62]. Excretion of organic mercury compounds occurs in the bile via the liver, but most MeHg is eliminated from the body by demethylation, and excretion of the inorganic form in faeces and urine [60,62,64].

The major toxic effects of MeHg are on the central nervous system [62]. In adults it is characterized by a latent period from weeks to months between exposure and onset of symptoms, depending on the dose and exposure period [61,62]. Physical lesions may appear, such as tingling and numbness in fingers and toes, loss of coordination, difficulty in walking, generalized weakness, impairment of hearing and vision, tremors and finally loss of consciousness leading to death [60]. Massive prenatal exposure may induce a form of cerebral paralysis, whereas lower exposure doses lead to neurodevelopment delays and cognitive deficits [61].

1.2. Analytical methods for the determination of mercury in aqueous samples

Mercury determination in natural waters is not an easy task due to the low concentrations of this metal, which are typically in the low-ng L^{-1} range, as previously shown in Table 1.3, making their reliable determination a major analytical challenge [46,66].

1.2.1 Instrumentation

A wide range of detection techniques can be used for Hg detection in aqueous samples, such as atomic emission spectrometry (AES) or atomic absorption spectrometry (AAS), but one of the most sensitive is provided by atomic fluorescence spectrometry (AFS) [67]. On the other hand, cold vapour is an efficient method for separating Hg from natural waters. Thus, cold vapour atomic fluorescence spectrometry (CV-AFS) is probably the most common technique used to determine Hg in natural water since it is recommended by regulatory bodies, such as the USEPA or the European Union (EU) [46]. Although this technique allows low limit of detection (LOD) [67], it presents considerable drawbacks, such as the complex and time-consuming procedures involved [46].

Inductively coupled plasma mass spectrometry (ICP-MS) is widely used for the determination of trace metals [68], and also allows low detection limits for Hg determination [66]. However, it also presents some problems when analysing Hg, such as long washout time, non-linear calibration curves or a decrease in sensitivity with time, which are associated to the so-called mercury memory effect induced by Hg adhesion to the walls of the spray chamber and the transfer tubing of the sample introduction system [68]. Even so, these problems can easily be overcome improving the washing efficiency of the system, for example, using L-cysteine as a washing reagent [68].

Inductively-coupled plasma optical emission spectrometry (ICP-OES) is also used for Hg determination from water samples. Since its LOD is higher compared to other techniques (in a range of µg L^{-1} instead of ng L^{-1}) [69], it usually requires preconcentration techniques due to the low Hg levels in natural samples. In addition, Hg usually needs to be separated from the matrix to simplify the sample analysis

Thus, specific instruments for mercury determination, such as the Advanced Mercury Analyser (AMA), are being used quite widely [70]. They use catalytic combustion of the sample, preconcentration by gold amalgamation, thermal desorption and detection by AAS.

1.2.2 Separation processes for sample preparation and preconcentration

Sample preparation is the analytical step between sample collection and sample analysis. The function of sample preparation methods is to transform the real sample matrix into a suitable sample for analysis [71]. Current analytical methods for sample preparation are commonly based on four main goals (i.e. to clean up, to extract, to preconcentrate, and to adapt the analytes and the sample form to the instrumental technique used), and aim to provide a robust and reproducible method that is independent of sample matrix variations [71,72].

Currently, several methods for sample preparation allow the extraction and pre-concentration of the analytes from both aqueous and solid samples and the choice of the most appropriate separation method in each case is usually conditioned by the amount of available sample, the required resolution, applicability, selectivity and sensitivity of the separation method.

Liquid-liquid extraction

Liquid-liquid extraction (LLE) is a separation technique based on the distribution of a solute between two immiscible phases between them (organic phase and aqueous phase). This process involves the transfer of the metal (M) to an organic phase (E) in the form of a complex (ME), normally including an extractant agent. The process requires the balance to be shifted to the formation of the metal complex.

$$\mathrm{M_{aq}} + \mathrm{E_{org}} \leftrightarrow \mathrm{ME_{org}} \qquad \text{(Equation 1)}$$

The LLE process normally consists of two stages. In a first step, called extraction, the analyte is transferred to an organic phase, in the form of ME. The second step, the so-called back-extraction or elution, allows recovery of the metal in another aqueous solution by contacting the extracted metal with an appropriate complexing agent, for example. One of the important aspects of being considered in liquid-liquid extraction is the choice of the extractant, which must be insoluble in water, but soluble in common organic solvents and selective for the analyte of interest. [73].

In the case of Hg, the commercial extractants trioctylmethylammoiun chloride (Aliquat 336) [74] and Cyanex 923 [75] have been investigated to remove mercury species from acid chloride solutions. Indeed, Cyanex 923 has proven to be a selective reagent for Hg(II) over other metal ions, such as Zn(II), Cd(II), Ca(II), Fe(III), in a pH range of 1-2. Also, triphenylphosphine sulphide (TPPS) was used as extractant for Hg extraction from salicylate solutions [76]. This method allowed the separation of Hg(II) from Zn(II), Bi(II), Cd(II), Pb(II) and Cr(VI). A LLE process involving 1-phenyl-3-hydroxy-4-dodecyldithiocarboxylate-5-pyrazolone (HDTC-12) as extractant enabled the extraction of Hg(II) from aqueous solutions of 0.1 M KNO_3 at a wide rage pH of 1-12 [77].

Solid phase extraction (SPE)

Recently, SPE have been widely and successfully used for the preconcentration and separation of Hg in aqueous samples since this procedure presents some advantages compared to solvent extraction, such as faster operation, easier manipulation, lesser use of organic solvent [78] or higher preconcentration factors [79]. The basic principle of SPE is the transfer of the analyte from the aqueous phase to bind to active sites of the adjacent solid phase and, in the case of Hg, the most successful SPE-extractors are those containing sulphur, nitrogen or phosphorous compounds [79]. Some techniques, allow the direct analysis of the solid phase, but other methodologies require the elution of the target of interest to another aqueous phase. Different strategies are used for the recovery of Hg species from the solid phase, such as lowering the pH with HCl or HNO_3, the use of sulphur-containing ligands (e.g., thiourea) or even organic solvents can be used if the target analyte is a Hg complex [67]. Figure 1.7 shows a scheme of the general procedure of solid-phase preconcentration methods, but it has to be highlighted that the sample transportation to the laboratory can be performed before or after the enrichment of Hg onto a sorbent.

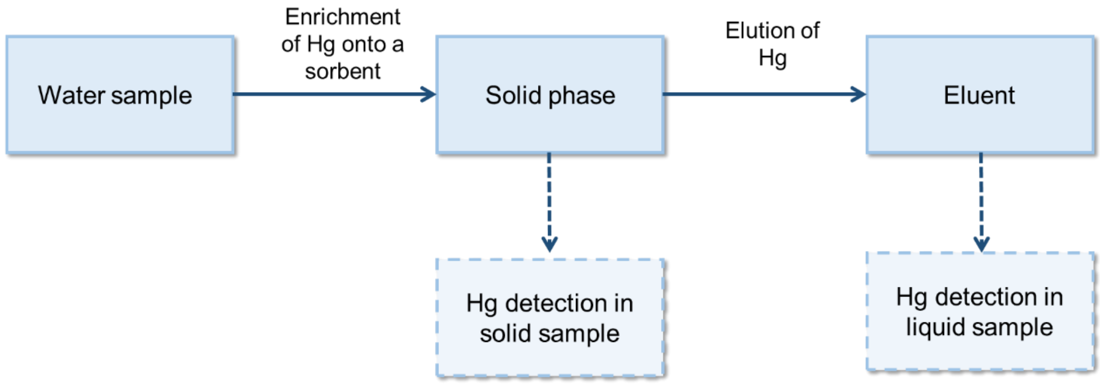


Figure 1.7. General procedure for solid-phase preconcentration methods. Adopted from [67].

Activated carbon is a commonly used material in general SPE processes. In recent years, it has gained considerable attention both in research-based studies and practical industrial applications for Hg extraction and removal from aqueous samples. Also, the manipulation and modification of the surface functional groups of activated carbon has been of great interest, becoming the application of sulphur-functionalized activated carbons one of the most common practices to promote mercury adsorption [80]. Another possibility is the modification of coal gangue with (3-mercaptopropyl) trimethoxysilane (MPTMS), which results in a mercapto-modified coal gangue for the removal of heavy metal cations such as Pb(II), Cd(II) and Hg(II) from aqueous solutions [81]. The same agent, MPTMS, has been used for the modification and optimization of the surface of bentonite clay raw material for the removal of Hg ions from aqueous media [82].

Nanoparticles (NPs) are also attractive solid sorbents useful for separation processes due to their special chemical, electrical, optical and thermal properties, as well as adsorption capacity [83]. Concretely, titanium dioxide (TiO_2) nanoparticles have been widely used due to their physical and chemical properties, low cost, non-toxicity and high specific surface area [83,84]. Similarly, NPs based on TiO_2 have been efficiently used to remove Hg(II) from aqueous samples when working with a photocatalytic process [84], also with the combination of ultrasound-assisted dispersive micro solid-phase extraction (USA DMSPE), which also allows its preconcentration in 1M HNO_3 [83].

Other common support materials for Hg preconcentration are alumina and silica and commercially-available ligands are sometimes bound to this kind of support materials [67]. Octadecyl silica cartridge as a sorbent and 1,4-bis(4-pyridyl)-2,3-diaza-1,3-butadiene (4-bpdb) as a ligand have been used as a simple and reliable method for the preconcentration and separation of trace amounts of Hg(II) [78]. That particular study demonstrated that the silica cartridge did not show any tendency to retain Hg(II) ions in the absence of the ligand, but it was capable of retaining the mercury 4-bpdb complex. Despite the absence of thiol groups in its structure, 4-bpdp has a pair of nitrogen atoms located in a position that allows each to form a covalent bond with Hg ions. The reagent chosen for Hg recovery from the sorbent was 0.01M HNO_3. Silica sorbents have also been functionalized using mercaptopropyl (MP), achieving selective Hg recovery of around 97% [85]. In this case, Hg elution from the support material was performed with 0.2M HNO_3 and low energy assisted digestion. Other studies using silica as a support material have been focused on silica as a gel, incorporating a ligand by physical or chemical attachment [67].

Also, the inorganic magnesium silicate (MgSi) sorbent has been modified with 2-mercaptobenzoxazole (MBO) for the development of a methodology to successfully determine ultra-trace level amounts of Hg ions in estuary waters [86].

The use of Ionic Liquids (IL) as extractants

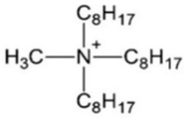
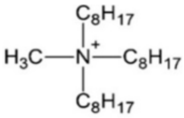
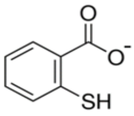
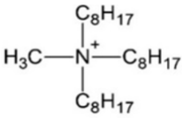
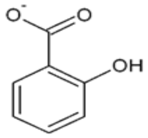
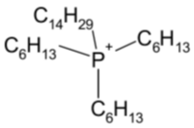
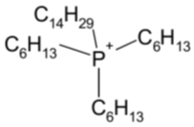
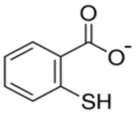
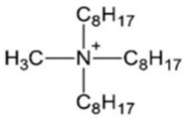
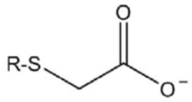
Among the different extractants available for metal extraction processes, the use of ionic liquids (ILs) for this purpose has been the focus of this study.

IL are a class of organic non-molecular solvents that are liquid at room temperature (and under 100ºC). They are formed by an organic cation, which contains nitrogen (N) or phosphorus (P) and an organic or inorganic anion, and they possess unique properties such as extremely low vapour pressure and high thermal stability [87], which makes them a good alternative for green chemistry. The IL Aliquat 336 has been widely evaluated as an extracting agent for different metals from aqueous solutions, but, despite its successful application, its extraction mechanism is based on anion exchange and therefore, its efficiency strongly depends on the composition of the aqueous phase and the pH [88]. To overcome this drawback, the properties of IL can be manipulated by

proper selection of the anion, to improve affinity to the target analyte [87,89–91]. Thus, appending a functional group onto the IL offers the possibility of increasing the diversity of target metals and also improving versatility. Numerous studies have been developed following this proposal. In Table 1.4, some of the most common IL are represented, as well as their target analytes.
A wide variety of Aliquat 336-based ionic liquids can be easily prepared by means of the simple replacement of the chloride anion for the desired one [92]. Common ligands used for metal ion extraction are functional groups, such as carboxylic acids, or contain donor atoms like crown ethers that can interact or even form complexes with metal ions [87–89].

The capacity of different Aliquat 336-based IL for the extraction of metals from communal and industrial waste water was studied by Fischer et al., testing the use of the anions thiosalicylate (TS), 2-(methylthio) benzoate (MTBA), benzoate (BA), hexanoate (Hex) and thiocyanate (SCN) [87] in combination with the cationic part of Aliquat 336. In the same study, the IL Cyphos IL 101 was tested for the extraction of the same metalloids with the incorporation of TS, salicylate and MTBA groups. It was found that the chemical characteristics differed from each prepared IL and also the extraction efficiency of metalloids was different depending on the IL as well as on which anion was incorporated. For industrial wastewater samples containing high concentrations of Zn, [A336][SCN] IL is recommended, whereas the application of TS or MTBA functionalized IL is recommended for communal wastewater treatment. Similarly, Leyma et al. studied the extraction of heavy metals from aqueous solutions using different TS-based ionic liquids using Aliquat 336 and Cyphos IL 101 as precursors [89]. Good extraction rates were observed for Cd(II) and Cu(II) for all the IL tested but were moderate for Zn(II). Another study demonstrated that Aliquat 336-based IL incorporating benzoate or hexanoate in their chemical composition, presented an extraction efficiency of Cu(II) between 11 and 16% from ultrapure and river water, without remarkable differences between the aromatic and the aliphatic carboxylic IL [91]. However, when TS was incorporated as the anion, the Cu(II) extraction capacity increased to nearly 100%, showing the important influence of the thiol group in the extraction process. Transition metals such as Fe(II) and Cu(II) have been efficiently extracted from aqueous solution with the Aliquat 336 derivative IL trioctylmethylammonium salicylate (TOMAS), which contains the salicylate anion [93].

Table 1.4. Chemical structures of commonly used IL for metal extraction from aqueous solutions.

TSIL / IL	Cation	Anion	Target metal	Reference
Tricaprylymethylammonium chloride [A336][Cl]		Cl^-	Hg(II) Co(II), Ni(II)	[74] [94]
Tricaprylymethylammonium thiosalicylate [A336][TS] / TOMATS			Pt(II) Cu(II) Zn(II) Cd(II) Hg(II) Cd(II)	[90] [89] [95] [91]
Tricaprylymethylammonium salicylate [A336][Sal]			Fe(III), Cu(II), Ni(II), Mn(II)	[93]
Trihexyl(tetradecyl)phosphonium cloride [PR$_4$][Cl]		Cl^-	Pd(II) Fe(III), Ni(II)	[96] [97]
Trihexyl(tetradecyl)phosphonium thiosalicylate [PR$_4$][TS]			Pt(II)	[90]
Methyltrioctylammonium butylsulfanyl acetate [N$_{1888}$][C$_4$SAc]			Cd(II), Cu(II)	[98]

A336: Tricaprylymethylammonium
PR$_4$: Trihexyl(tetradecyl)phosphonium
N$_{1888}$: Methyltrioctylammonium

In the specific case of Hg, different sulphur-containing groups, such as TS, MTBA or SCN, are some examples of functionalized groups that, appended to ILs, improve their affinity towards Hg [87]. An IL-based liquid phase micro-extraction (LPME) procedure was used to evaluate their capacity as extracting agents. When working with an aqueous phase of 0.01M $CaCl_2$ with a mix of different metals ([Hg] approximately 7.5 µg L^{-1}), the extraction efficiencies of Hg ranged from 80 to 100% for all the tested IL, with a better tendency for those incorporating a specific functional group containing sulphur (MTBA>SCN>TS). However, no general conclusions concerning the most important parameters affecting Hg extraction could be made because, as proved when testing wastewater, various parameters influence the extraction mechanism, such as the composition of the matrix or the physico-chemical properties of the IL.

Metal extraction has also been accomplished using ILs incorporated in supports such as alginate, chitosan or mineral materials [99]. For example, microdroplets of the IL A336-MTBA were immobilized and entrapped in a polyvinyl alcohol (PVA)-alginate matrix gel for Hg(II) recovery from aqueous samples [99]. Alginate material has also been used as a biopolymer capsule for the immobilization and encapsulation of Cyphos 101 IL to recover Hg(II) from HCl solutions [100]. Polystyrene microcapsules have also been reported as an another interesting approach for IL immobilization [88]. Efficient Hg removal from polluted surface waters was achieved with poly(styrene-co-divinylbenzene) microcapsules containing immobilized Aliquat 336 [101].

Due to the good characteristics of trioctylmethylammonium thiosalicylate (TOMATS) for extracting Hg, in this thesis we have focused on the use of this IL to develop novel methodologies to facilitate Hg detection. A few years ago, this IL was commercially available as a novel task-specific ionic liquid provided by Sigma-Aldrich. However, it can easily be prepared by using Aliquat 336 as a source of the cation trioctylmethyl ammonium and adding the thiosalycilate anion by an ion-exchange reaction [90]. The chemical structure of this compound is represented in Table 1.4, and its physical characteristics are presented in Table 1.5.

Ismaiel et al., explored the possibility of using impregnated activated carbon, obtained from palm shell, with TOMATS with the aim of removing Hg(II) from synthetic aqueous solutions with high levels of Hg(II) [95]. The modified activated carbon achieved a maximum adsorption capacity of 83.3 mg Hg(II) g^{-1}, and was able to work at pH range of 6-8, which proved this IL to be a suitable extractant for Hg(II) from aqueous solutions. Nonetheless, no desorption of Hg from the surface was possible, indicating that the process of adsorption was almost irreversible.

Table 1.5. Physical characteristics of TOMATS [90].

	TOMATS
Appearance	Greenish-yellow viscous liquid
Solubility	Soluble in ethyl acetate, ethanol, methanol, acetone, toluene, dichloromethane, chloroform, ACN; insoluble in water, ether, hexane
Melting point	Liquid at room temperature
Refractive index (N_D^{20})	1.5180
Viscosity (cP)	3220 (25ºC)
Density (g cm^{-3})	0.9556

The same research group developed an electrochemical sensor for Hg(II) determination in aqueous solution taking advantage of this activated carbon impregnated with TOMATS [102]. The proposed electrode was able to efficiently determine Hg(II) from aqueous solutions over the pH range of 3-9 and it was also successfully applied to drinking water samples. Additionally, TOMATS has also been explored by various authors as an extracting agent for other different metals [90,91,103]. This IL has performed well for Cd(II) extraction from ultrapure and river waters [91] as well as for Pt(II) from a 0.01M $CaCl_2$ aqueous phase [90] achieving extraction efficiencies of nearly 100 and 85%, respectively. Furthermore, TOMATS has been incorporated into magnetic nanoparticles that have been successfully applied for the determination of Cd(II) from aqueous solutions as well as in fruit samples [104]. This IL has also been used in IL-based ultrasound-assisted dispersive liquid-liquid microextraction for the preconcentration of Cd(II), Co(II) and Pb(II) in tea samples and its subsequent dissolution with methanol for its analysis by liquid chromatography with UV detection [105].In another study, it was explored in ultrasound-assisted dispersive liquid-liquid microextraction as an excellent technique for the preconcentration of Co(II) from very different complex matrices, such as water samples, herbs or vitamin supplements [106].

1.3. Polymer Inclusion Membranes (PIMs)

Among the different sample treatments, membrane-based processes have attracted noticeable attention as a valuable alternative to conventional separation processes. In general, a membrane can be defined as a semipermeable barrier between two phases [107]. The membrane restricts the movement of the different molecules through various mechanisms, such as size exclusion, differences between diffusion coefficients, and electric charge.

In this thesis we have focused on the preparation of a kind of liquid membranes (LM) incorporating IL as carriers to allow Hg extraction and separation. The LM concept combines solvent extraction and membrane-based technologies, enabling both extraction and back-extraction in a single step with reduced consumption of extractants and diluents, involving mass transfer of a target chemical species between two solutions separated by an immiscible LM [108].

Polymer inclusion membranes (PIMs) are a type of self-supporting LMs for the extraction and separation of metallic and non-metallic ionic species and small organic molecules from aqueous solution [109]. This kind of membranes were introduced by Sugiura in the early 1980s [110] and, even though they were initially used as the sensing component in ion-selective electrodes and optodes, interest around them has been growing exponentially as a PIM-based separation system [111]. Some of the advantages of this technique are its easy operation, minimum use of hazardous chemicals, and flexibility in membrane composition to achieve the desired selectivity as well as separation efficiency [112]. PIMs entrap an extractant, often called carrier, in a base polymer matrix and, in some cases, may contain plasticizers [109]. The simplicity of PIM fabrication makes it particularly attractive for developing low-cost separation/analytical technologies.

In order to successfully cast a PIM, a good miscibility between the membrane components is imperative. Poorly miscible combinations will phase separate upon drying, resulting in a brittle and inhomogeneous membrane, which will consequently contribute to the poor extractive performance of the PIM-based system. PIM components may only be miscible in specific or limited formulations, or exclusively in the presence of particular plasticizers or modifiers. If the PIM composition has been well designed, the resulting film should be mechanically stable, hydrophobic and safe to handle. An image of a transparent and homogeneous PIM can be observed in Figure 1.8, and their structure is represented in Figure 1.9 by means of Scanning Electron Microscopy (SEM) images from surface and cross-section.

PIMs constituents are explained in detail below.

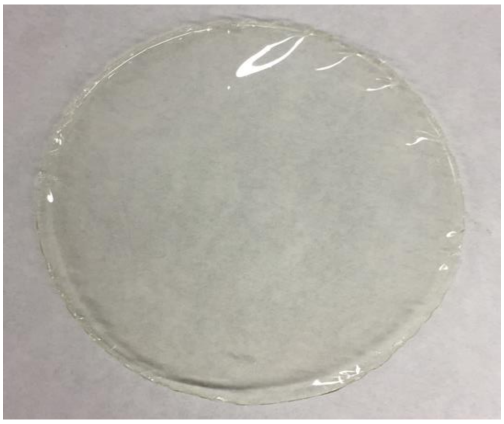


Figure 1.8. Image of a PIM.

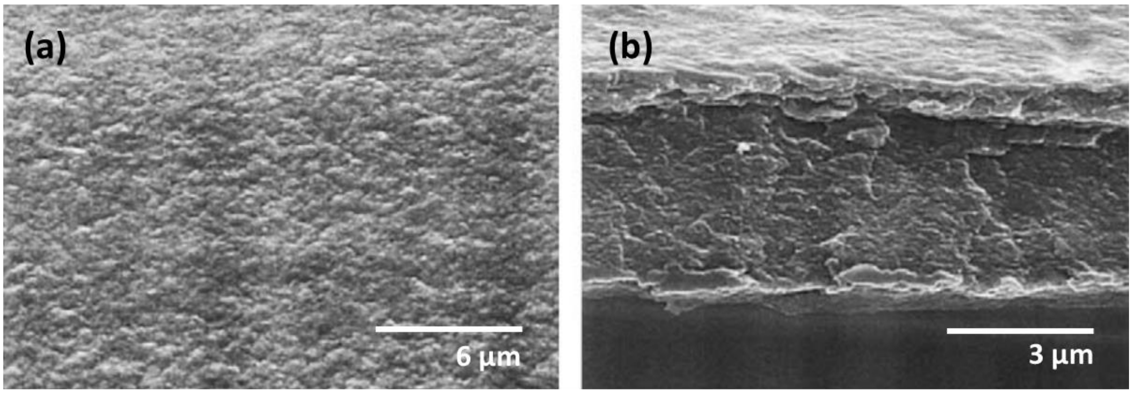


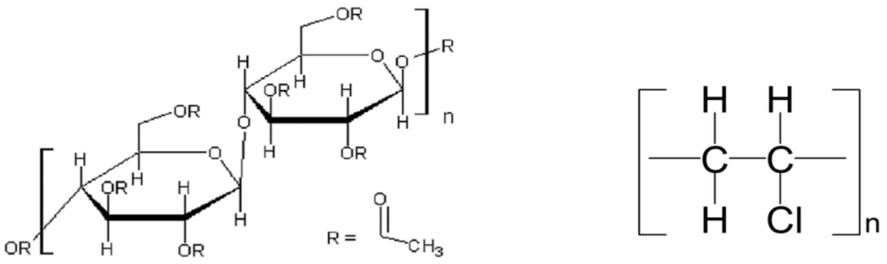
Figure 1.9. Scanning Electron Microscopy (SEM) images of the surface (a) and cross-section (b) of a PIM. Image obtained from [113].

1.3.1 PIMs constituents

Polymers are structures formed by basic units called monomers that provide mechanical strength and form a long chain polymer network and high molecular weight. Their properties vary from one polymer to another so the polymer used must be chosen based on the application and the need for thermal stability, flexibility, and cost, among others [114].

Polymers that make up the skeleton of a PIM are thermoplastics, consisting of linear polymer strands without any cross-link between them, and soluble in a suitable organic solvent where the polymer strands become separated [112]. They are responsible for providing mechanical strength to the membrane, which is a combination of intermolecular forces and the process of entanglement [112,115]. Intermolecular forces determine the flexibility of the material with high intermolecular forces resulting in a rigid membrane, whereas entanglement is the result of random diffusion of the polymer strands in the PIM [112].

The two major polymers used for PIMS are cellulose triacetate (CTA) and polyvinyl chloride (PVC), not only because they provide high mechanical strength and are compatible with a large range of carriers and plasticizers [108], but also because thin films can be easily prepared with a simple procedure based on their dissolution in an organic solvent [112]. As can be observed in Figure 1.10, these two polymers present different chemical structures. CTA is a polar polymer with a number of hydroxyl and acetyl groups that are capable of forming highly orientated hydrogen bonding, giving CTA a crystalline structure [108]. In contrast, PVC is relatively polar due to the presence of a C-Cl functional group and non-specific dispersion forces dominate the intermolecular interactions, resulting in an amorphous structure with a small degree of crystallinity [112]. The main characteristics of these two polymers are presented in Table 1.6. It is essential to correctly choose the polymer when preparing PIMs, because they can play an essential role in the transport of the target analyte. PIMs using different ILs as a carrier and containing nitrophenyl octyl ether (NPOE) as a plasticizer allowed divalent zinc (Zn(II)) transport when the polymer used was CTA, but no transport was performed when using PVC [116]. However, As(V) transport was achieved with PIMs using CTA or PVC polymer indistinctly [117,118].



Cellulose triacetate **Poly(vinyl) chloride**

Figure 1.10. Chemical structures of the most frequently used polymers for PIM.

Another important parameter that must be taken into account is the state of the polymer, defined as the phase in which the polymer appears, and this has an influence on its mechanical, chemical, thermal and permeation properties [114]. One typical characteristic of amorphous polymers is the glass transition temperature (T_g). Below T_g, the polymer is rigid and glassy and individual polymers strands are unable to change their configuration, whereas beyond this temperature value the rubbery state of polymer occurs [112,114]. For crystalline polymers, this change in physical and chemical properties occurs beyond the melting temperature (T_m). In this sense, when polymers are found at temperatures below T_g they are rigid and, since they do not allow the formation of flexible membranes, they are unfavourable for metal ion transport in membranes [112]. To overcome this issue, plasticizers are often added to the polymer to lower its T_g value and create more flexible and less brittle membranes [112].

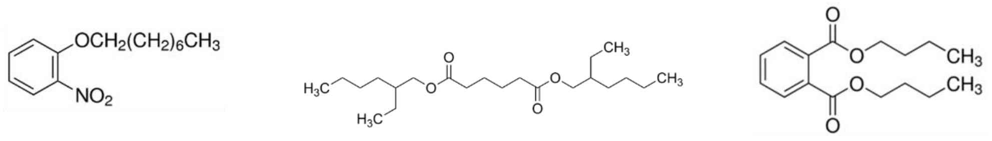
Table 1.6. Physical properties of polymers most frequently used in PIMs. Data obtained from [112].

Polymer	MW (kDa)	T_g (ºC)	T_m (ºc)	Characteristics
Cellulose triacetate (CTA)	72-74	na	302	High degree of crystallinity, excellent strength
Poly(vinyl chloride) (PVC)	90-180	80	na	Slightly crystalline, mostly amorphous

na: not available

The individual molecular chains in PIMs are held together by a combination of different types of attractive forces, such as the weak and non-specific van der Waals forces or stronger polar interactions at the polar centre of the molecules, resulting in the formation of a rigid membrane structure unfavourable for the diffusive flux of material within the polymer matrix [108,112]. Thus, **plasticizers** are added to the PIM composition to penetrate between polymer molecules and neutralize their polar groups and/or to increase the distance between the polymer molecules and hence reduce the strength of the intermolecular forces [115,119]. Consequently, and as stated above, the addition of plasticizer implies a decrease in the T_g and leads to an increase in membrane softness and flexibility [108], which generally improves the flux between the polymer matrix [112].

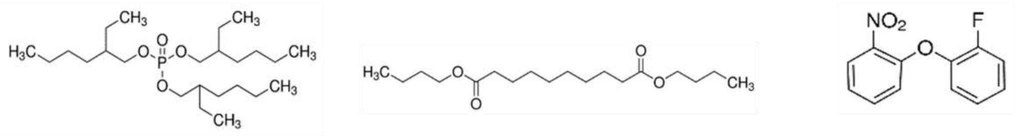
Although there are a large number of commercially available plasticizers, the most commonly used are NPOE and 2-nitrophenyl phenyl ether (NPPE), but others, such as bis(2-ethylhexyl) adipate (DEHA) or dibutylsebacate (DBS), have also been tested [120]. The chemical structure of different plasticizers are represented in Figure 1.11.



2-nitrophenyloctyl ether (NPOE)

Bis(2-ethylhexyl) adipate (DEHA)

Dibutylphtalate (DBP)



Tris (2-ethylhexyl) phosphate (TEHP)

Dibutylsebacate (DBS)

2-fluorophenyl 2-nitrophenyl ether (FPNPE)

Figure 1.11. Chemical structures of some of the plasticizers used in PIMs

Plasticizers are generally organic compounds containing a hydrophobic alkyl backbone with one or several highly solvating polar groups that interact with the polar groups of the base polymer neutralizing them [112]. On the one hand, a longer alkyl chain results in higher viscosity and hydrophobicity, which supresses the polar properties of the plasticizer complicating transport across the membrane. In contrast, an increase in the number of polar groups implies an increase in plasticizer hydrophilicity and a decrease in its viscosity, thus leading to loss of the plasticizer to the aqueous phase [121].

Therefore, a balance between the polar and non-polar group of the plasticizer is an important factor [108].

The increase in the amount of NPOE in PVC-based PIMs allowed an improvement in Cu(II) transport efficiency. However, this improvement was slight when the plasticizer content was below 30 wt%, but a great enhancement was found above this value [122]. Therefore, not only does the type of plasticizer affect transport efficiency, but also its concentration in the PIM [123]. A too low plasticizer content results in rigid and brittle membranes due to the antiplasticization effect. The minimum concentration of plasticizer required varies widely depending on both the plasticizer and the polymer used, being, for example, around 20 wt% of plasticizer for PIM with PVC as a polymer [108]. If the plasticizer concentration is too high, the plasticizer could migrate or exude to the PIM surface and form a film in the membrane/aqueous interphase, creating an additional barrier to the transport across the membrane. Moreover, an excess of plasticizer usually reduces the mechanical strength of the PIM making it a difficult membrane to work with [112].

Another parameter that can influence the flux across the PIM is the viscosity of the plasticizer. It was first stated that viscosity can even be a parameter governing transportation rates [112], but it is important to highlight that, as shown in Table 1.7, most of the plasticizers used in PIMs have similar viscosity values [120]. A recent study with cellulose triacetate membranes testing plasticizers with different viscosity values, found that this parameter does not appear to have a dominant effect [124].

Table 1.7. Physicochemical parameters of some PIM plasticizers.

Plasticizer/Modifier	Dielectric constant (ε_r)	Viscosity (cP)	Ref.
NPOE	24	11.1	[108]
DBS	4.5	9.5	[125]
TEHP	4.8	13.1	[108]
FPNPE	50	13	[125]

Dielectric constants of plasticizers also play an important role in the diffusion process [112], as plasticizers with relatively high polarity are preferentially applied as a solvent in PIMs [126]. PIMs with CTA as a polymer using different plasticizers with similar viscosity values, but with totally different dielectric constants, obtained the best transport performances for metallic species with plasticizer presenting a moderate dielectric constant [126]. Also, two similar plasticizers were studied in a PIM made of CTA, just differing in the presence of an ester function and in the dielectric constant value (24.2 and 5.8) [127]. The plasticizer with a moderate dielectric constant allowed transport fluxes two times higher than the plasticizer with more common and lower values of this parameter. Nonetheless, it should be noted that the dielectric constant of the plasticizer is apparently different from the dielectric constant of the PIM itself because it is also dependent on the carrier and the base polymer [120], and thus a degree of caution must be taken when attempting to correlate initial PIM flux values with the dielectric constant and plasticizer viscosity [120].

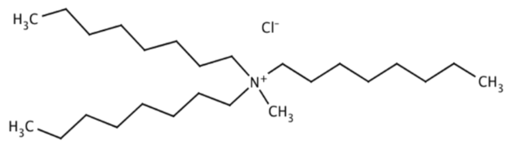
It is also remarkable that there are some carriers, such as IL, which, because of their physicochemical properties, can act as plasticizers and even as extracting agents at the same time [112].

The **carrier** or extractant is usually a complexing agent or an ion-exchanger responsible for binding the species of interest and thus, facilitating the extraction and transport of the complex or ion-pair within the PIM [108]. Solvent extraction reagents have been studied as carriers in PIMs, namely, basic, acidic and chelating, neutral or solvating, and macrocyclic and macromolecular [112]. Chemical structures of some of the most studied carriers in PIMs are represented in Figure 1.12 and some examples of each type of carriers are presented in Table 1.8. Although several studies have been conducted using commercially available solvent extraction reagents, carriers for PIMs can also be synthesized or tailor made [112].

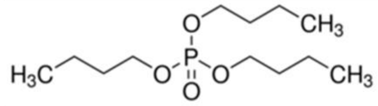
Table 1.8. Classification and examples of different types of carriers used in PIM systems [108,120].

Type of extractants		**Examples**
Basic	Quaternary amines	Aliquat 336
	Tertiary amines	TOA: tri-n-octylamine
	Thiadiazine derivatives	FFAT: 5-(4-phenoxyphenyl)-6H-1,3,4-thiadizin-2-amine
Acids	Alkyl phosphoric acids	D2EHPA: Di-(2-ethylhexyl)phosphoric acid
	Carboxylic acids	Lasalocid A
	Hydroxyquiinoline	Kelex 100
Neutral	Phosphoric acid esters	TBP: tributylphosphate
	Phosphonic acid esters	Dibutyl butylphosphate
	Ionic liquids	Cyphos IL 101
Macromolecules	Crown ethers	DC18C6: Dicyclohexano-18-crown-6
	Calixarenes	calix[4]arene
	Calix crowns	calix[4]-crown-6

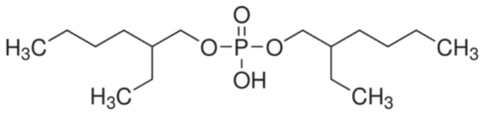
Figure 1.12. Chemical structures of examples of basic (Aliquat 336), acidic (D2EHPA), neutral (TBP) and macromolecular (DC18C6) carriers.



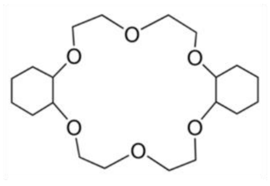
Trioctylmethylammonium chloride
(Aliquat 336)



Tributylphosphate
(TBP)



Di-(2-ethylhexyl)phosphoric acid
(D2EHPA)



Dicyclohexano-18-crown-6
(DC18C6)

Basic carriers consist mainly of amine-based compounds such as quaternary ammonium salts, tertiary amines and thiadiazine derivates [108,120]. If it is a fully substituted quaternary ammonium compound, it reacts as an anion exchanger forming an ion-pair with another anion from the feed phase or with a complex metal ion but, if the carrier is an amine or another weak base, it must first be protonated in order to allow anion exchange or even react directly with a protonated complex metal ion [108]. Aliquat 336 is a commercial solvent extraction reagent that has been extensively applied as a carrier in PIMs and, since it presents plasticizing properties, it is frequently used without the need for the addition of a plasticizer [120] and forms suitable membranes with CTA or PVC polymer indistinctly [128].

Acidic carriers generally include organophosphorous acids, sulfonic acids, carboxylic acids, and also presenting chelating properties, hydroxyoximes and quinolones. They are usually used for the extraction and transport of metal cations, involving an exchange of the metal ion with hydrogen ion from the carrier [108]. For example, D2EHPA is a widely studied carrier for PIM systems and has been successfully applied with PVC for the development of a passive sampler device [129].

Neutral carriers are commercially available phosphorous-based extractants with high selectivity towards actinides and lanthanides, but the previously explained ILs are also included in this group [108].

Finally, macromolecule carriers can be tailored to a particular metal ion. Even though they are not commercially available and their synthesis is often expensive, a considerable number of research papers have been focused on them [108,120].

1.3.2 Transport mechanism

The PIM mass transfer process is usually referred to as facilitated transport and it allows transport of the extracted species from an S solution with a lower concentration to an R solution with a higher concentration. Thus, it is possible to transfer the extracted species quantitatively. This transport process is schematically shown in Figure 1.13.

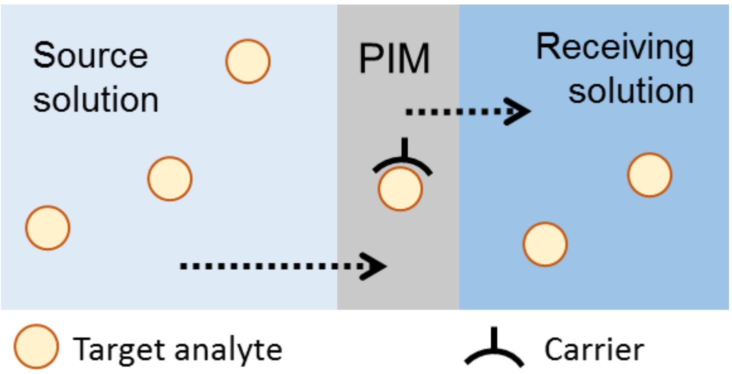


Figure 1.13. Facilitated transport across a PIM containing a carrier [111].

The overall transport mechanism across a typical PIM consists of the following three steps:

1. The extracted species diffuses across the stagnant diffusion layer at the membrane/S solution interface where it reacts with the carrier, and the reacted carrier species are replaced by another carrier species from the bulk of the membrane.

2. The product of the reaction between the extracted species and the carrier, which is usually a complex or an ion-pair, is transported through the membrane/R phase interface along the corresponding concentration gradient.

3. At the membrane/R phase interface, the extracted species are released into the R solution, which can be facilitated by a suitable solution reagent. The carrier species liberated in this process are transported back to the membrane/S solution interface.

1.3.3 Analytical application of PIMs

PIMs were first used about 50 years ago as the sensing membranes in ion-selective electrodes (ISEs) and optodes. From then onwards, PIMs have been used in different analytical applications, such as sample preparation, sample preconcentration, electro-driven extraction, passive sampling and speciation studies, and been incorporated into on-line and automated analysis systems and used as sorbents for different target compounds [111]. PIMs have been widely reported for their removal of heavy metals using diverse combinations of different types of carriers, plasticizers and base polymers [130].

Effective methodologies for the determination of metals from liquid samples have been developed with the combination of these membranes with their subsequent analysis with X-ray fluorescence (XRF) [131,132]. Preconcentration procedures using solids in combination with XRF allow the direct analysis of the solid and, consequently, the elution step is avoided, sample preparation time is reduced and the whole procedure is simplified [133]. Among the different solid-liquid extraction procedures used for metal preconcentration in combination with XRF [134], this instrumentation operates best on solid samples and gives optimal sensitivity and accuracy for thin, homogeneous targets [133], such as PIMs. A PVC-based membrane using Aliquat 336 as a carrier and NPOE as a plasticizer, allowed Cr(VI) extraction and its subsequent detection by energy dispersive XRF (EDXRF) [131]. Indeed, Cd(II) was efficiently removed from complex liquid samples such as sea water and satisfactorily detected with XRF, using a CTA-based PIM with Aliquat 336 as the carrier and NPOE as the plasticizer [135].

PIMs have also been successfully used to act as sorbents of organic compounds. A PIM made of 70% CTA and 30% NPOE efficiently extracted the pesticides chlorpyrifos, diazinon and cyprodinil from natural waters without the requirement of carrier content. After extraction, analytes were recovered from the membrane with 1 mL of acetonitrile and injected into the gas chromatography and mass spectrometry (GC-MS) detection [136].

PIMs have also been used in optical chemical sensors named optodes. Concerning Hg, the immobilization of a dye 4-(2-pyridylazo)resorcinol with Aliquat 336 in the tri-(2-ethylhexyl) phosphate-plasticized CTA matrix allowed the development of a colour-changeable optode for the detection of Hg(II) ions in aqueous samples [137]. Also a synthesized fluoroionophore; chloro phenyl imino propenyl aniline (CPIPA) was immobilized in plasticized PVC membrane for the development of a sensitive and highly selective disposable optode for Hg(II) ions [138].

However, most studies concerning PIMs are related to the use of these membranes in transporting metallic species, metalloids, anions, as well as some organic compounds from the source phase to a receiving phase. Some of these studies are collected in [112,120]. Moreover, when incorporating the PIM in a suitable device that contains a smaller volume of receiving solution than the source phase, not only the transport but the preconcentration of the analyte can be achieved. A PVC-based PIM containing Aliquat 336 allowed the separation and preconcentration of As(V) employing a device configuration using 100 mL of aqueous sample and 5 mL of 0. M NaCl [139], whereas a CTA-based PIM containing Aliquat 336 and NPOE as plasticizer performed excellent transport efficiency for different antibiotics with a PIM-device configuration involving 120 mL of sample solution and 4 mL of 0.5-1 M NaCl as a receiving phase [140].

The PIM-device used for As(V) preconcentration was also tested as for total ammonia monitoring using a PVC-based PIM composed of dinonylnaphtalene sulfonic acid (DNNS) and 1-tetradecanol (as modifier), and 0.8M HCl as the receiving solution [129]. After its previous calibration under laboratory conditions using underwater pumps to mimic the natural flow in environmental waters, a good agreement between spot and passive sampling was obtained. One of the advantages of using acidic extractants in PIMs, such as DNNS, is that the membrane surface is constantly acidic during the sampler period and it prevents the formation of biofilms, which is one of the main drawbacks of passive sampling [141]. Also, different PIM-based sampler device configurations were tested for the monitoring of sulfamethoxazole (SMX) using a PVC-based PIM containing Aliquat

336 and NPOE as a plasticizer, and 2M NaCl as receiving solution [142]. Even though it was demonstrated that the chemical composition of the PIM and the receiving solution minimized the matrix effect of the source solution, the flow pattern of the source solution influenced significantly the performance of the device. This issue was solved by attaching a plastic cylindrical container around the PIM in order to create a stagnant liquid layer.

Concerning Hg, a PIM composed of 30% CTA + 10% Cyanex 471X + 60% NPOE was successfully developed for its transport to a 0.05 M NaCl solution at pH 12.3 [143].

1.4. Passive sampling

The routine monitoring of conventional parameters in water bodies, such as organic matter, suspended soils, nutrients, microbial organisms, and inorganic pollutants, is required to control the quality and satisfy the requirements of legislation and regulations [141,144]. Effective monitoring of pollutants in waterways is challenging from both a sampling and analytical point of view [129] and can cause ongoing challenges for environmental and analytical scientists [141]. In this sense, environmental Hg monitoring has attracted special attention owing to its high toxicity and widespread occurrence [145].

The most common and widely accepted procedure used to monitor heavy metals and organometallic species in aquatic environments is the technique known as *spot sampling* that involves the collection of discrete water samples followed by their measurement [141]. However, a relatively large number of samples must be taken from a given location to establish the quality of the water body. Therefore, this method only reveals instant chemical concentration levels at the time of sampling, which may not reflect average conditions, and intermittent pollution events are likely to be missed. Increasing the frequency of spot sampling could solve this problem but, as a result, it could become costly and time consuming [129,141].

To overcome these shortcomings, passive sampling approaches have been developed and interest in them has been increasing [146]. Passive sampling can be defined in its broadest sense as any sampling technique based on the free flow of analyte molecules from a sampled medium to a receiving phase in a sampling device, as a result of a difference between the chemical potentials of the analyte in the two media [147]. Sampling proceeds without the need of any energy source other than this chemical potential difference, and continues until equilibrium is established in the system or until the sampling period is stopped [147]. Analytes are trapped or retained within the sampling device in a suitable medium, which is often referred to as the reference or receiving phase and can be a solvent, chemical reagent or porous adsorbent material. The receiving phase is exposed to the water phase without attempting to quantitatively extract the dissolved contaminants [146]. Instead, the information obtained as a result of the use of passive sampling technology depends on the accumulation regimes in which passive samplers operate during field exposure [141].

Passive samplers are designed to maximize the amount of analyte sampled with the goal of detecting low levels of analyte present in water, and at the same time ensuring a quantitative correlation between the collected mass of the chemical and its concentration in the sampled medium during the sampling period [146]. Different criteria must be taken into account for the proper design of passive samplers. The device, commonly called sampler, should be easy and not expensive to manufacture, easy to deploy, small enough to send inexpensively to and from remote locations, sensitive to the pollutants of interest as well as not affected by any interfering matrix component [148].

For practical proposes, calibration of passive sampler exchange kinetics is performed in the laboratory at known exposure concentrations [149]. The prediction of the time-weighted average (TWA), which is the concentration of a pollutant in the water phase, requires numerous calibration studies to establish the uptake of chemicals under different exposure conditions. The uptake kinetics of chemicals not only depends on the physicochemical properties of the analyte, but also on the sampler design and on different environmental variables, such as temperature, water turbulence and biofouling [146]. Biofouling is the colonization by bacteria and various flora and fauna on the active sampler surface that may ultimately form a biofilm, and is a potentially serious challenge for passive sampling, especially for long-duration deployments, because it can influence the mass transfer of pollutants and also damage the surface of membranes [141].

Over the last few years, several types of passive samplers have been developed for the monitoring of different pollutants in aquatic environments [146]. Among all the passive sampling techniques used to measure inorganic contaminants in water, Diffusive Gradient in Thin films (DGT) is one of the most widely known techniques.

1.4.1 Diffusive Gradient in Thin Film (DGT) technique

The DGT technique was developed in the mid-1990s by Davison and Zhang as an *in situ* technique for the determination of kinetically labile metal species in aquatic systems [150]. Later, it was developed as a general monitoring tool for transition and heavy metals [151]. Since then, DGT-based technologies have been used extensively in a variety of geochemical and environmental health disciplines [152], becoming a successful and reliable technique for the measurement and determination of cesium (Cs) and strontium (Sr) [153], uranium (U) [154], dissolved phosphates [155,156] and sulphates [157], major cations such as Ca^{2+} and Mg^{2+}[158] or the simultaneous determination of dissolved inorganic nitrogen and phosphates [159]. In fact, a DGT with a resin gel based on Chelex 100 demonstrated its capabilities for the measurement of 55 elements [160].

The DGT technique is based on the diffusive transport of solutes across a well-defined concentration gradient, typically established within a layer of hydrogel of a known thickness and outer filter membrane directly exposed to the deployment solution [151]. DGT units comprise a binding agent, which is separated from the solution to be analysed by an ion-permeable gel and a filter membrane, all assembled into a plastic sampling device consisting of a piston base and a cap which is represented in Figure 1.15 [141]. The filter membrane is exposed directly to the deployment solution and acts as a protective layer for the diffusive gel and once solutes have diffused through these outer layers, they are irreversibly removed from the solution and chelated at the back side of the diffusive gel by a selective binding agent, which is immobilized in a second layer of hydrogel [151].

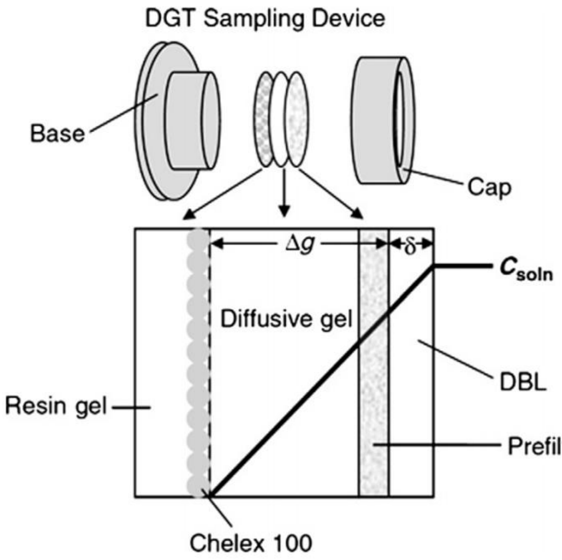


Figure 1.14. Schematic representation of a DGT sampling device obtained from [141].

The DGT technique is based on Fick's first law of diffusion. As represented in Figure 1.14, the resin gel is separated from the bulk solution by a permeable membrane of thickness Δ*g*. Between this gel layer and the bulk solution there is a diffusive boundary layer (DBL) of thickness δ which is negligibly small compared to Δ*g* and thus, the flux *(F)* of metal ions diffusing through the gel layer to the resin can be expressed by (Equation 2):

$$F = D\frac{(C_b - C')}{\Delta g} \qquad \text{(Equation 2)}$$

Where *F* is the flux of ions per time unit, D is the diffusion coefficient in the diffusive gel, C_b the free concentration of a metal ion in bulk solution, and C' the free concentration of the metal ion in the resin gel layer. If the free metal ions are in rapid equilibrium with the resin, and the resin is not saturated, *C'* can be effectively considered zero. Following the definition of flux (*F=M/At)* the mass (*M*) diffused through and area (*A*) after a given time (*t*), *M* can be calculated by Equation 3.

$$M = \frac{DC_b tA}{\Delta g} \qquad \text{(Equation 3)}$$

As the mass of ions that have diffused into the resin layer, *M*, can be analytically determined, the concentration in the bulk solution can be quantified by Equation 4.

$$C_b = \frac{M\,\Delta g}{DtA} \qquad \text{(Equation 4)}$$

Prior to their deployment in the environment, preliminary experiments are conducted in the laboratory to establish DGT performance characteristics, including the capacity of the resin layer, the diffusion coefficient of metals, the elution factor or the effect of the solution composition [141].

To date, DGT have been deployed in a wide range of natural waters. In 2000, Davison et al. reviewed the applications of the DGT technique in water, soils and sediments [161]. Development and application for aqueous systems including speciation measurements, bioavailability studies and routine environmental monitoring were reviewed in 2007 by Warnken and collaborators [151]. In 2015, Zhang and Davison reviewed the use of DGT for studies of chemical speciation and bioavailability, discussing key publications over the last 20 years [162]. Menegário and coworkers recently reviewed the progress in chemical fractionation, speciation and bioavailability of metals in water using DGT for *in situ* measurement.

Nowadays, it is possible to sample any labile species for which a suitable binding agent can be embedded into the receiving phase gel [141]. The resin Chelex 100, with iminodiacetic groups, with high affinity for divalent and trivalent cations is one of the most binding layers used for heavy metal measurements. However, other kinds of more selective resins have been used, such as ferrihydrite for the measurement of phosphates [155], or even a combination of ferrihydrite and Chelex 100 for the determination of cations and anions in one assay [163]. Other examples are the use of silver iodide resin gel for sulphide determination [164], ammonium molybdophosphate (AMP) for Radiocesium [165] or polystyrene sulfonate for Cu(II) and Cd(II) [166]. Also, commercial products have been used as binding layers, such as the granular adsorbent Metsorb® for ZnO measurement [167], or cellulose membrane Whatman® DE81 for the detection of Cr(VI) [168] or Ba(II) [169].

The DGT technique has been used by different authors as a passive sampling technique for in situ determination of kinetically labile mercury species in aquatic systems for the monitoring, determination and detection of Hg in environmental systems. Typical binding agents used in DGT include selective resins bearing thiol groups, as shown in Table 1.9.

Table 1.9. Binding agents for DGT used for the determination of Hg species.

Binding agent	Hg species	References
3-mercaptopropyl-functionalized silica gel	MeHg	[170–173]
	MeHg, Hg(II)	[174]
Spheron-thiol	THg	[175]
	Hg	[176]
Thiol-modified carbon nanoparticle suspension (SH-CNPs)	Hg(II)	[177]
Ambersep GT74	Hg(II)	[178]
Ambersep GT74 / Duolite GT33	Hg(II), MeHg, EtHg, PhHg	[179]
Saccharomyces cerevisiae	MeHg	[180]
TOMATS	THg	[70]

Even though Chelex 100 can also be used as a binding phase in DGT to determine Hg measurement in aquatic environmental systems, DGTs containing Spheron-thiol are more efficient for the determination of Hg in river water [1]. This fact is attributed to the higher affinity of thiol groups to Hg(II) when bound in non-labile complexes. Clarisse et al. studied the use of 3-mercaptopropyl functionalized silica gel as a binding phase in a polyacrylamide gel to measure the dissolved MeHg in natural waters [170]. It was demonstrated that this type of DGT provided the time-averaged mean concentration when DGT units were deployed close to the sediment water interphase with a MeHg concentration of 0.308 ng L^{-1} and at pH 8.1. A similar homemade DGT was successfully tested for the assessment of labile MeHg in freshwaters after photodegradation experiments [181].Likewise, Fernández-Gómez et al. investigated three different types of samplers (commercial, manufactured with agarose diffusive gel and manufactured with polyacrylamide diffusive gel) in laboratory assays using the same binding agent, 3-mercaptopropyl functionalized silica gel [173]. Although this binding agent was shown to be efficient for measuring both organic and inorganic Hg species, the best performance was obtained when polyacrylamide was used as a diffusive gel. However, agarose gel resulted more suitable for the measurement of Hg at a very low concentration in water due to its slightly larger uptake rate as well as its low resin blank values. Interestingly, when DGTs were used in freshwaters containing DOM for MeHg determination [172], the diffusion coefficients for DGTs containing both agarose and polyacrylamide gels were significantly different, with higher values for ones with agarose gels. This fact revealed that this gel might perform better in waters dominated by DOM. The same resin gel was used for the simultaneous determination of MeHg and Hg(II) in fresh, estuarine and saline water environments [174]. Other resins such as Duolite GT73 and Ambersep GT74 have also been studied as binding phases for the determination of different Hg species in spiked river water [179]. Furthermore, Wu et al. developed a new DGT device using thiol-modified carbon nanoparticle suspension as the liquid binding phase and cellulose acetate membrane as the diffusive layer for the determination of Hg(II) in water [177]. It was found that this new device could work efficiently in a wide range of ionic strength and that it was not affected by possible interfering ions. Recently, Turull et al. have evaluated the possibility of using the IL TOMATS as a binding agent of DGT when incorporated in a PIM using CTA as the polymer [70]. Although it was not possible to correlate the metal extracted with the metal present in the river water, a high performance in terms of THg extraction was achieved, showing TOMATS as a suitable binding agent for the DGT technique.

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Chapter 2

Objectives

The overall aim of this study is to develop innovative analytical methodologies to facilitate mercury detection in natural waters using ionic liquids. Therefore, this general aim will be met by achieving the following specific objectives:

- To investigate the possibility of obtaining the ionic liquids trioctylmethylammonium thiosalicylate (TOMATS) and trioctylmehtylammonium salicylate (TOMAS) from the commercial extractant Aliquat 336 and the corresponding sodium salts of the anions.

- To prepare and characterize polymer inclusion membranes (PIMs) containing TOMATS and TOMAS, and to evaluate their capacity to extract Hg(II) from natural waters.

- To develop a new analytical methodology to determine Hg(II) in natural waters based on a preconcentration using a PIM containing TOMATS acting as a sorbent followed by Energy Dispersive X-ray Fluorescence (EDXRF) analysis.

- To investigate the effect of the PIM components, *i.e.* polymer and plasticizer, using TOMATS as the carrier, on both the membrane's characteristics and the efficiency of the separation system in terms of Hg(II) preconcentration from natural waters.

- To develop a global preconcentration system based on a PIM with TOMATS to facilitate the detection of Hg(II) when contained in any type of natural water, and to evaluate the effect of the presence of other toxic metals on Hg(II) transport.

- To explore the use of TOMATS as a potential new binding phase for the diffusive gradient in thin film (DGT) technique and its application for Hg(II) monitoring.

Chapter 3

Results

Chapter 3

SECTION 1

A polymer inclusion membrane as an effective sorbent to facilitate mercury storage and detection by X-ray fluorescence in natural waters

3.1.1 Abstract

A novel and simple method is presented for the preconcentration and determination of mercury (Hg) from natural waters through its extraction into a polymer inclusion membrane (PIM) containing the task-specific ionic liquid trioctylmethylammonium thiosalicylate (TOMATS) followed by Energy Dispersive X-ray fluorescence (EDXRF) analysis. The determination was made directly on the membrane without any treatment or elution step, and due to the characteristics of the PIM no matrix or thickness corrections were required in EDXRF analysis. Under the best extracting and EDXRF operating conditions a Hg limit of detection of 0.2 µg Hg L^{-1} was obtained. Moreover, no water matrix effect was observed when Hg was extracted from different types of water such as river, seawater, groundwater and tap water, showing this extraction system as a global solution when dealing with natural waters. Interestingly, this Hg collected in the PIM has shown to be stable for at least 6 months without the use of any preservative. This fact is of prime importance taking into account usual stability problems of Hg during sample storage.

3.1.2 Background and aims

At present, there is a considerable interest in Hg monitoring due to its widespread occurrence and the high toxicity of most of its compounds [1].

In this sense, the European Water Framework Directive (2008/105/EC) [2] classifies Hg as one of the 33 “Priority pollutants”, and the World Health Organization (WHO) as well as the Council Directive 98/83/EC [3] on the quality of water intended for human consumption has established a limit of 1 µg L^{-1}. Taking into account the low concentration levels of this metal and the complexity of some natural waters the reliable determination of Hg is a major analytical challenge [4]. Moreover, it is well known that Hg suffers from stability problems during sample storage, which can lead to serious systematic errors of mercury determination. Sample stability is a critical prerequisite if analytical data obtained from the analysis of the sample are to provide valid information about the metal present in the original sample. Several possible mechanisms have been hypothesized as assisting the loss of Hg, including adsorption on the container walls or metal volatilization. Different strategies have been developed to prevent this loss, such as the addition of preservatives (acids and complexing agents) or containers pre-treatment [5,6].

Commonly used techniques for Hg determination include cold vapor atomic absorption spectrometry (CVAAS) [7], atomic fluorescence spectrometry (AFS) [8], direct analysis by thermal decomposition [9] or direct mercury analyzer (DMA), which integrates thermal decomposition, gold amalgamation preconcentration, followed by atomic absorption spectrometry [10]. Hallmarks of these techniques include limits of detection at ng L^{-1} levels and a high dynamic range. However, these techniques are specially designed for Hg determination and this fact limits their use in multipurpose environmental laboratories. Inductively coupled plasma mass spectrometry (ICP-MS) has also been used for Hg determination in low salinity water samples [11]. Nevertheless, despite of the excellent detection limits, Hg memory effects really hamper ICP-MS measurements at ultra trace levels [12].

Another alternative to determine Hg in water samples is X-ray fluorescence spectrometry (XRF). Most XRF techniques also comply with the desired feature of multi-elemental capability but their use has been restricted mostly to the analysis of solid samples [13,14]. In the case of natural water samples, XRF is not widely used because the sensitivity of the conventional XRF spectrometers such as Energy Dispersive XRF (EDXRF) and Wavelength Dispersive XRF (WDXRF) is lower than other instruments. Moreover, direct XRF analysis of solutions entails technical difficulties related to the bubbles released from solutions and the heating process during the measurement. This fact is especially problematic in the case of volatile elements determination such as Hg. To cope with these problems, in the last years, a wide range of preconcentration procedures have been used in combination with XRF spectrometry [15]. In principle, any preconcentration and separation method developed for any analytical technique could be used in combination with XRF. Nevertheless, taking into account that XRF operates best on solid samples and gives optimal sensitivity and accuracy for thin and homogeneous targets, preconcentration procedures leading to solid thin targets are very suitable in combination with XRF [16].

Accordingly, organic functionalized membranes have been applied in the past to entrap metallic species as a preconcentration procedure before XRF analysis. In our previous works, the use of a membrane containing the anionic exchanger Aliquat 336 (a quaternary ammonium salt) allowed the isolation of different metals such as Cr(VI) [17], Cd [18], and Pd(II) and Pt(IV) [19] contained in liquid samples prior XRF analysis. The use of these membranes as a sample pretreatment offers important advantages compared to other proposed preconcentration methodologies such as simplicity and high throughput. Moreover, this is a particularly convenient means of separation for the X-ray fluorescence method because the metal loaded-membrane can be directly mounted in the spectrometer for its analysis after the separation process.

The determination of Hg by XRF has been allowed by means of different strategies such as collecting the metal in selective membranes produced directly on thin film substrates [20] prior the detection by EDXRF or on the surface of quartz reflectors used in total reflection X-ray fluorescence (TXRF) [21]. In these studies, membranes consisted of the polymer polyvinyl chloride (PVC) and different complexing agents such as dithizone or thiourea, among others. This methodology was useful to determine low-level (µg L^{-1}) concentration of Hg in different types of water samples. TXRF spectrometry was also used by Marguí et al. [22] to detect Hg in different wastewater samples, using thiourea at pH=10 as a means to form a stable Hg-complex to avoid volatilization during traditional sample preparation for TXRF analysis. Another approach to facilitate Hg determination by WDXRF is the use of activated carbon impregnated with the extractant Aliquat 336 [23]. To allow metal extraction by this anion-exchanger, metal samples were prepared in HCl, to take benefit of the formation of Hg anionic chloro complexes that are present in chloride solutions [24–26].

The high affinity of Hg towards S-containing reagents, has promoted the development of selective preconcentration systems such as those based on thiol-containing electrospun nanofibrous mercury filter (ENMF) [27], thiourea derivative polymers [10] or liquid membranes containing benzoylthiourea derivatives as extractants [28], for example.

Recently, ionic liquids (ILs), which are salts made of a combination of organic cations with organic/inorganic anions, have attracted increasing attention due to their significant physical and chemical properties such as negligible vapor pressure, good thermal stability and high conductivity [29]. Task specific ionic liquids (TSIL) are a unique subclass of IL with a functional group covalently tethered to the cationic or anionic part that has the ability to coordinate to the metal ions [30,31]. The thiol-containing IL trioctylmethylammonium thiosalicylate (TOMATS) has successfully been used to extract Hg in water samples when impregnated in an activated carbon [32]. In a recent work, we used this TSIL to prepare a polymer inclusion membrane (PIM), a type of functionalized membrane, to act as a binding phase in a Diffusive Gradients in Thin films (DGT) device for Hg monitoring in river water [33]. In PIMs, a suitable carrier is immobilized within the chains of a plasticized thermoplastic polymer, such as PVC or cellulose triacetate (CTA). The polymer provides the membrane with mechanical strength whereas the carrier is the responsible for the extraction. It is worth mentioning that when IL are used as carriers suitable PIMs can be prepared using only a polymer and the IL [34,35] due to the plasticizing characteristics of these reagents. In general, PIMs are easy to prepare, possess good mechanical proper-ties (e.g. strength and flexibility), and are versatile in terms of the target chemical species they can extract and transport [36].

The aim of the present study is to develop a simple methodology based on a PIM containing TOMATS to allow Hg preconcentration from natural waters prior its analysis by a low power benchtop EDXRF system. Factors affecting the extraction system such as water matrix, sample volume or membrane area have been investigated. Also, instrumental parameters have been optimized to enhance the sensitivity of analysis. The developed methodology has successfully been applied to the determination of Hg at low µg L^{-1} in waters intended for drinking purposes. Moreover, we have investigated, for the first time, the use of PIMs as a promising approach for the storage of Hg in the analysis of natural waters without the use of any preservative.

3.1.3 Materials and methods

Reagents and solutions

The TSIL trioctylmethylammonium thiosalicylate was prepared using the commercial reagent Aliquat 336 (mixture of quaternary ammonium compounds, tri-C8-10-alkylmethyl chlorides) (Sigma-Aldrich, USA), and sodium thiosalicylate (TCI, Japan). The polymer cellulose triacetate (CTA) from (Fluka, Switzerland) and the organic solvent chloroform were used to prepare the PIM. Mercury working solutions were prepared daily by the appropriate dilution of Hg standard of ICP 1000 mg L^{-1} ± 2 mg L^{-1} in 12% (w/w) HNO_3 (Fluka, Switzerland) in the tested water.

Water Samples

In this study, different types of natural waters were sampled and used in Hg extraction experiments. The chemical composition of these waters can be consulted in Table 3.S-1. The ionic composition of water samples was determined by an ion chromatograph (IC) DIONEX IC5000 equipped with an autosampler As-AP, a conductivity detector and a IonPac® AS18 anion-exchange column (4x50 mm). The pH and conductivity values were determined with a Crison Model GLP 22 pH meter and Ecoscan, Entech Instruments portable con-ductimeter, respectively. Total organic carbon (TOC) was determined by a Shimadzu TOC-V CSH (230V) Total Organic Carbon Analyzer. Zinc content in groundwater (GW) was found to be 266 µg L^{-1} determined by an inductively coupled plasma optical emission spectroscopy spectrometer system (Agilent Technologies, Agilent 5100 Vertical Dual View ICP-OES, Agilent Technologies, Japan).

All samples were collected in different areas belonging to Girona region (Catalonia, in the northeast of Spain). Water samples were used without any previous treatment such as filtration or pH adjustment.

TSIL synthesis and PIM preparation

TSIL was prepared by exchanging the chloride anion present in the formulation of Aliquat 336 by thiosalicylate anion. For that, 5 g of Aliquat 336 were transferred in a separation funnel and dissolved in 50mL of chloroform. A 30%-fold excess of sodium thiosalicylate over Aliquat 336 were slowly added to this solution. This mixture was vigorously stirred for 5 h at room temperature. Afterwards, it was rinsed with water to remove both the excess of sodium thiosalicylate as well as sodium chloride formed. Washing was repeated until no chloride was detected in the water sample (by the addition of silver nitrate) and the organic solution was dried with magnesium sulphate. Finally, chloroform was evaporated under reduced pressure until a brownish viscous liquid was obtained.

The procedure to prepare the PIM was the following: 200 mg of the polymer CTA were dissolved in 20 mL of chloroform, and after 5 h under magnetic stirring, 200 mg of TOMATS was added to have a PIM composition of 50% CTA+50% TOMATS (% in mass). This mixture was continuous magnetically stirred for 2 h. Then, the solution was poured into a 9.0 cm diameter flat bottom glass Petri dish, which was set horizontally and loosely covered. The solvent was allowed to evaporate overnight at room temperature and the resulting film was peeled of the Petri dish. The scanning electron microscopy (SEM) observations of the membrane samples were made using a FE-SEM Hitachi, S-4100 (Japan). The samples were placed on a stub and coated with carbon (model K950 turbo evaporator, Emitech Germany). Digital images were collected and processed by Quarz PCI program. Figure 3.1 shows that the resulting membranes are dense and homogeneous. Circular segments were cut from the center of the membrane to ensure uniform thickness (43±3 µm, measured using a Digimatic Micrometer 0-25 mm, Mitutoyo, Japan) and were used for extraction experiments.

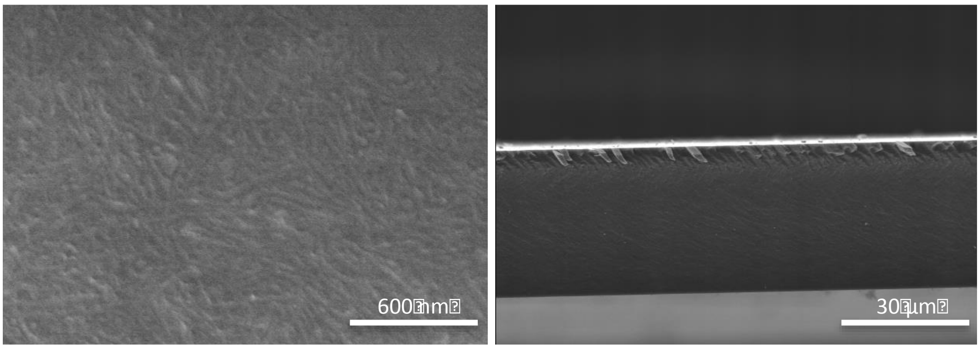

Figure 3.1. SEM images of the PIM. Left: surface; right: cross-section.

PIM-assisted Hg-extraction

To check the effectiveness of PIM containing TOMATS for Hg extraction solid-phase extraction experiments were conducted in beakers containing 25 mL of 1 mg L^{-1} Hg in

GW and a membrane piece of 4 cm^2. Solutions were stirred with orbital agitation at a constant rate with a basic KS250 multiple stirrer (Ika, Labortechnik, Germany). Hg concentration was monitored at different time intervals and each sample was analyzed by ICP-OES and the extraction efficiency was calculated by Equation (1):

$$Extraction\ efficiency\ (\%) = \frac{([Hg^{2+}]i - [Hg^{2+}]f)}{[Hg^{2+}]i}\ x\ 100 \qquad \text{(Equation 1)}$$

Where [Hg^{2+}]i and [Hg^{2+}]f are the metal concentration in the aqueous phase before and after the extraction, respectively.

PIMs analysis by EDXRF

To check the suitability of PIMs for XRF analysis, a first set of experiments was carried out by contacting circular segments (3 cm diameter) of PIMs and 100 mL of GW with Hg at spiked concentrations ranging from 0 to 500 µg L^{-1}. PIMs were left in contact 24 h under orbital agitation. Afterwards, membrane segments were withdrawn, rinsed with ultrapure water, and let to dry at room temperature.

Experiments performed to optimize EDXRF parameters were done using similar segments of membrane (3 cm diameter) previously contacted with 100 mL 500 µg L^{-1} Hg in GW, whereas the effect of water matrix was investigated using a Hg concentration of 100 µg L^{-1} in the different water samples tested (GW, tap water, river and seawater).

In order to increase the amount of Hg per cm^2 of PIM, some experiments were performed varying the area of the membrane. Thus, circular segments with diameters ranging from 1 to 3 cm were used which represented and amount of TOMATS varying from 4x10^{-5} to 5x10^{-6} moles, respectively. These values ensure an excess of TOMATS, extending over about 2 orders of magnitude as minimum with regards to Hg amount in water samples. Considering a 1:1 extraction stoichiometry [37] the extraction capacity of the membrane is 6 µmoles Hg/cm PIM.

For EDXRF analysis, PIM segments were directly placed between two 4.0 µm thick Prolene X-ray foils (supplied by Chemplex Industries, Inc., Palm City. USA) mounted in special sample holders, which incorporates snap-on ring at the end of the cell for attachments of thin-film supports. On top of the cell, a piece of Teflon (1.5 cm height) was placed as a back stopper. Afterwards, samples were sealed in the sample holder of the equipment for EDXRF analysis.

A commercially available benchtop EDXRF spectrometer (S2 Ranger, Bruker AXS, GmbH. Karlsruhe, Germany) was used in the present study. This instrument is equipped with a Pd target X-ray tube (max. power 50 W) and a XFLASH™ LE Silicon Drift Detector (SDD) with an ultra-thin beryllium window (0.3 µm thickness) and a resolution lower than 129 eV at Mn-Kα line for a count rate of 100000 counts per second (counts/s). The instrument is also equipped with nine primary filters and it can operate under vacuum conditions. An additional advantage of the EDXRF system used includes low operating costs because it does not require cooling media for function.

The software used to control the equipment and to perform the data treatment was SPECTRA EDX (Bruker AXS, GmbH, Germany). This software can perform the full line profile fitting, deconvolution when lines are overlapped, intensity correction for inter-element effects and full-quantitative routines. Measuring conditions were carefully evaluated in order to obtain the best sensitivity for Hg determination.

3.1.4 Results and discussion

Testing PIM abilities to extract Hg

First, the extraction abilities of the PIM to extract Hg contained in GW was investigated. The results in terms of metal extraction are presented in Figure 3.2. As it can be seen, the extraction of Hg increases with time, reaching a quantitative extraction after 15 h. For practical reasons, further experiments were done fixing 24 h as time of contact.

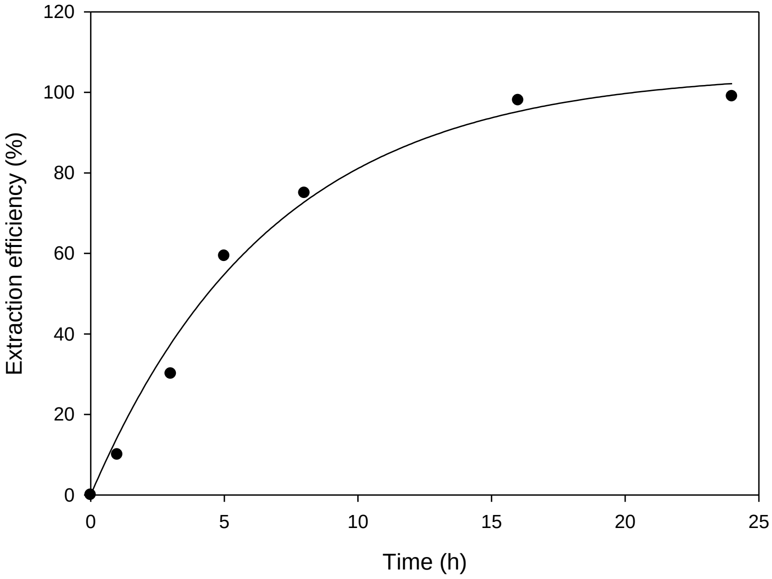

Figure 3.2. Hg(II) extraction efficiency vs time.

Analysis by EDXRF

In a first stage, the analytical capability of PIMs for Hg extraction in combination with EDXRF analysis was investigated by using different segments of a PIM, namely: without contact (blank), in contact with GW (no spike), and immersed in GW with Hg at spike levels from 100 to 500 µg L^{-1}. Once dried at room temperature, the corresponding PIM was simply analyzed by EDXRF without any treatment. Resulting EDXRF spectra are displayed in Figure 3.3. As it is shown, in all the spectra (Figure 3.3 (A)), a prominent sulphur peak arising from the PIM matrix (TOMATS) can be observed. In addition, since the EDXRF system is equipped with a Pd X-ray tube, characteristic radiation from this source can also be identified in the resulting EDXRF spectra. Small peaks of Cu, Fe and Zr due to the sample holder used are also present in all spectra as well. The presence of a peak at 8.638 KeV, characteristic of Zn, for the PIM contacted with GW, points out the feasibility of TOMATS to extract this metal from natural waters. However, as it can be seen in the zoom view of the region where Hg-L_{α} line is placed (see Figure 3.3 (B)), there are no spectral overlaps and Hg peak can be properly fitted. The comparison of EDXRF spectra obtained for the three (100, 200 and 500 µg L^{-1}) spiked GW samples reveals that Hg signal increases when increasing the metal concentration initially present in the aqueous solution. The representation of the peak area obtained for each membrane vs. the initial Hg content in the water sample shows a very good linearity (R2=0.998).

This result indicates that there is a strong relationship that can be used as a calibration curve for future determination of Hg when contained in water samples. Moreover, the characteristics of PIMs (organic thin layer) avoids the need of matrix and thickness effect corrections commonly required in EDXRF analysis since, in this case, the attenuation of the incident primary and emergent secondary spectral line radiation is almost negligible and thus the analyte intensity is directly proportional to its concentration [38].

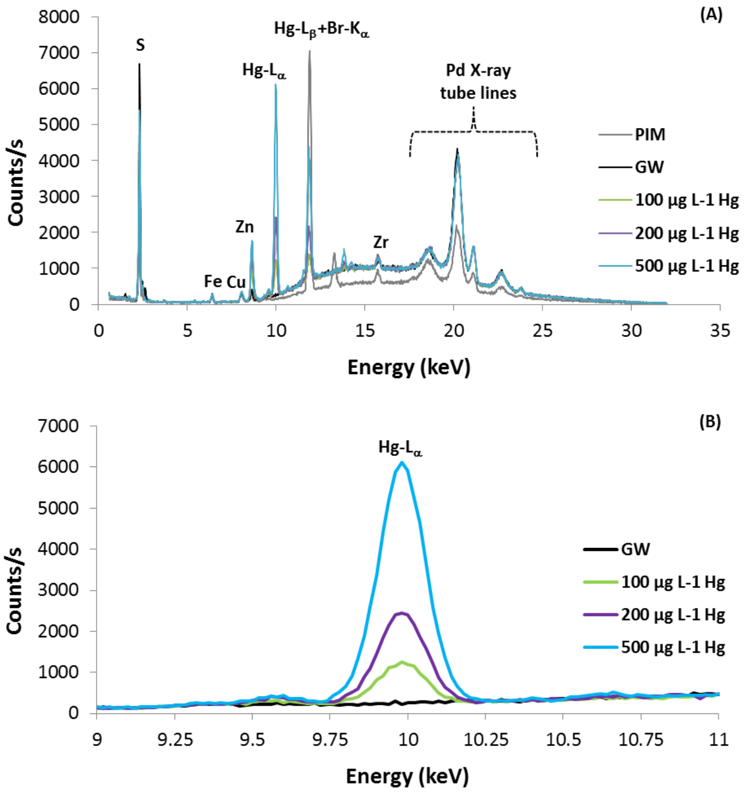

Figure 3.3. EDXRF spectra for a PIM (blank) and PIMs contacted with groundwater (GW) and GW spiked at different Hg concentration levels. EDXRF experimental conditions: 40 kV, Al 500 µm primary filter, 100s measurement time.

Once demonstrated the potential of PIMs for Hg extraction in water samples, a careful evaluation of the EDXRF measurement conditions was carried out in order to obtain the best sensitivity for Hg determination at trace and ultra trace levels.

Effect of water matrix on Hg extraction

Mercury extraction can be affected by other compounds present in natural waters such as metals or ions that can be competitive species. Moreover, Hg speciation is highly influenced by the media. In the case of natural water, at neutral pH, predominant species are neutral or cationic, whereas the anionic complex $HgCl_4^{2-}$ is the prevailing inorganic Hg(II) species in seawater. For example, the use of Aliquat 336 to functionalized the membrane only allows the extraction of Hg in the anionic form, and thus, cannot be used as a general extractant for all water samples. We investigated the ability of the formed PIM with TOMATS to collect Hg from very different water composition such as tap water, river and seawater besides GW (see Supplementary Material Table 3.S-1 for chemical composition). The obtained results are shown in Fig. 3.4 in terms of Hg signal acquired from PIMs contacted with different spiked water samples (100 µg L^{-1} Hg). It is worthy to mention that water samples were used without any previous treatment such as filtration or pH modification. As it can be observed, similar results were obtained for the four waters tested. Indeed, no statistical significant differences (*P* >0.05) in Hg signal were observed for different waters.

These results suggest that PIMs made of 50% TOMATS and 50% CTA is a suitable sorbent to ensure Hg preconcentration from any type of natural water with a notorious sample handling minimization.

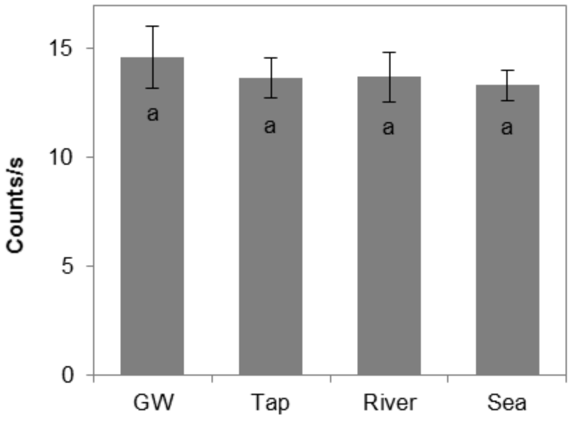

Figure 3.4. Matrix effect on Hg extraction. PIMs were contacted with 100 mL of different water samples containing 100 µg L^{-1} Hg. Error bars represent mean ± SD (n = 2). Different letters within the graph indicate significant differences.

Evaluation of EDXRF measuring conditions

As aforementioned, EDXRF measurement parameters including the X-ray tube potential, the use of primary filters, the sample holder type and the measurement time were evaluated to select the best conditions for Hg determination at trace levels.

Firstly, the influence of excitation conditions was tested. The target PIM was analyzed at 30 kV, 40 kV and 50 kV and in all cases the intensity was adjusted automatically to obtain a maximum count rate of 100.000 counts/s. A potential of 40 kV was selected as a trade-off between an acceptable net signal for Hg peak and a good signal-to-noise ratio.

The influence of the placement of primary beam filters (Al 20µm, Al 500µm, Cu 100µm, Ag 5µm) between the X-ray source and the measured sample on Hg response was also evaluated. The role of these filters is to reduce the intensity of interfering elements and/or the continuum of the X-ray tube, improving in this way the signal-to-noise ratio for element determination. The metal and thickness of the primary filter depends on the region where the element of interest is located in the EDXRF spectra [39]. EDXRF spectra obtained when using the different filters can be seen in Figure 3.5.

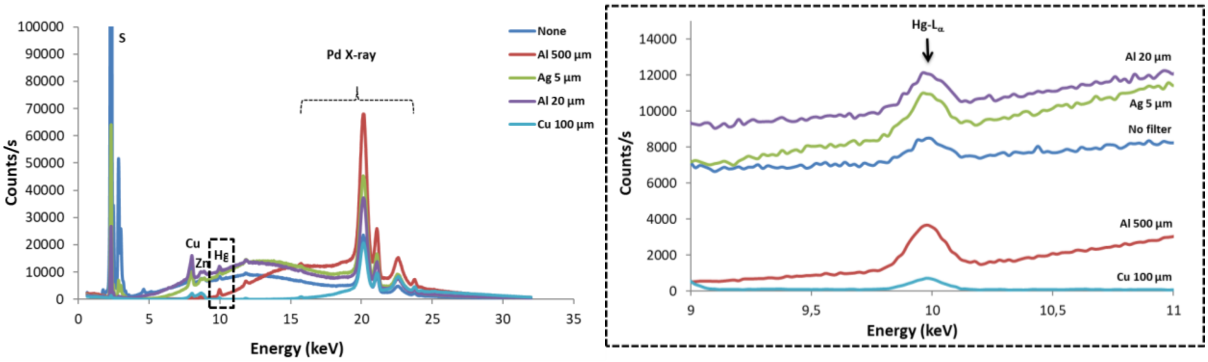

Figure 3.5. EDXRF spectra showing the influence of primary filters on Hg determination (40 kV, 100s measurement time).

As can be observed, for elements with emission lines lower than 5 keV (including S), the best choice is the use of a voltage of 30 kV without the addition of any primary filter. Otherwise, for elements with higher energy emission lines (including Hg) the strategy to get the best signal to noise ratio and a better Hg response is the use of an Al foil of 500 µm thickness as primary filter.

In EDXRF instruments, the sample is usually placed into a metal holder cup. Hence, radiation coming from these materials can interfere to the spectrum shape and therefore, may modify its determination. This fact is particularly relevant when thin layers or light matrices are analyzed. For this reason, different sample holder types were evaluated for loaded PIM samples analysis such as a commercial sample holder designed for filter analysis (Bruker AXS) (a), Teflon cups of 3.5 cm (b) and 2.0 cm (c) which incorporate snap-on ring at the end of the cell for attachments of thin-film supports (Table 3.1). In the case of sample holders b and c, the use of a Teflon or a boric acid disk back stopper at the bottom of the cup was also evaluated for scattering reduction. Teflon and boric acid possess low transmittance at 20 kV that makes them a proper mask material for this application. As it is shown in Table 3.1, using the Teflon cups as sample holders (b and c), the signal to noise ratio (S/N) and the Hg response were clearly improved in comparison with the commercial sample holder (a) designed for filter analysis. Similar results were obtained in terms of S/N and Hg response when using sample holders 2 and 3 with and without any back stopper. Finally, the combination of a Teflon cup of 2.0 cm (c) and a Teflon disk as back stopper was selected to carry out the analysis of the loaded PIM samples as a compromise between a good Hg signal and S/N ratio.

Table 3.1. Influence of sample holder types tested for PIMs measurements on Hg EDXRF responses (40 kV, Al 500 µm primary filter, 100s measurement time).

Sample holder	Back stopper	S/N	Counts/s
(a) 	None	2.9	22.9
(b) 	None	20.0	50.3
	Boric acid pellet	18.2	50.4
	Teflon disk	17.6	49.7
(c) 	None	18.5	55.8
	Boric acid pellet	17.2	53.7
	Teflon disk	19.3	54.0

Measuring time has also a significant influence on the precision of the results and limits of detection (LOD) [40]. For that, the influence of measuring time on the relative standard deviation, RSD (n=5), of the results obtained when analyzing two PIMs contacted with 100 mL of GW at two Hg levels (e.g. 10 and 500 µg L^{-1}), was investigated. As it is shown in Figure 3.6 (A), RSD values depend on the measurement time but also on the Hg concentration level. For high concentration levels (500 µg L^{-1}) RSD values as low as 2% can be obtained even using very short measurement times (50 s). In the case of low Hg levels (10 µg L^{-1}) RSD values are higher (10% to 13%) with almost no differences between the results obtained at different measurement times (50s vs. 300s). On the other hand, LOD (Figure 3.6 (B)) was also not really influenced by measuring time. For in-stance, increasing the measuring time three times (from 100 to 300s), only a LOD decrease of around 20% was estimated. In view of the achieved results, further analysis were performed fixing a measuring time of 100 s.

As it is shown in Figure 3.6 (B), using the best measuring conditions (40 kV, Al 500µm primary filter and 100s measuring time) a LOD for Hg of around 10 µg L^{-1} was estimated. This value is ten times higher than the maximum permissible limit of Hg in drinking waters [3]. Thus, in order to diminish the LOD and expand the application of the developed methodology, some parameters that should improve the capacity of PIMs to uptake Hg were studied, such as water sample volume and the size of the membrane.

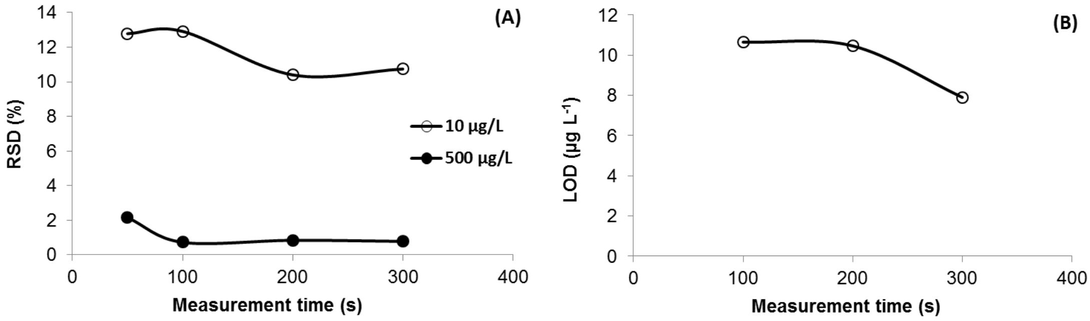

Figure 3.6. Effect of measurement time on Hg loaded PIMs: (A) precision (RSD (%), n=5) and (B) Hg detection limit (µg L^{-1}).

Influence of PIM size and sample volume

In a previous work it has been pointed that the diameter of the analyzed sample is a critical parameter in XRF analysis [41]. It has to be ensured that the whole sample is irradiated and the resulting element characteristic radiation is acquired by the detector system. For that, smaller segments of PIMs were cut to cover the range of 1-3 cm diameter of membrane and were contacted with the same amount of Hg. Afterwards, these PIM segments were mounted in the holder and analyzed. It was observed that the sensitivity increased while decreasing the area of the membrane (Figure 3.S-1). This fact reveals the high capacity of the PIM to extract Hg that allows obtaining a higher ratio Hg/PIM in the 1 cm diameter segment.

With the aim of applying the developed methodology to a more realistic Hg concentration in natural waters, the effect of sample volume was also investigated, using PIMs of 1cm diameter and 100 mL, 500 mL and 1 L of GW spiked with 6 μg L^{-1} Hg. Increasing the solution volume increases the total Hg mass in solution, and as a result the metal signal is expected to rise up. However, it was observed that a volume of 500 mL gave a similar signal to that obtained with 1000 mL for 24 h of contact (Figure 3.S-2). Other experiments were run with 1000 mL sample and increasing the contact time to 48 h, and a higher Hg signal was obtained when analyzing the PIM. In order to simplify the methodology, we choose 500 mL and 24 h contact time for further experiments.

Limit of detection, dynamic range, precision, and accuracy

Applying the best conditions to extract and determine Hg, analytical figures of merit of the proposed methodology were evaluated and obtained results are summarized in Table 3.2.

LOD was estimated by analyzing a PIM contacted with GW spiked at the level of 3 μg L^{-1} and using the 3σ criteria [40]. It was found that the minimum Hg content that can be detected in a water sample was 0.2 μg L^{-1} which satisfies the possibility of determining Hg in drinking waters at the permissible level of 1 μg L^{-1}.

For calibration purposes, GW samples (n=5) containing Hg concentration in the range 0.6-10 μg L^{-1} were taken throughout the whole methodology described above. Intensities of characteristic Hg-Lα line obtained under the selected operating conditions for each membrane were plotted versus known concentrations of Hg in the initial aqueous solutions and a straight line was fitted to measured points by the least-square method (confidence limit 95%). As it is shown in Table 3.2, the regression coefficient was higher than 0.99, indicating a good linearity throughout the studied working range.

Global precision of the PIM-EDXRF method was tested by analyzing 8 independent replicates (i.e. 8 different PIM segments) previously contacted with 500 mL of GW (6 μg L^{-1} of Hg). Besides, one of the replicates was measured 8 times and the RSD associated was also calculated. This uncertainty is related to the instrument stability and counting statistics. Therefore, by means of error propagation, the uncertainty due to sample preparation (extraction of Hg using PIMs) can also be estimated. Results obtained are displayed in Table 3.2. As it is shown, global precision is acceptable with a RSD value of 16.5 %. From the obtained RSD values, it can also be deduced that uncertainties in sample preparation have a significant contribution to the global precision of the obtained results.

Table 3.2. Analytical characteristics of the developed method for Hg determination.

Parameter	Analytical feature
LOD	0.2 μg L^{-1}
Linear range	0.6-10 μg L^{-1}
R^2	0.997
Method precision: n=8. 1 measurement (6 μg L^{-1})	RSD: 16.5%
Instrumental precision: n=1. 8 measurements (6 μg L^{-1})	RSD: 3 %

The accuracy of the developed methodology was investigated by analyzing two types of drinking water with spiked Hg(II) (1 μg L^{-1}). Results obtained with the developed methodology of PIM preconcentration and EDXRF analysis were also compared in Table 3.3 with those obtained from the direct analysis of waters by ICP-MS (Agilent 7500c Inductivity Coupled Plasma Mass Spectrometer, Agilent Technologies, USA). No significant differences ($p>0.05$) are observed within the values of Hg added or determined by ICP-MS and Hg found by PIM-EDXRF revealing the good accuracy of the new methodology.

Table 3.3. Hg determination in drinking waters using the developed methodology

Sample	Hg added	ICP-MS (recovery)	PIM-EDXRF (recovery)
Tap water (µg L^{-1})	1.018	1.176 (115%)	1.079 ± 0.21[a] (106%)
Mineral water (µg L^{-1})	1.009	1.119 (111%)	0.0907 ± 0.16[a] (90%)

[a]SD, n=2.

Evaluation of PIM as a sorbent to store Hg from natural waters

Nowadays, the appropriate techniques for the preservation and storage of aqueous samples for ambient-level Hg (ng L^{-1} to µg L^{-1}) are well-documented. Despite that, risk of contamination due to minor contamination of the sampling materials, additives, and the storage containers is always probable. Moreover, Hg may be lost to the walls of the container regardless of acidification samples [5], and thus, can affect the reliability of its final determination. This analyte loss due to adsorption on surface of the containers can be minimized using Teflon vessels, but is not economical. Moreover, especially during long-time field campaigns, it is sometimes difficult to conduct a rapid determination or even the preservation of Hg before access to an adequate laboratory. This fact is especially relevant for studies done in remote regions. Hence, it is of paramount importance to provide a trustworthy media to store and preserve mercury samples. In this sense, PIMs can act not only as a sorbent to extract and preconcentrate Hg, but also to be a suitable material to preserve the metal until the appropriate analysis can be done. To explore this possibility, we performed the analysis of the PIMs previously loaded with Hg from GW samples (concentrations ranging from 10 to 500 µg L^{-1}) after 6 months of the extraction. PIMs were kept in the laboratory without any special precaution (i.e., at room temperature and without any film to protect them). Metal signals obtained for each membrane from EDXRF analysis were compared with those obtained when PIMs were analyzed immediately after the extraction process (Figure 3. 7). As it can be observed, no significant differences ($P>0.05$) were obtained for the same PIM analyzed the day 1 or after 6 months, showing that the concentration of Hg extracted in PIMs does not change over time. This finding can lead to the development of suitable sorbents that can be used to preserve sample information of natural waters. Moreover, considering that water matrix did not affect the extraction efficiency of the membrane, the developed PIMs can offer a robust and inexpensive solution for Hg extraction, preservation and storage for all types of natural waters in one step.

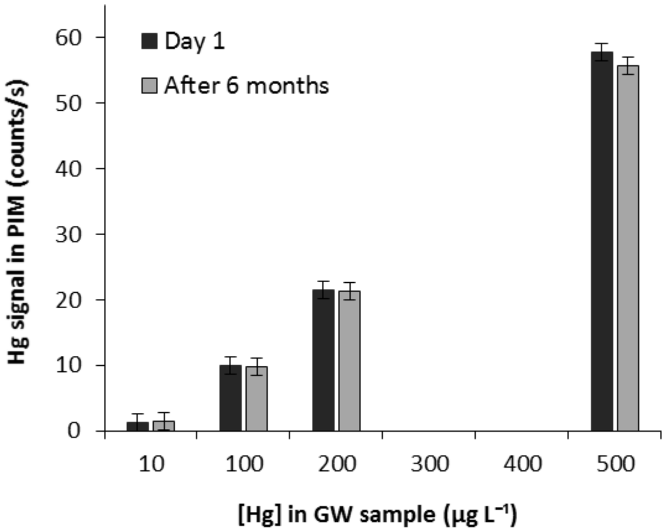

Figure 3.7. Effect of time on metal signal collected in PIMs contacted with 100 mL GW with 10-500 µg L^{-1} Hg and membrane diameter 3 cm (n=2).

3.1.5 Conclusions

The methodology presented here is based on a PIM containing TOMATS in combination with EDXRF spectrometry enabled the determination of Hg at low µg L^{-1} level from natural waters. This methodology exhibited excellent repeatability and accuracy with a LOD of 0.2 µg L^{-1} when using 500 mL of sample volume and a PIM segment of 1 cm diameter, and has been successfully applied to the detection of Hg in drinking waters at the legislated levels. Some important advantages should be highlighted from the proposed methodology, such as the simplicity of the preparation of the PIMs, low cost, high selectivity exhibited towards the metal, and the fact that no analyte elution step is required. Moreover, PIMs have revealed to be a suitable material to extract and preserve Hg for long periods of time without any alteration on its concentration, which is of paramount importance for a reliable quantification in environmental samples. Finally, since no matrix effect was observed when Hg was detected in different types of water, this simple method seems to be an appropriate global solution when dealing with natural waters.

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3.1.7 Supplementary data

Table 3.S.1. Characteristics of the water samples used for this study (expressed in mg L-1 for ions and TOC and µS cm-1 for conductivity)).

Source	pH	conductivity	Na^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	SO_4^{2-}	Br^-	F^-	NO_3^-	TOC
Groundwater	8.3	527	17.9	17.1	30.1	96.1	15.4	63.9	<0.01	nt	1.2	nt
Mineral water	7.9	237	11.7	1.3	4.5	30.3	7.5	11.6	<0.01	0.82	1.7	0.30
Tap wàter	7.8	394	20.0	3.5	8.8	52.8	28.3	42.3	<0.01	0.10	4.9	1.84
River	7.2	462	11.8	3.1	14.9	119.5	16.5	26.2	nt	nt	7.6	1.15
Seawater	8.1	66100	11764	428	1412	443	21075	2920	77.6	1.06	<0.02	4.82

Nt: not tested.

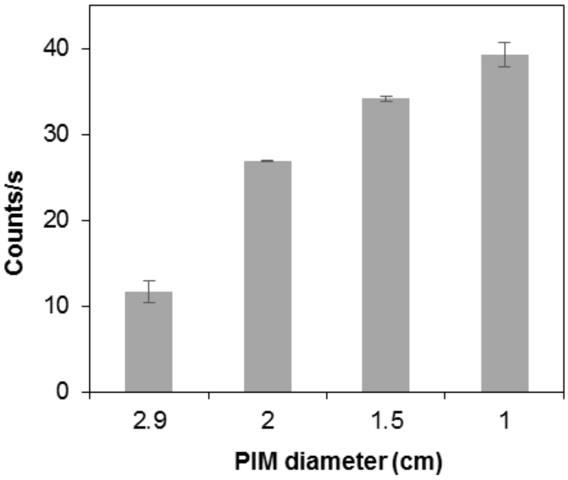

Figure 3.S. 1. Effect of PIM size on Hg signal. PIMs were contacted with 100 mL GW with 100 µg L-1 Hg (n=2).

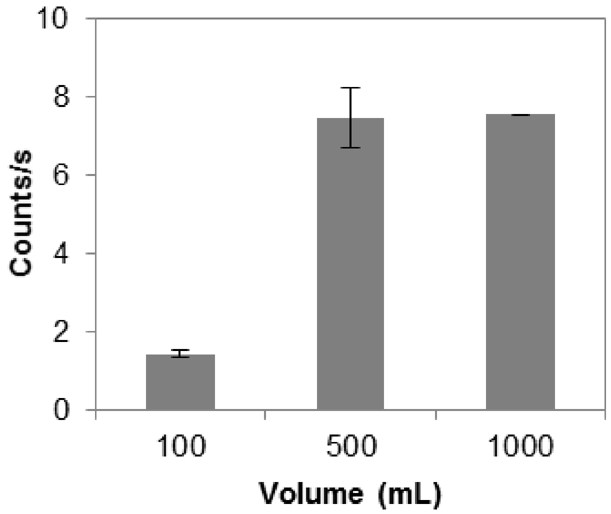

Figure 3.S. 2. Effect sample volume on Hg signal using a 1 cm diameter PIM contacted with GW with 6 µg L-1 Hg (n=2). Contact time: 24h.

Chapter 3

SECTION 2

System for mercury preconcentration in natural waters based on a polymer inclusion membrane incorporating an ionic liquid

3.2.1 Abstract

In this study, we have evaluated two different ionic liquids (IL) as extractants based on the same cation (trioctylmethylammonium) but bearing the anion thiosalicylate (TOMATS) or salicylate (TOMAS). Both IL have been incorporated as carriers in polymer inclusion membranes (PIMs), and mercury (Hg) has been preconcentrated using a special device. Results show that among the tested IL, TOMATS has given better results. A PIM made of 50% cellulose triacetate, 30% TOMATS and 20% nitrophenyl octyl ether as a plasticizer enabled the effective transport of Hg to a 10-3M cysteine solution used as a stripping phase. This novel and simple PIM-device system allows the transport of Hg at low concentration levels in different types of natural waters such as rivers, groundwater and seawater without any previous treatment. Since no matrix effect was observed on Hg transport efficiency with different waters, this newly developed PIM-system could be used as a global detection system for this metal. The effect of biofilm growth on the surface of PIMs has been investigated for the first time, and no significant differences on Hg transport have been found when using a fresh PIM and a PIM deployed 7 days in a pond.

3.2.2 Background and aims

Mercury (Hg) is a toxic metal naturally present in the environment, and is considered a global pollutant given that it has been released into atmospheric, aquatic and terrestrial ecosystems as a result of human activity [1]. Hg occurs in the environment in different chemical forms, but the mercuric ion (Hg^{2+}) is the one that presents high cell toxicity [2] and can be transformed into methylmercury ($MeHg^{+}$) that tends to bioaccumulate and biomagnify through aquatic trophic chains [3]. Given its high toxicity and hazardous effects on wildlife and human health [4], monitoring it in aquatic ecosystems has become a requirement for environmental purposes. In this sense, different international regulations have become aware of it, such as the European Water Framework Directive (WFD) [5] which has identified Hg and its compounds as a priority hazardous substance, or the Minamata Convention on Mercury [6], which is the best prospect seen so far for reducing risks from direct and indirect exposure to Hg at a global level.

The use of extractants, which are able to interact with metals, can be used to facilitate their analysis [7,8]. In this sense, ionic liquids (IL) can be employed as heavy metal extracting agents [9–11]. ILs are a class of organic non-molecular solvents that are liquid at room temperature (and under 100ºC). They are formed by an organic cation, which contains N or P atoms, and an organic or inorganic anion, and possess unique properties that makes them a good alternative for green chemistry [9]. The application of ILs as extracting agents for efficient removal of metal ions can be manipulated by proper selection of the anion, which is supposed to bind to the respective metal ions. Trioctylmethylammonium chloride (Aliquat 336) or trihexyl(tetradecyl)phosphonium chloride (Chypos IL 101) are common sources of cations to form new ILs derivatives [9,10,12–14] as shown in [15] where novel thiosalycilate-based ionic liquids from Aliquat 336 and Cyphos IL 101 are prepared and investigated as heavy metals extractants.

The thiol-containing IL trioctylmethylammonium thiosalicylate (TOMATS) shows a high affinity to extract Hg. This reagent has successfully been used to impregnate activated carbon for Hg removal through a batch-adsorption technique [16]. TOMATS was found to effectively remove Hg from synthetic aqueous solutions at pH 6-8 with a maximum adsorption capacity of 83.33 mg/g. However, the desorption process was not possible. This IL has also been incorporated into a polymeric matrix of cellulose triacetate (CTA) to form a polymeric sorbent used as a binding phase in a Diffusive Gradient of Thin Films (DGT) device for Hg monitoring in river water [17]. Moreover, this CTA-TOMATS sorbent was also investigated to extract Hg from different natural water samples to facilitate its detection by EDXRF [18]. It was found that the extraction of Hg was not affected by the type of water tested (river, sea, tap, and groundwater) and, remarkably, that its content in the polymeric sorbent was stable up to 6 months after extraction. This fact reveals the great stability of the complex formed within TOMATS and Hg.

As a step forward in these recent investigations, it seems appropriate to use polymeric films incorporating TOMATS as functionalized membranes to allow not only the extraction of the metal, but its transport and release to a receiving solution to preconcentrate the metal. These membranes are known as polymer inclusion membranes (PIMs). Besides the extractant and the polymer, PIMs can also incorporate a plasticizer to enhance the mechanical properties of the film as well as to facilitate the diffusion of the extracted species [19,20]. These components are held inside the polymer matrix by a combination of physicochemical interactions [21] and the process of polymer entanglement [22]. Generally, PIMs are stable, homogeneous and dense films, with the absence of pores in their structure, and exhibit good mechanical properties (e.g., strength and flexibility). These membranes are not currently commercially available but can be easily prepared. It is important to point out that the specificity of the membrane relies on the characteristics of the extractant (called carrier when incorporated in the PIM) [23]. The carrier reactively interacts with the compound of interest, acting as the phase-transfer agent and thus extracting the compound from the aqueous phase (or feed phase) into the membrane phase by forming a hydrophobic ion-pair or a complex, which is finally released into a receiving solution (stripping phase) [24]. PIMs have allowed the separation and transport of different species such as metals, metalloids, ions and small organic compounds [19,24]. Moreover, when PIMs are incorporated in a special device containing a small reservoir for the receiving phase, the target analyte can also be preconcentrated into an adequate solution to facilitate its analysis, as is the case of As in groundwater [8], Cd in seawater [25] or the antibiotic sulfamethoxazole [26] in natural waters. The same PIM-based device has also been successfully used as a passive sampler to monitor Zn [27] or ammonia [28].

In the present study, we have taken the opportunity to use this PIM-based device for the preconcentration of Hg from different natural water samples to facilitate its detection. The IL TOMATS has been chosen as a carrier, based on its good performance for extracting Hg. We have also tested trioctylmethylammonium salicylate (TOMAS), with a similar chemical structure to TOMATS, but bearing an alcohol group instead of a thiol (SH-) group. This IL has shown to be a good extractant for some metals such as Cu(II) and Cd(II) [29], but it can also interact with Hg when incorporated in an electrode designed for the potentiometric determination of Cd(II) [30].

With this aim, we have first prepared and characterized both ILs to produce, afterwards, PIMs with TOMATS and TOMAS as carriers to investigate Hg extraction and elution. A device incorporating PIMs has been used for the transport and preconcentration of Hg from different environmental waters with the purpose of obtaining a low-cost and simple system to facilitate Hg monitoring in the environment.

3.2.3 Materials and methods

Reagents, solutions and apparatus

TOMATS and TOMAS were prepared using the commercial reagent Aliquat 336 (Sigma-Aldrich, UEA), chloroform stabilized with ethanol (Panreac, Spain), and the sodium salts of thiosalicylate (TCI, Japan) and salicylate (Fluka, Switzerland), respectively. The polymer cellulose triacetate (CTA) (Fluka, Switzerland), and the plasticizer nitrophenyl octyl ether (NPOE) (Sigma-Aldrich, UEA) were used to prepare PIMs.

Simulated natural water (SNW) with a composition 2 mM $NaHCO_3$, 1 mM $CaCl_2 \bullet H_2O$, 0.5 mM Na_2SO_4 was prepared by dilution of sodium nitrate, sodium bicarbonate, calcium chloride hexahydrate (Panreac, Spain) and sodium sulphate (Merck KGaG, Germany) in Milli-Q water (18.2 MΩ cm). Working mercury solutions were prepared daily by the appropriate dilution of Hg standard of ICP 1000 mg L^{-1} ± 2 mg L^{-1} c(HNO_3)= 12% w/w (Fluka, Switzerland) in the tested water. Milli-Q water used in this work was obtained by a Milli-Q Plus system (Millipore, France). L-Cysteine (MercK KGaA, Germany) and ethylenediaminetetraacetic (EDTA) (Panreac, Spain) were used for elution experiments.

Hg content in the different aqueous samples was measured using an inductively coupled plasma optical emission spectrometer (Agilent Technologies, Agilent 5100 Vertical Dual View ICP-OES) for Hg concentration in the range of 25 – 7000 µg L^{-1} (λ= 253.652 nm). For lower Hg concentrations (1 – 200 µg Hg L^{-1}) an inductively coupled plasma mass spectrometry (ICP-MS) system (Agilent Technologies, Agilent 7500c ICP-MS) was employed. The isotope ^{202}Hg was selected since it is the most abundant. In both cases, standards were specially prepared with the corresponding matrix to avoid the possibility of interferences during sample analyses.

An optical microscope BX-40 (Olympus, Japan) was used to observe the surface of PIMs in biofouling studies.

IL preparation and characterization

ILs were prepared by exchanging the chloride anion present in the formulation of Aliquat 336 by the thiosalicylate or the salicylate anion, for TOMATS and TOMAS preparation, respectively [18,31]. In this study, 3 g of sodium thiosalicylate or salicylate were added for 5 g of Aliquat 336. The chemical structure of ILs is represented in Fig. 3.8.

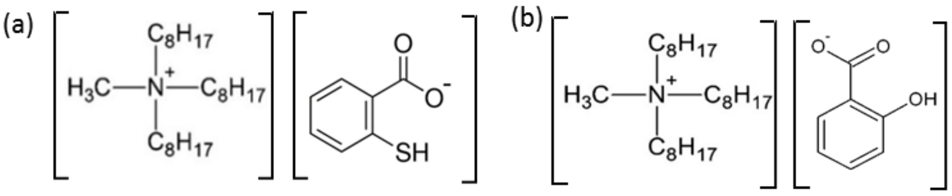

Figure 3.8 Chemical structure of TOMAT (a) and TOMAS (b).

ILs were characterized by elemental analyses with a Perkin Elmer EA2400 instrument and results are shown in Table 3.4 which shows that the experimental values of N, C, H and S are similar to the expected ones, indicating that this procedure is a simple way to obtain the desired ILs.

Table 3.4. Elemental analysis (N, C, H, and S, in %) of both ILs prepared (n=2).

	TOMATS		**TOMAS**	
Element	Theoretical	Analysed	Theoretical	Analysed
N	2.70	2.54 ± 0.01	2.80	2.67 ± 0.04
C	73.60	72.16 ± 0.37	75.90	75.38 ± 0.01
H	11.30	11.76 ± 0.16	11.70	12.69 ± 0.15
S	6.00	6.02 ± 0.13	0.00	0.82 ± 0.09

In addition, proton nuclear magnetic resonance (1H NMR 400 MHz Bruker) was performed for both ILs, using deuterated chloroform (CDCl3) as a reference. The corresponding description of the spectrums confirmed the expected results and can be found in Supplementary Material (Figure 3.S.3).

PIM preparation

PIMs with a composition of 50% CTA - 50% IL (% in mass) were prepared by the solvent casting method as described in [23]. For PIMs with a composition of 50% CTA – 30% IL – 20% NPOE, the same amount of CTA (200 mg) was used, but 120 mg of IL and 80 mg of plasticizer were added. The stability of the PIM was studied in terms of mass loss, which is related with the loss of the carrier [32]. For that, pieces of PIM about 4 cm^2 were immersed in 25 mL of Milli-Q water, sea water or 10^{-3}M cysteine solution, with agitation during 24 h. PIMs were weighted before and after the experiment, and the mass loss was then calculated.

PIM extraction and elution experiments

To evaluate the effectiveness of IL incorporated in PIMs, some experiments were conducted by contacting 4 cm^2 pieces of tested PIMs with 25 mL of 1 mg L^{-1} Hg in SNW under orbital agitation at a constant rate (basic KS250 multiple stirrer Ika, Labortechnik, Germany). Extraction efficiency (EE) was evaluated by Equation 3.1,

$$EE\ (\%) = \frac{([Hg^{2+}]i - [Hg^{2+}]f)}{[Hg^{2+}]i}\ x\ 100 \qquad (Eq. 3.1)$$

where $[Hg^{2+}]$i denotes the Hg initial concentration in the aqueous phase and $[Hg^{2+}]$f is the final Hg concentration in the water sample after contacting with the PIM.

The same procedure was conducted to evaluate the possibility of recovering the extracted metal. To this end, PIMs loaded with Hg were contacted with 25 mL of the tested eluent during 24 hours under orbital stirring. Recovery efficiency (RE) was calculated using Equation 3.2,

$$RE\ (\%) = \frac{[Hg^{2+}]aq}{[Hg^{2+}]i - [Hg^{2+}]f}\ x\ 100 \qquad (Eq. 3.2)$$

where $[Hg^{2+}]$aq denotes the Hg concentration in the eluent after the deployment time of the loaded membrane and $[Hg^{2+}]$i and $[Hg^{2+}]$f are the initial and final Hg concentration in the initial aqueous solution, respectively.

PIM-preconcentration experiments

Figure 3.9 shows the scheme of the PIM-device used in this work [33]. This system consists of a glass tube with a bottom opening that contains a circular piece of PIM fixed with a screw cap especially designed to expose 1.5 cm^2 of membrane to the feed solution. The glass tube is then filled with 5 mL of receiving phase and the top opening is closed with a standard screw cap. To carry out the experiments using the device, 100 mL of water spiked with Hg (1-500 µg L^{-1}) were poured into a glass beaker and placed on a magnetic stirrer Multistirrer 15 (Fisher Scientific, USA). As a receiving phase, 5 mL of 10^{-3} M cysteine or 5 mL 10^{-2} M EDTA were investigated.

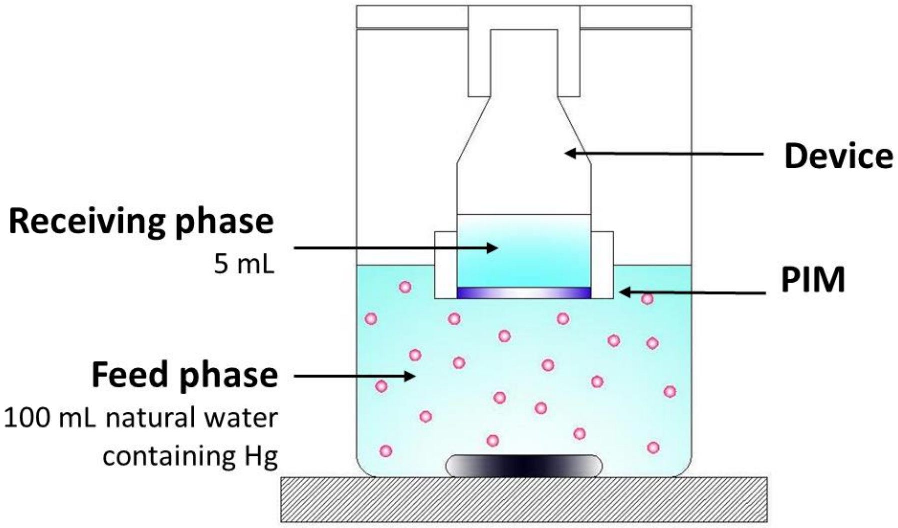

Figure 3.9. Scheme of the PIM-device system used for Hg(II) preconcentration experiments.

After 24 h of deployment time, the PIM-device was removed from the feed solution and the receiving phase was taken for analysis. The performance of the system was studied in terms of transport efficiency (TE) calculated following Equation 3.3,

$$TE(\%) = \left(\frac{Vs}{Vf}\right)\left(\frac{[Hg^{2+}]_{rec_{(t)}}}{[Hg^{2+}]_{feed_{(0)}}}\right)x100 \qquad (Eq. 3.3)$$

where $[Hg^{2+}]_{rec(t)}$ denotes the Hg concentration in the receiving phase at time t, $[Hg^{2+}]_{feed(0)}$ is the initial Hg concentration in the water sample and V_s and V_f are the volume of stripping and feed solution, respectively.

4.3.6 Water samples

To evaluate the effect of water composition on Hg transport, several natural water samples with different chemical composition were tested. Samples were collected at different locations in Catalonia (NE Spain): groundwater (GW) was collected from Pujarnol (Pla de l'Estany, Girona), seawater samples from the Mediterranean Sea (Tossa de Mar, Girona), and river water was sampled from the Ter River (Girona). For application purposes, hot spring water from Caldes de Malavella (Girona) and bottled mineral water from Viladrau (Girona) were also included. The chemical composition of these samples is shown in Table 3.5.

The ionic composition of the water samples was determined by an ion chromatograph (IC) DIONEX IC5000 equipped with an autosampler AS-AP, a conductivity detector, and a IonPac® AS18 anion-exchange column (4x250mm) with the AG Guard column (4x50 mm). The pH values were determined with a Micro pH 2002 (Crison, Spain), and conductivity was obtained with a hand-held Eutech instrument TDS 6+. Total organic carbon (TOC) was determined by a Shimadzu TOC-V CSH (230V) analyzer.

Table 3.5. Chemical characteristics of the water samples used for this study (concentrations are expressed in mg L^{-1} and conductivity in µS cm^{-1}). * Data from [20].

Source	pH	conductivity	Na^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	SO_4^{2-}	HCO_3^-	NO_3^-	TOC
GW*	8.3	527	17.92	17.12	30.10	96.10	15.43	63.86	268.5	1.17	0.22
River*	7.2	462	11.78	3.09	14.91	119.54	16.52	26.19	460	7.57	1.15
Sea*	8.1	66100	11764.13	427.87	1411.86	442.95	21075.16	2919.51	145.26	<0.02	4.82
SNW	7.45	459	70.18	0	0	40.25	71.19	47.35	120	0	0
Hot spring	7.36	3500	1190	2.7	8.8	24.4	570.1	41.4	1998.5	<0.02	nt
Mineral	7.3	242	12.2	Nt	4.2	28.5	6.0	11.2	114	nt	nt

Biofouling studies

To evaluate possible grow of biofilm on PIM's surface, different membranes were deployed for 7 and 30 days (Spring 2017) in a pond of a farm located in Pujarnol (Girona), where GW is sampled (chemical characteristics depicted in Table 3.5). This pond contains natural freshwater algae floating and growing underwater on rocks and mud.

PIMs were observed by means of an optical microscope before and after the deployment exposure time and, afterwards, membranes deployed for 7 days were used for Hg transport experiments using 100 mL of SNW with 100 µ L^{-1} Hg as a feed phase and 5 mL of 10^{-3} M cysteine as a receiving solution.

3.2.4 Results and discussion

PIM with TOMATS or TOMAS: extraction and elution experiments

The effectiveness of TOMATS incorporated in a polymeric matrix was demonstrated in our previous studies, where a PIM with a composition of 50% CTA-50% TOMATS extracted Hg from natural waters [17,18]. In the present study, with the aim of comparing the extraction efficiency of both IL, we used PIMs containing 50% TOMATS or 50% TOMAS to test their ability to extract Hg from a SNW sample. Experiments were designed in order to ensure that PIMs had enough extraction capacity to completely extract the Hg present in the solution. Results are presented in Figure 3.10, which shows that even though TOMATS is the most efficient IL, allowing a quantitative extraction of Hg, the derivative incorporating the salicylate anion is also able to extract more than 50% of the metal initially present in the solution. In the study by Zhang et al. [34] using the IL tricarpylylmethylammonium 2-(methylthio)benzoate, in which the S atom is bearing a methylene group instead of the H of TOMATS, it was reported that the extraction of Hg(II) occurred via an interaction of the metal with the S atom along with the contribution of the carboxylate group.

In the case of TOMAS, it is reported in [30] that the extraction of Cd(II) is based on the chelating effect of the carboxylate group in addition to the formation of metal hydroxylates that forms a coordinate interaction between TOMAS and the metal ion. Given this, a similar extraction mechanism can be proposed for the extraction of Hg with this IL.

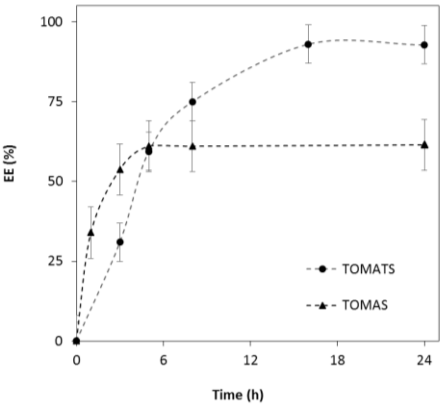

Figure 3.10. Hg extraction efficiency vs. time with PIMs made of 50% CTA-50% IL (25 mL of SNW 1000 µg L^{-1} Hg; n = 5).

The fact that the value of %EE is higher for the PIM incorporating the derivative bearing a thiol group instead of the alcohol is in agreement with the well-known high affinity of Hg for S-containing reagents. However, it is important to consider that the formation of very stable complexes can hamper further elution, if the extracted metal needs to be recovered in an aqueous solution.

Hg-loaded PIMs were used to investigate the elution efficiency (in terms of recovery, RE) using chelating agents such as EDTA, which form stable complexes with many metals, and cysteine, bearing both a thiol and a carboxylic acid group. Results are presented in Table 3.6. It could be observed that, no matter whether the concentration of cysteine was 10^{-3}M or 0.1M, it allowed the recovery of the extracted Hg for both IL with good efficiencies. However, the fact that 69% of metal extracted with the PIM with TOMAS could be eluted using a 0.01 M EDTA solution, compared to only 6.6% for TOMATS, revealed that the complex formed between Hg ions and TOMAS was probably weaker than the complex formed with TOMATS.

Table 3.6. Hg recovery efficiencies (RE, %) using Hg-loaded PIMs (50% CTA + 50% IL).

	Hg recovery efficiency (%)	
	TOMATS	TOMAS
0.01 M EDTA	6.6	69.0
0.1 M cysteine	100	71.5
10^{-3} M cysteine	76.8	Nt

nt: not tested

Hg preconcentration using a PIM-device

Optimization of PIM composition

When a PIM is used for transport purposes, first the metal has to be extracted from the feed phase, followed by its facilitated transport though the membrane, and the final release of the metal to the receiving solution. All these steps take place simultaneously, and, therefore, a proper receiving solution is needed to avoid the accumulation of the metal in the membrane that turns into a decrease in efficiency. Likewise, in transport experiments, the composition of the PIM is of paramount importance since not only does the metal have to be extracted but also the complex (carrier and metal) must diffuse through the polymeric matrix. Given this, we tested different membrane compositions as well as receiving solutions to evaluate Hg transport using the PIM-device (Figure 3.9). For both IL we tested membranes with and without a plasticizer, and also, in the case of TOMATS, PIMs with different amounts of the three components were also investigated. It is worth mentioning that all the formed membranes were dense, with no pores in the structure (as shown in [23] and in Figure 3.S.4), and that their characterization by means of FT-IR spectrometry revealed the typical bands of the main components of the PIM (see Fig.4. S.5).

As a receiving phase for the PIM-device, an EDTA solution was only tested for PIMs with TOMAS, whereas cysteine was investigated for membranes with both IL. Even though a 0.1M cysteine solution gave high recovery values in batch experiments, for transport experiments we only tested a 10^{-3} M solution of this reagent, since it is known that organic compounds at quite high concentration can be transported to a small degree across functionalized membranes by passive diffusion. This fact leads to a decrease in the efficiency of the separation system [35].

Table 3.7 shows the effect of both PIM and receiving solutions composition on Hg TE(%) after 24 h of experiment. In the case of TOMATS, when the membrane was made with only the polymer and the IL (70/30 or 50/50 CTA/TOMATS), the transport was very low. Even though the PIM with 50% TOMATS exhibited a very good extraction performance [23], it was not effective for transport purposes. Hence, we also tested PIMs with a plasticizer. It is known that the addition of a plasticizer increases the fluidity and plasticity of a PIM, improving its permeability due to the plasticization effect [20,36]. NPOE is one of the most commonly used plasticizers in PIMs [19,37]. Accordingly, PIMs containing 20% or 40% NPOE were investigated in the case of TOMATS. As can be observed in Table 3.7, the presence of NPOE dramatically increased Hg TE(%), with similar values for both NPOE content.

In the case of PIMs containing 50% TOMAS, the transport of Hg was ineffective in either EDTA or cysteine solutions. The addition of the plasticizer in the membrane increased the TE(%), but only reaching a value of 34%.

Consequently, PIM with a composition 50% CTA-30% TOMATS-20% NPOE was fixed for further experiments. To ensure the homogeneity of this PIM, five different replicates of transport experiments were done using membranes prepared on different days, and the relative standard deviation (RSD, %) of the TE(%) was 8, demonstrating the precision of this methodology. Moreover, this PIM composition was found to result in a stable membrane, since an insignificant mass loss was found for each solution tested (1.4% for milli-Q water, 1.8% for a 10^{-3}M cysteine solution and 2.5% for sea water). These results are in agreement of those reported in [17] where a mass loss of 2.4% was found for a PIM made of 50%CTA-50% TOMATS immersed in 0.1 M NaCl solution.

Table 3.7. Effect of PIM composition and receiving phase on Hg transport efficiencies (TE, %). Feed phase: 100 ml of SNW, spiked value=500 µg L^{-1} Hg (n=2 and n=5 for *).

	PIM composition				
	CTA (%)	**IL (%)**	**NPOE (%)**	**Receiving phase**	**TE (%)**
TOMATS	70	30	-	10^{-3}M cysteine	4 ± 5
	50	50	-		38 ±2
	50	30	20		84 ±7*
	30	30	40		80 ±5
TOMAS	50	50	-	10^{-3}M cysteine	9 ±0
	50	30	20	10^{-3}M cysteine	34 ± 6
	50	50	-	0.1M EDTA	2 ±1

Water matrix effect

The presence of other components existing in natural waters can negatively affect the efficiency of the membrane. On the one hand, some major ions could compete with the target species for the active sites of the carrier, becoming an interference. This effect was observed when studying the transport of As(V) using a PIM-device containing the IL Aliquat 336, since hot spring water samples, bearing high conductivity values (3,450 µS), hampered the transport of arsenate [8]. On the other hand, some of these major ions can act as complexing agents and affect metal speciation. In the case of Hg, different species can be found in natural waters depending on chloride concentration at a neutral pH. Taking into account the pH and both chloride and hydrogencarbonate anions content in each water (shown in Table 3.5) we calculated the predominance species of Hg (at a value of 500 µg L^{-1}) using the MINTEQ software. It was found that the predominant species in river, SNW and groundwater were neutral or cationic, whereas in seawater the predominant inorganic species was the anionic complex $HgCl_4^{2-}$. These differences may influence Hg extraction and transport depending on the carrier. For instance, if the ion exchanger Aliquat 336 is used, Hg can only be extracted if it is present as an anionic form [38] and, therefore, can be useful for seawater or high salinity water samples, but not for GW or river water samples, for example.

With the aim of testing whether TOMATS can provide a wider use in different natural waters, transport experiments were performed using river, GW and seawater, as well as SNW, and results are presented in Figure 3.11 As shown, despite the different species' dependence on the water's characteristics, no statistical significant differences ($p > 0.05$) were observed on transport efficiency for the different waters tested. This fact reveals the great robustness of the preconcentration method based on a PIM incorporating TOMATS as a carrier.

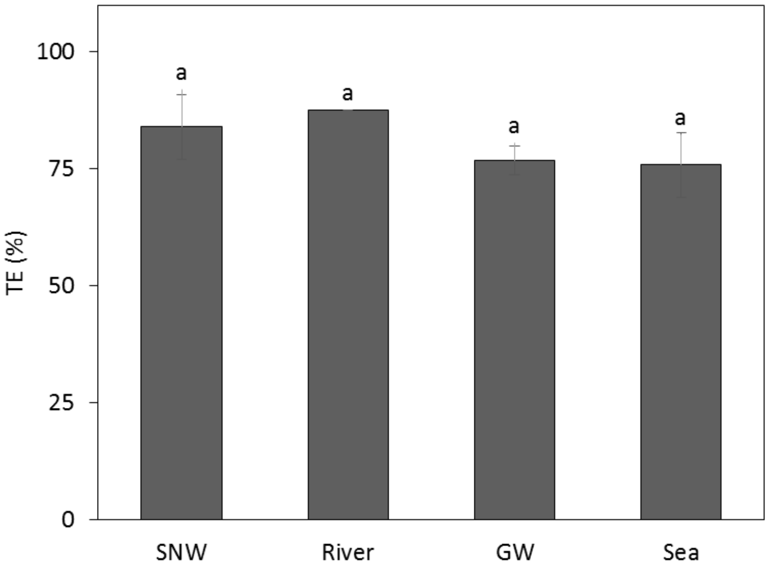

Figure 3.11. Matrix effect on Hg TE using the PIM-device system. Feed phase: 100 mL 500 µg L^{-1} Hg ($n = 3$). Different letters within the graph indicate significant differences.

Calibration

The effect of metal concentration in different natural waters was investigated in a range of 25 – 500 µg L^{-1} for both river and seawater while the range for GW was 1 - 500 µg L^{-1}. Also, three replicates using milli-Q water as a feed phase (with no Hg added) were done to calculate the limit of detection (LOD) and quantification (LOQ) of the PIM-system by analysing the receiving phase by ICP-MS. Then, LOD was found to be 0.29 µg L^{-1} and LOQ was 0.74 µg L^{-1}. Figure 3.12 shows the calibration curves obtained for the analysis of Hg concentrated in the receiving phase plotted vs. the initial Hg present in the water sample. As shown in Figure 3.12a, a straight line was fitted to measured points by the least-square method. Parameters of the resulting calibration curve are also included in Figure 3.12a. The fact that the regression coefficient was higher than 0.99 in all cases indicates good linearity throughout the working range, whereas similarities within the slopes of the regression lines reveal the lack of matrix effect within the waters studied. Figure 3.12b depicts the calibration curve obtained for GW containing Hg in the range 1 to 10 µg L^{-1}. As shown, good linearity is also obtained, and the value of the slope (14.5) is in concordance with the calibration curves obtained with a higher amount of Hg.

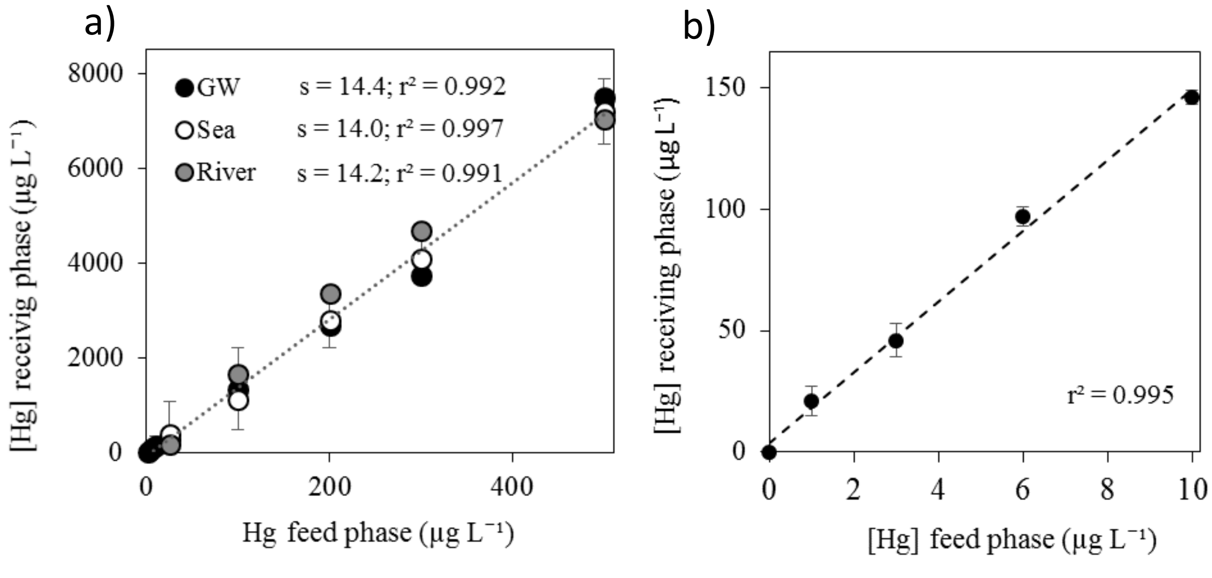

Figure 3.12. Calibration curves obtained with PIM-device using different natural waters (n=2). (a) Feed phase: 100 mL water with 25 - 500 µg L^{-1} Hg and (b) 100 mL of GW 1 – 10 µg L^{-1}.

These results are highly noteworthy since they demonstrate that a single calibration curve is enough to determine the initial amount of Hg present in natural water, after its preconcentration by the PIM-device.

Hg determination using the PIM-device

The PIM-device was used to determine the amount of Hg in natural waters spiked with 5 µg L^{-1} of metal (Table 3.8). To this end, Hg preconcentrated in the receiving phase was interpolated in the calibration curve in Figure 3.12(b) and the Hg initially present in water samples was determined. As shown in Table 3.8, a good agreement was found between both the spiked level and the Hg content using the PIM-device. It is worth mentioning that even though the metal was present at a very low content in such complex samples (e.g. hot spring water, seawater), the use of this methodology enabled its detection.

Table 3.8. Application of the PIM-device to determine Hg content in natural waters (n=3).

Sample	[Hg] added (µg L^{-1})	[Hg] in the receiving phase (µg L^{-1})	[Hg] found* (PIM-device) (µg L^{-1})
Spring	5	78 ± 5	5.1± 0.2
Mineral	5	89± 5	5.8 ± 0.3
Groundwater	5	78 ± 6	4.8± 0.2
Sea	5	74 ± 4	5.1± 0.3

*Calculated from calibration curve of Figure 3.12

PIM biofouling characteristics and effect on Hg transport

Taking into account the good performance exhibited for this PIM system, and with the aim of using this device as a passive sampler to facilitate Hg monitoring in water bodies in possible future studies, we also tested (for the first time) the possible biofouling growth on the surface of the membrane. Biofouling is the colonization by bacteria and various flora and fauna that can form a biofilm which can damage the surface of the membrane [39] and can even affect the transport of the compounds of interest, as happens in DGT devices [39,40].

PIMs were observed by an optical microscope before and after deployment in the controlled water reservoir. Two different deployment times were investigated: 7 days, which is a normal time for passive sampling studies with PIM devices [27], and 37 days, to better investigate whether or not the biofilm could grow. PIMs surface images are shown in Figure 3.13. It can be seen that PIMs immersed for 7 days were not colonized by biofilm, but after a month of deployment, some diatoms appeared on the surface of the membrane, the main orders being Naviculate and Pennate. After 7 days of deployment, the transport of Hg in the PIM was investigated and compared with new PIMs. No statistical differences ($p > 0.05$) were found for the TE(%) values. However, further experiments should be performed at longer deployment times to better establish the likely role of biofilm growth on PIMs.

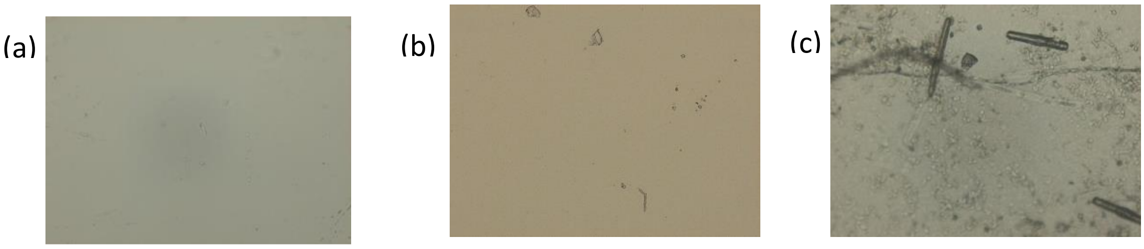

Figure 3.13. Optical microscope images (10x) of the PIM surface (a) before deployment, (b) after 7 days, and (c) after 37 days of deployment

3.2.5 Conclusions

This novel, low-cost and simple method to facilitate mercury determination presented here is based on a PIM containing CTA as a polymer, TOMATS as a carrier, and the plasticizer NPOE, with an optimized composition of 50%CTA-30%TOMATS-20%NPOE. Since the efficiency of the system was not affected by the composition of the waters tested, this method may be a suitable global solution when dealing with Hg monitoring in natural waters. Furthermore, since no biofilm growth was observed for about a week of deployment in the field, the PIM-system would appear to be a very promising tool for the design of a suitable passive sampler device for eutrophic freshwaters.

3.2.6 References

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3.2.7 Supplementary material

Figure 3.S. 3. Description of 1H-RMN spectrum of IL synthesized (δ (ppm)).

TOMATS: 8.1 (dd, J = 21.2 Hz, J’ = 1.6 Hz, 1H), 8.05 (dd, J = 143.6 Hz, J’ = 2 Hz, 1H), 7.65 (dd, J = 40 Hz, J’ = 0.8 Hz, 1H), 7.55 (dd, J = 1.2 Hz, J’ = 0.4 Hz, 1H), 7-7.15 (m, 3H), 6.8 (td, J = 6.8 Hz, J’ = 1.2 Hz, 1H), 3.6 (t, J = 6.8 Hz, 1H), 3.25 (t, J = 8 Hz, 9H), 3.15 (s, 4H), 1.6 (d, J = 2.4 Hz, 9H), 1.25 (d, J = 18 Hz, 56H), 0.89 (td, J = 6.8 Hz, J’ = 4.8 Hz, 15H).

TOMAS: 7.91 (dd, J = 7.6 Hz, J’ = 1.6 Hz, 1H), 7.21 (ddd, J = 9.2 Hz, J’ = 7.2 Hz, J’’ = 2 Hz, 1H), 6.81 (dd, J = 8 Hz, J’ = 0.8 Hz, 1H), 6.7 (td, J = 7.6 Hz, J’ = 1.2 Hz, 1H), 3.65 (t, J = 6.4 Hz, 1H), 3.25 (dd, J = 12 Hz, J’ = 8.4 Hz, 5H), 3.15 (s, 3H), 1.6 (d, J = 3.6, 6H), 1.25 (t, J = 3.2 Hz, 36H), 0.8-0.9 (m, 9H).

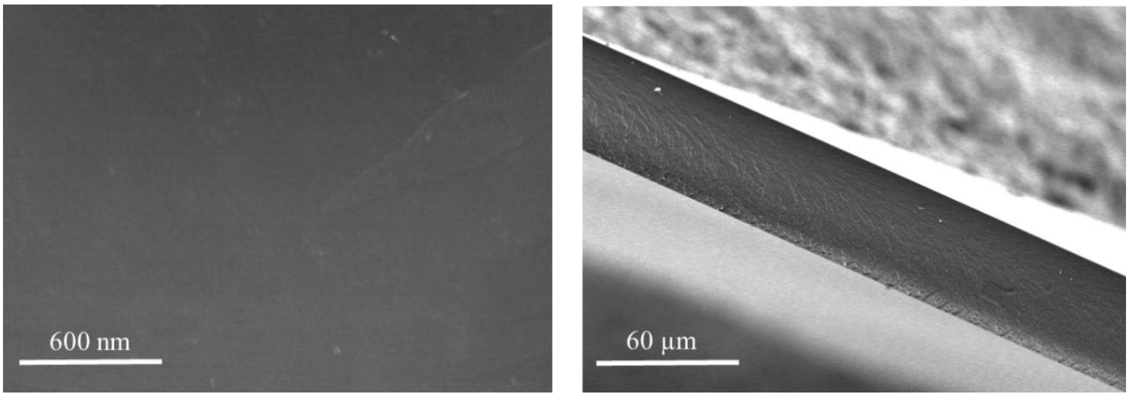


Figure 3.S. 4. SEM images of PIM with a composition 50% CTA + 50% TOMAS obtained with a FE-SEM Hitachi, s-4100 instrument (Japan). Images were collected and processed by Quarz PCI program.

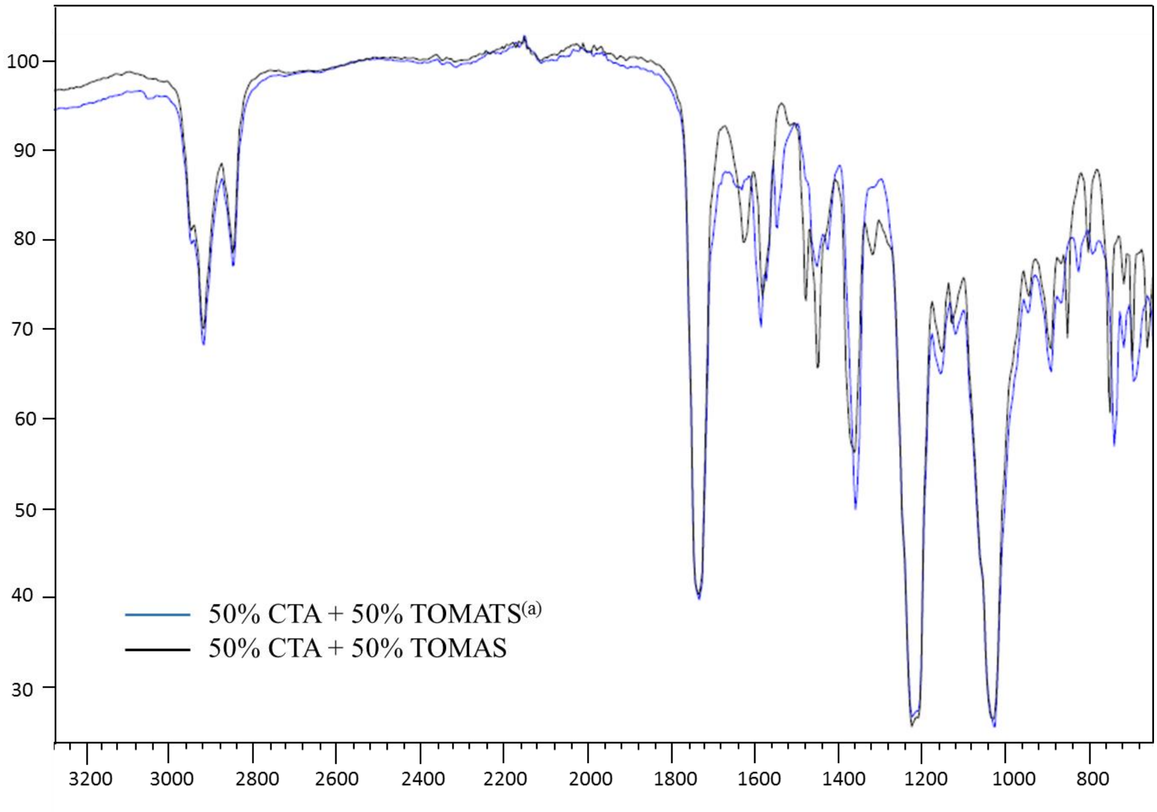


Figure 3.S. 5. FTIR spectra for PIMs with a composition 50% CTA + 50% IL, recorded using an Agilent Cary 630 FTIR spectrometer.

(a) From our previous work [17].

The bands at 1747 and 1041cm $^{-1}$ correspond to the C=O and C-O-C groups presents in the CTA polymer. The stretching vibrations of CH_3 and CH_2 from both TSIL appear in the bands at around 2850 and 2925 cm^{-1}. Also, the bands at 1598 cm^{-1} in both spectra, correspond to the COO-group stretching vibrations. The SH group of thiosalicylate could not be observed due to the low intensity that it generally presents.

Chapter 3

SECTION 3

Polymer inclusion membranes for mercury transport: effect of the membrane components and new applications

3.3.1 Abstract

The influence of the base-polymer and plasticizer of a polymer inclusion membrane (PIM) incorporating the task specific ionic liquid (TSIL) trioctylmethylammonium thiosalicylate (TOMATS) as the carrier was investigated with respect to its transport efficiency for Hg(II). The optimum PIM composition was found to include cellulose triacetate (CTA) and nitrophenyl octyl ether (NPOE) as the base-polymer and plasticizer, respectively. A solution of 10^{-3} M cysteine was used as a receiving phase. Furthermore, the developed PIM also was found to be suitable for the preconcentration of Cd(II), Ni(II) and Pb(II) contained in different natural waters. These results demonstrate the suitability of this PIM as a potential preconcentration tool to facilitate the detection of the toxic metals mentioned above at trace levels in natural waters.

3.3.2 Background and aims

Polymer inclusion membranes (PIM), a kind of liquid membranes (LMs) used for separation processes, are commonly prepared by casting a solution of a base-polymer and an extractant (also called carrier). In some cases, a plasticiser can be added to the PIM composition. The extractant, and the plasticizer, when present, form the so-called membrane liquid phase which is entrapped within the base-polymer matrix by a combination of physicochemical interactions [1], thus forming a flexible, thin and stable film.

The role of the polymer is to provide the membrane with a mechanical stability, and among the different polymers that can be used for PIMs, the most extensively studied are cellulose triacetate (CTA) and poly(vinyl chloride) (PVC) [2]. Both CTA and PVC are thermoplastic polymers consisting of linear polymer chains with no cross-linking between them that can be easily dissolved in a suitable organic solvent for casting PIMs [3].

The carrier is responsible for the interactions with the target chemical species, and in principle, all the extractants used in solvent extraction can be used as carriers in PIMs.

Plasticizers are compounds added to increase the softness and flexibility of PIMs [3,4] by means of a mechanism of polymer chain separation inducing the corresponding decrease in the glass transition temperature (T_g). The main responsibility of a plasticizer is to provide flexibility to the PIM, but also to improve the compatibility between the membrane components [5] by reducing the intermolecular polymer interactions [2,4]. Plasticizer molecules penetrate between the polymer chains and neutralize the polar groups or increase the distance between such groups in order to reduce the intermolecular forces [1]. Moreover, these molecules can act as a solvating medium of carriers, creating continuous pathways between the two interfaces of the membrane [6], which, sometimes, lead to an improvement in the transport of the target chemical species [7–9]. Several authors have investigated the effect of the plasticizer on PIM extraction and transport, and it is usually explained in terms of chemical structure, dielectric constant or viscosity. It is of general agreement that the inclusion of a plasticizer of high viscosity leads to a slow PIM diffusion, and, in turn, to a low efficiency of the membrane transport. The dielectric constant is expected to affect the dissociation of the adduct of the extracted species with the carrier which together with the size and lipophilicity of these two species may determine the trans-membrane transport mechanism, i.e., diffusion of the adduct or 'hopping' of the extracted species between carrier molecules [10]. Due to its high dielectric constant and low viscosity, ortho-nitrophenyl octyl ether (NPOE) is one of the most commonly used plasticizers [11]. Wang et al. demonstrated that the addition of NPOE facilitated the transport of Cu(II) across CTA-based PIMs [8]. Also PIM compositions incorporating NPOE as plasticizer and Aliquat 336 as a carrier improved the transport of sulfamethoxazole when the polymer used was CTA instead of PVC [12]. Pont et al. studied the effect of three plasticizers for CTA-based PIMs with Aliquat 336 as the carrier on Cd(II) transport from chloride solutions [9]. Cd(II) removal was more effective with NPOE than when using dibutyl sebacate (DBS) or tris-(2-ethylhexyl) phosphate (TEHP) as plasticizers. Sometimes the plasticizer may also assist in the solubilization of the extracted adduct of the target species and the carrier in the organic phase [2], thus acting as a modifier. Organic compound with hydroxyl functional groups such as 1-dodecanol are often used for such purpose.

PIMs are versatile considering the diversity of target compounds that they can extract [5] and are adaptable to different analytical applications such as sensing, sample pre-treatment [13] and chemical speciation [14]. In our previous studies, we demonstrated that a CTA-based PIM loaded with the ionic liquid trioctylmethylammonium thiosalicylate (TOMATS) as its carrier was able to efficiently extract Hg(II) from natural waters, without the use of any plasticizer [15]. A detection system for this metal based on its preconcentration in the PIM with subsequent analysis of the membrane by Energy Dispersive X-ray Fluorescence (EDXRF) [16] was also developed. It is important to mention that PIM was used as a sorbent to collect Hg and no elution of the extracted metal was performed.

Moreover, it was demonstrated that, using a similar PIM but containing the plasticizer NPOE, Hg(II) was transported to a receiving phase consisting of 10^{-3} M cysteine [17]. The PIM-system was tested in different natural waters and the results showed that Hg(II) transport was not affected by the water matrix. However, the influence of the polymer chosen or the nature of the plasticizer on the Hg(II) transport efficiency was not studied, as well as the possibility of using this preconcentration system for other toxic metal ions that can be present in contaminated waters.

The aim of this study was to evaluate the Hg(II) transport efficiency of a PIM containing TOMATS as a function of its base-polymer and plasticizer. We also studied whether the PIM system developed for Hg(II) was also effective for the transport of other toxic metal ions such as Cd(II), Ni(II) and Pb(II), included together with Hg(II) in the list of 33 “priority pollutants” by the European Water Framework Directive (2008/105/EC) [18]. Hence, due to the high toxicity of these metals there is a substantial interest in the development of reliable techniques to facilitate their determination in aquatic systems.

3.3.3 Materials and methods

Reagents and solutions

The polymers CTA (Fluka, Switzerland) and PVC (Sigma-Aldrich, UEA) were used for PIM preparation. Chloroform stabilized with ethanol (Panreac, Spain) was used to dissolve CTA, whereas tetrahydrofuran (THF) (Panreac, Spain) was used in the preparation of the PVC-based PIMs. TOMATS, used as a carrier, was prepared as described by Elias et al. [16] from the commercial reagent trioctylmethylammonium chloride (Aliquat 336) (Sigma-Aldrich, UEA) and sodium thiosalicylate (TCI, Japan). NPOE, 2-fluorophenyl 2-nitrophenyl ether (FPNPE), bis (2-ethylhexyl) phthalate (BEHP), butyl stearate (BTS), bis

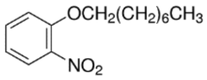
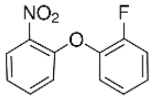
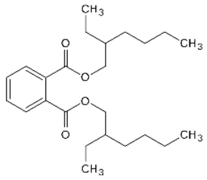
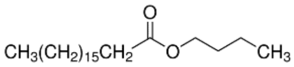
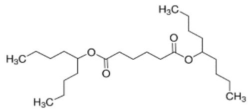
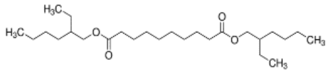
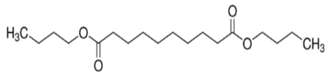
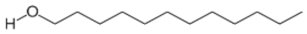
(1-butylpentyl) adipate (BBPA) (Sigma-Aldrich, UEA), di (2-ethylhexyl) sebacate (DOS), dodecanol (Merck, Germany), and dibutyl sebacate (DBS) (Fluka, Switzerland) were used as PIM plasticizers. The main characteristics and chemical structure of these compounds are shown in Table 3.9.

Mercury working solutions were prepared daily by appropriate dilution of a Hg ICP standard of 1000 mg L^{-1} ± 2 mg L^{-1} in HNO_3 (12% w/w) (Fluka, Switzerland) with the tested water. The working solutions, containing a mixture of Cd(II), Pb(II) and Ni(II), were prepared by proper dilution of each metal ICP standard of 1000 mg L^{-1} in HNO_3 (2% w/w) (Fluka, Switzerland) with the corresponding aqueous solutions.

A 10^{-3} M solution of L-Cysteine (Cys) (MercK KGaA, Germany) solution was used as the receiving phase of the PIM separation system and it was prepared daily. Other solutions tested were 0.1 M EDTA, 0.1 M HNO3 and 0.05 M sodium citrate (Panreac, Spain).

Experiments were done using different types of water: simulated natural water (SNW) containing 2 mM $NaHCO_3$, 1 mM $CaCl_2 \bullet H_2O$, 0.5 mM Na_2SO_4 and natural waters such as river, mineral, tap, sea, and ground water with chemical compositions described by Elias et al. [16]).

Table 3.9. Characteristics of the plasticizers studied.

Plasticizer	Chemical Structure	Viscosity (cP)	Dielectric Constant (ε_r)	Density (g/mL)
NPOE		11.1 [2]	24 [2]	1.04
FPNPE		13 [6]	50 [6]	1.315
BEHP		40.4 [2]	5.22 [2]	0.985
BTS		Na	na	0.861
BBPA		14 [6]	4 [6]	0.91
DOS		16.7 [5]	3.9 [5]	0.914
DBS		9.5 [6]	4.5 [6]	0.936
Dodecanol		Na	5.82 [2]	0.833

(n.a.= not available)

Preparation of PIMs

Different PIMs were prepared following the procedure described earlier by us [19]. The PIMs compositions investigated in the present study are presented in Table 3.10.

Table 3.10. Compositions of PIMs studied.

Polymer/carrier/ Plasticizer (% mass)	**Polymer**		**TOMATS**	**Plasticizer**
100/0/0	CTA	400 mg	0	0
60/0/40		267 mg	0	133 mg (NPOE)
50/50/0		200 mg	200 mg	0
50/30/20		200 mg	120 mg	80 mg (Plasticizer)
50/50	PVC	400 mg	400 mg	0
50/30/20			240 mg	160 mg (NPOE)

Metals transport experiments

PIMs were incorporated in a device to allow the preconcentration of the metals, as shown in [17,20]. It consists of a glass tube with two openings, and the piece of PIM is placed at the bottom opening and fixed with a special screw cap that allows a membrane area exposed to the aqueous phases of 1.5 cm^2. The opening at the top enables to fill up the device with an appropriate volume of solution and once filled, it can be closed with a standard screw cap. In this work, the feed phase consisted of 100 mL of the waters tested spiked with Hg(II) or other metal ions, and stirred with a magnetic stirring bar. The transport experiments were conducted for 24 h, except for kinetics studies, where different PIM-devices were stopped at different times.

The extraction efficiency (EE) of the PIM-based device was calculated by Equation 3.4.

$$EE\ (\%) = \frac{[M]i - [M]f)}{[M]i}\ x\ 100 \qquad (Eq. 3.4)$$

where $[M]_i$ and $[M]_f$ denotes the initial and final metal concentrations in the feed phase, respectively.

The transport efficiency (TE) was calculated by Equation 3.5.

$$TE(\%) = \left(\frac{V_r}{V_f}\right)\left(\frac{[M]_{r,t}}{[M]_{f,i}}\right)x100 \qquad (Eq.3.5)$$

Where V_r is the volume of the receiving phase, V_f is the volume of the feed phase, $[M]_{r,t}$ denotes the metal concentration in the receiving phase at time t and $[M]_{f,i}$ is the initial metal concentration in the water sample (feed phase).

Apparatus

The concentration of the metal ions in the aqueous samples was measured using an inductively coupled plasma optical emission spectrometer (Agilent Technologies, Agilent 5100 Vertical Dual View ICP-OES) (25-7000 µg L^{-1}) or an inductively coupled plasma mass spectrometer (ICP-MS) system (Agilent Technologies, Agilent 7500c ICP-MS) (isotope ^{202}Hg) for low levels of Hg(II) (5-200 µg L^{-1}). Standards were specially prepared with the corresponding matrices to avoid the possibility of matrix interferences during sample analysis.

Milli-Q water was obtained by a Milli-Q Plus system (Millipore, France).

The scanning electron microscopy (SEM) images of the membranes were obtained with a FE-SEM Hitachi, S-4100 (Japan), after placing PIM samples on stubs and coating them with carbon (Model K950 turbo evaporator, Emitech Germany). Quartz PCI program was used to collect and process the images.

A DSSA25 drop-shape analyser from Krüss GmbH (Hamburg, Germany) was used to measure contact angles and was controlled with the Krüss Advance software.

3.3.4 Results and discussion

Membrane composition

To first test the effect of the polymer and plasticiser on Hg(II) transport, membranes were prepared using either CTA or PVC as the base-polymer with or without NPOE as the plasticizer. Table 3.11 shows the effect of the PIMs composition on the membrane Hg(II) extraction efficiency (EE, Eq. (3.4)) as well as Hg(II) transport efficiency (TE, Eq. (3.5)).

Table 3.11. Influence of PIM composition on EE and TE. Feed phase: 100 mL of simulated natural water (SNW) with 500 µg L^{-1} Hg(II); receiving phase: 5 mL of 10^{-3} M cysteine.

PIM composition	EE (%)	TE (%)
50% PVC + 50% TOMATS	91 ± 5	3 ± 0.1
50% PVC + 30% TOMATS + 20% NPOE	94 ± 7	5 ± 2
50% CTA + 50% TOMATS*	92 ± 1	38 ± 2
50% CTA + 30% TOMATS + 20% NPOE*	96 ± 5	84 ± 7

* TE as in [17].

As can be seen, Hg(II) was effectively removed from the feed phase independently of the PIM composition. However, relatively small fractions of the extracted into the PIM Hg(II) were found in the receiving solutions when using PVC membranes, pointing out that the membranes acted as a sorbent and did not transport Hg(II) into the receiving phase. Better transport was achieved when the PIMs contained NPOE which agrees with the results of Wang et al. who improved back-extraction efficiencies of Cu(II) from ammoniacal solutions with PVC-based membranes by the addition of 30% of NPOE [8]. As observed, in the case of CTA membranes Hg(II) was effectively preconcentrated in the receiving solution only in the case of the PIM made of 50% CTA, 30% TOMATS and 20% NPOE. When the CTA-membranes did not incorporate the plasticizer, only 38±2 % of Hg(II) was transported to the receiving solution.

To better understand these results, the morphology of both PVC- and CTA-based PIMs incorporating NPOE was studied. Figure 3.14 shows the SEM images of the membranes studied. In the case of PVC, the inner structure of the membrane appears to be inhomogeneous (Fig. 3.14-A2) unlike in the case of CTA (Fig. 3.14-B2). The surface images indicate that both polymers form smooth PIMs (Figs. 3.14-A3 and 3.14-B3).

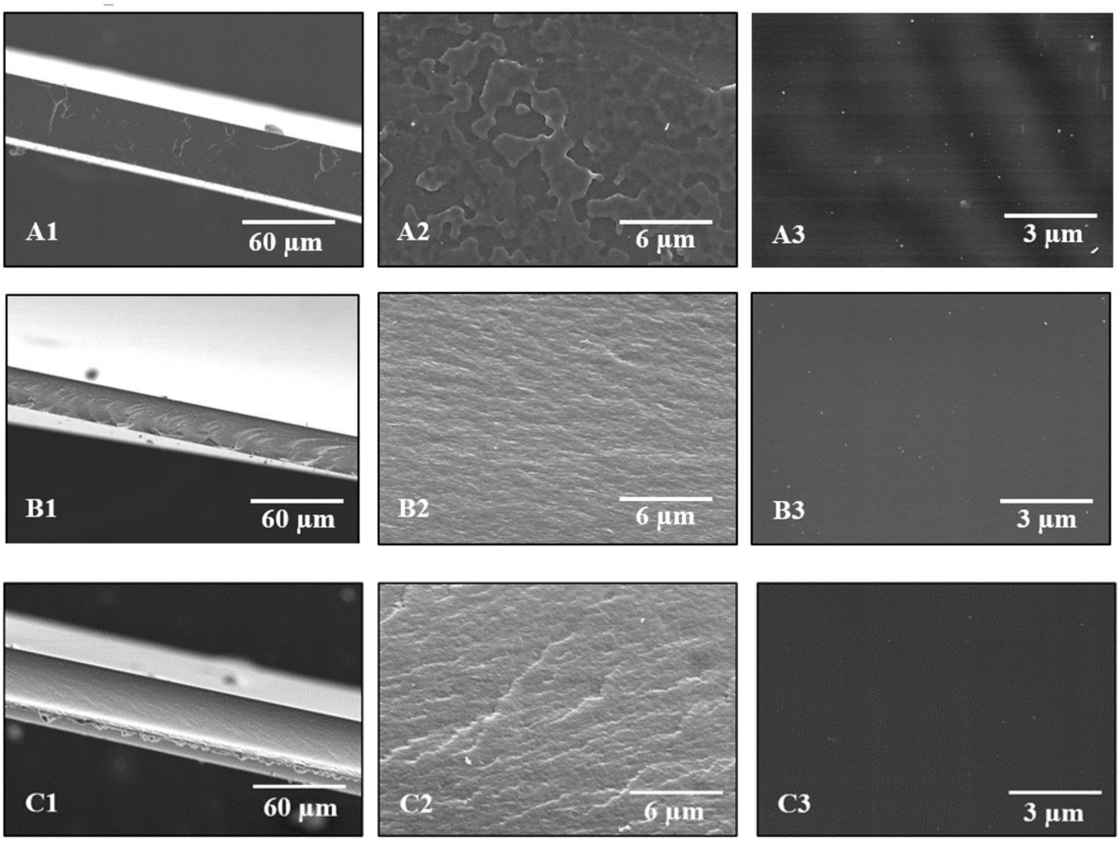


Figure 3.14. SEM images of PIMs with a composition (A) 50% PVC + 30% TOMATS + 20% NPOE, (B) 50% CTA + 30% TOMATS + 20% NPOE and (C) 50% CTA + 30% TOMATS + 20% DBS (1 and 2: cross-section images; 3: surface images).

Based on the extraction results (Table 3.11), and taking into account the morphology of the membranes, it seems that TOMATS ion-pairs are available on the surface of both the PVC- and CTA-based PIMs. However, the transport of Hg(II) through the PVC-based PIM is likley to be interrupted due to its inhomogeneity which may have resulted in blocked transport pathways between the two PIM surfaces (e.g., blocked membrane liquid phase channels). This different morphology between the CTA- and PVC-based PIMs can be due to different compatibilities between the liquid phase components of the membrane (i.e., carrier and plasticizer) and its polymer backbone, being more pronounced in the case of the PVC-based PIMs.

Moreover, to ensure that the transport was due to the interaction between Hg(II) and the carrier TOMATS, transport experiments were also performed using membranes prepared without carrier. Accordingly, a 100% CTA and 60% CTA and 40% NPOE membranes were tested and no metal ions were extracted from the feed phase, confirming that TOMATS was responsible for Hg(II) extraction.

Thus, further experiments were performed with PIMs using CTA as the base-polymer.

Effect of the plasticizer nature

The influence of the PIM composition was investigated by adding different plasticizers to the CTA-TOMATS casting solution. Contact angle measurements were performed for each membrane to evaluate the hydrophobicity of the surface. Figure 3.15 shows the TE(%) for Hg(II) for each membrane composition tested and the value of the contact angle measured.

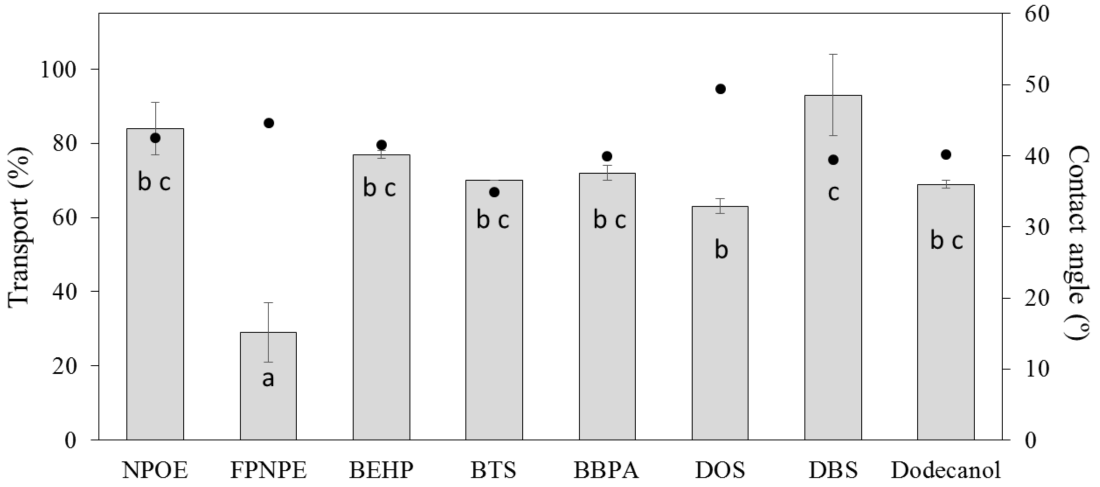


Figure 3.15. Effect of the plasticizer on Hg TE (%) and the contact angle (●) of the PIMs made of 50% CTA + 30% TOMATS + 20% plasticizer. Feed phase: 100 mL of SNW with 500 µg L^{-1} Hg; receiving phase: 5 mL 10^{-3} M cysteine. Different letters within the graph indicate significant differences ($p<0.05$) in TE (%) for Hg.

As it can be observed, contact angle values of the membranes were in the range 35º-49.5º (the lowest for BTS and the highest for DOS). For comparison, the contact angle of a film made of pure CTA was 61.2º while for a PIM made of 50 % CTA and 50% TOMATS it was 49.3º. Hence, the addition of the plasticizer increased the hydrophilic character of the membrane surface.

The addition of a plasticizer to the PIM had a significant effect on the transport efficiency of the membrane. As it can be seen in Fig. 3.15, plasticizers as different as NPOE (viscosity= 11.1 cP, dielectric constant= 24) and DBS (viscosity= 9.5 cP, dielectric constant= 4.5) showed the best results in terms of Hg(II) transport.

Considering the chemical structure, NPOE, FPNPE and BEHP are molecules with phenyl groups while the other plasticizers tested are aliphatic. In the case of the first two, they have also a nitro functional group in common and the highest dielectric constant and density values and lower viscosity (Table 3.9). However, the dielectric constant of FPNPE is much higher than that of NPOE and, as a result of this, TOMATS may not mix properly with FPNPE, resulting in a lower PIM transport efficiency for Hg(II) (Fig. 3.15). These results agree with a previous work of Fontàs et al. [6] where the metal flux was found to be inhibited when using FPNPE as a plasticizer in a CTA-based PIM incorporating Aliquat 336 as a carrier. Another interesting observation was that the hydrophobicity of PIMs containing NPOE or FPNPE were very similar which suggested that the hydrophobicity of a PIM was not the governing parameter for its Hg(II) transport.

Both BEHP and NPOE have phenyl groups but the dielectric constant of BEHP is much lower than that of NPOE and it has the highest viscosity value. However, despite these differences the TE(%) of the PIM prepared with BEHP was not significantly different ($p>0.05$) from that of the PIM containing NPOE thus suggesting that in the case of BEHP the low dielectric constant compensated for its high viscosity.

All PIMs with the four aliphatic plasticizers studied (i.e., BTS, BBPA, DOS and DBS), exhibited high TE values (Fig. 3.15). However, DBS and DOS exhibited significantly different Hg(II) TE values, but the results for the four aliphatic plasticizers where similar to the TE value of the NPOE-based PIM. The fact that the PIM containing DOS was the most hydrophobic supported the fact that the hydrophobicity of a PIM was not linked to its TE. The morphology of the PIM with the highest TE, which contained DBS as plasticizer (Fig. 3.15), was studied. Similarly, to the other PIM with high TE, which contained NPOE as plasticiser (Fig. 3.15), both the cross-section (Fig.3.14-C2) and surface (Fig. 3.14-C3) morphology of the DBS membrane was found to be homogeneous.

The use of 1-dodecanol as a plasticizer was also tested and as it can be observed in Fig. 3.15, there were no significant differences between TE of this PIM and those containing plasticizers such as NPOE, BTS, BBPA, and BEHP. This result is of a great interest if it is taken into account that all these reagents are expensive in comparison with 1-dodecanol, that allows the production of PIMs with good transport efficiency for Hg(II).

The kinetics of the TE of the two best performing PIMs, i.e., those containing DBS or NPOE, were also investigated and no statistically significant difference ($p>0.05$) between the results presented in Fig. 3.16 were observed. Consequently, further experiments were done with a PIM composition of 50% CTA + 30% TOMATS + 20% NPOE.

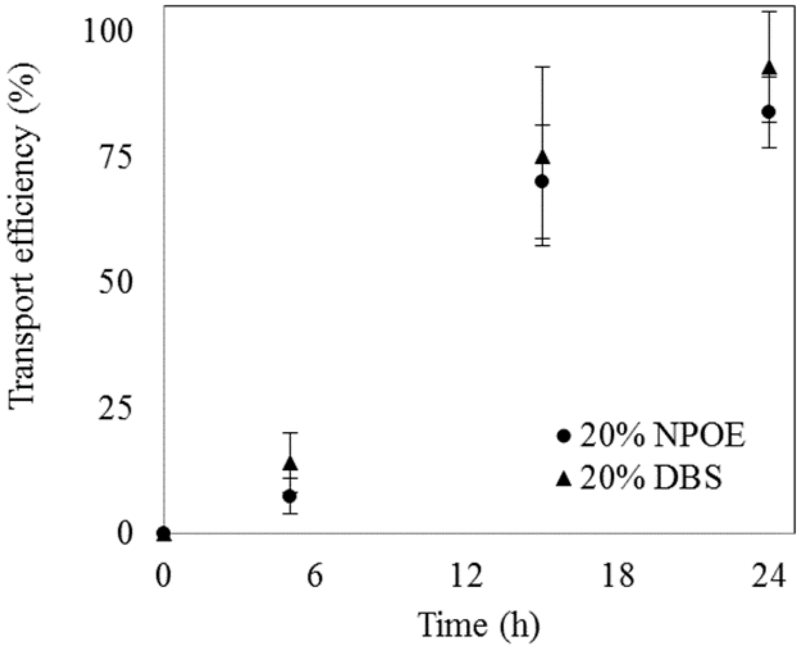


Figure 3.16. Kinetics of mercury transport across PIMs containing NPOE or DBS as plasticizers. Feed phase: 100 mL of SNW containing 500 µg L^{-1} Hg; receiving phase: 5 mL 10^{-3}M cysteine.

Evaluation of the PIM for the preconcentration of other toxic metals

The IL TOMATS has been previously found by other authors as a suitable carrier for the extraction of different metal ions from aqueous solutions [21–24]. For example, Kogelnig et al. demonstrated that more than 99.9% of the Cd present in ultrapure water and river water was eliminated when using TOMATS in a solvent extraction method [23]. Liquid phase micro extraction (LPME) was also tested with this IL to eliminate different metals from an aqueous solution of 0.01 M $CaCl_2$ obtaining results of 85% of extraction efficiency for Pt(II) [22], as well as 94%, 95% and 79% for Ag(I), Cu(II) and Sn(II) respectively [21]. Thus, in this work, we studied whether the PIM system developed for Hg(II) was also effective for the transport of other toxic metal ions such as Cd(II), Ni(II) and Pb(II) contained in different natural waters of different complexity. For that, in the first stage, we investigated the effect of the receiving solution composition using as a feed phase a tap water sample spiked with 500 µg L^{-1} of each metal. Aqueous solutions of 0.1 M HNO_3, 0.1 M EDTA or 0.05 M sodium citrate were tested as receiving phases. With this approach, better results were obtained when using the EDTA solution in which case the TE was found to be 43% for Cd(II), 25% for Pb(II) and 14% for Ni(II). Nitric acid only allowed the transport of Cd(II) (15%), whereas citrate was not effective for any of metal ions studied. These results show that the PIM developed for Hg preconcentration is also suitable for the transport of other toxic metal ions.

The effect of water characteristics on the TE for Cd(II), Pb(II) and Ni(II) was investigated with different natural waters (i.e., sea, river, tap and mineral) using a 0.1 M EDTA solution as the receiving phase (Figure 3.17).

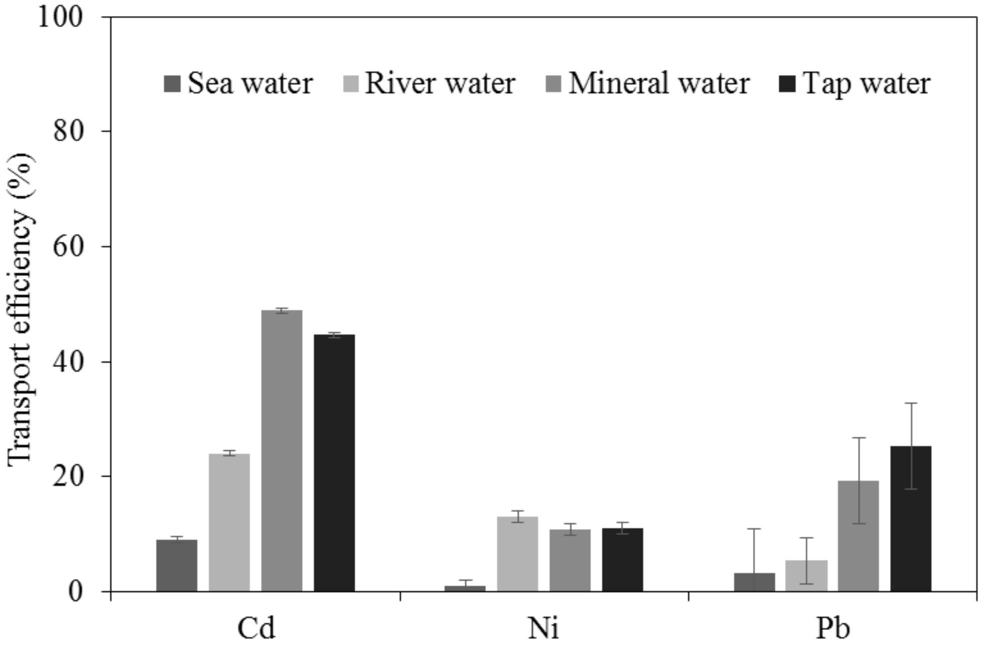


Figure 3.17. Effect of water matrix on the TE of Cd(II), Ni(II) and Pb(II). PIM composition: 50% CTA + 30% TOMATS + 20% NPOE. Feed phase: 100 mL of the water tested spiked

with 500 µg L^{-1} of each of the heavy metal mentioned above. Receiving phase: 5 mL of 0.1 M EDTA

The results in Fig. 3.17 show the good performance of the PIM studied for Cd(II) transport, since this metal ion was efficiently transported (>40%) from both mineral and tap water. Even though to a lesser extent, Cd(II) was also preconcentrated in the receiving phase from river and sea water, although with lower TE values, both Pb(II) and to a lesser extent Ni(II) were also transported across the PIM. It is important to point out that the extraction of Ni(II) is difficult [25]. Separation processes involving this metal are designed to transport the other metals and leave Ni(II) in the initial solution. This is the case of a study using a PIM made of PVC and Aliquat 336 as a carrier designed for the separation of Ni(II) from Co(II). It was found that under the experimental conditions, there was no extraction of Ni(II) and, thus, allowed the complete separation of these two ions [26]. On the contrary, results from our study show the possibility of using the carrier TOMATS as a good candidate to extract Ni(II) and allow its separation and preconcentration.

To evaluate the preconcentration system when Hg(II) was also present, an experiment was performed using sea water and ground water spiked with 5 µg L^{-1} of Hg(II) and 100 µg L^{-1} of Cd(II), Ni(II) and Pb(II). Since preliminary results testing EDTA to strip Hg(II) were not successful [17], a 10^{-3} M cysteine solution was used as the receiving phase (Fig. 3.18).

Under these conditions, Hg(II) was effectively transported from both water samples into the receiving phase, and hence, the presence of the other metal ions did not affect the performance of the PIM system. The TE values for Cd(II), Ni(II) and Pb(II) followed the same trend (i.e., Cd > Pb > Ni) as in the experiments when Hg(II) was not present. It should be highlighted that the use of cysteine, instead of EDTA, lead to similar TE values for all the metal ions, independently of the type of water used as a feed solution.

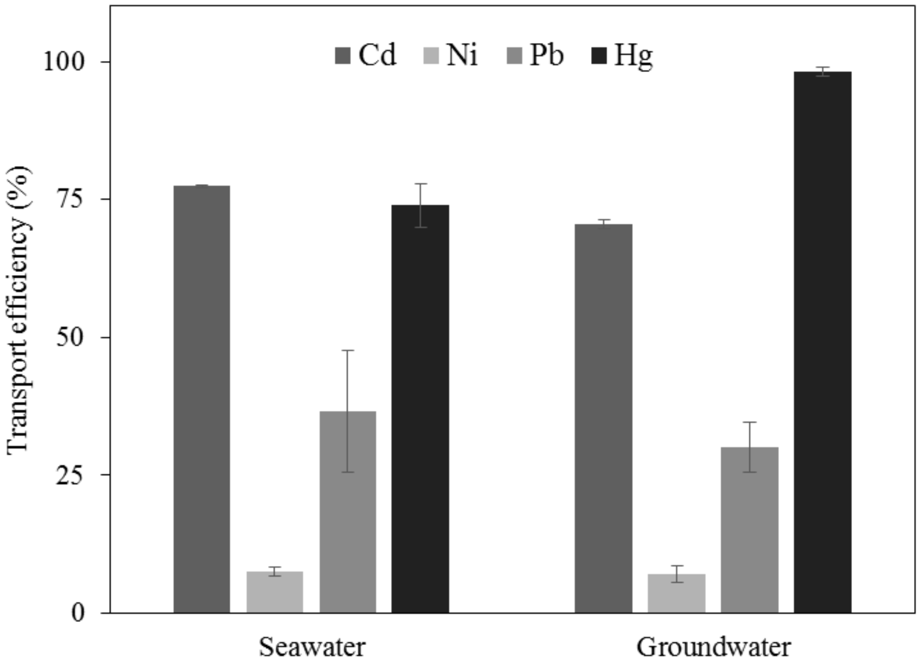


Figure 3.18. TE for toxic metals using the PIM composed of 50% CTA + 30% TOMATS + 20% NPOE. Feed phase: 100 mL of sea water or groundwater spiked with 5 µg L^{-1} of Hg(II) and 100 µg L^{-1} of Cd(II), Ni(II) and Pb(II). Receiving phase: 5 mL 10^{-3} M cysteine.

Table 3.12 shows metal ion concentrations in the receiving phase, and as it can be seen, all metal ions were preconcentrated. In the case of Hg(II), it was still preconcentrated even though the concentration of the other metal ions was 20 times higher than that of Hg(II). Hence, the PIM system seems not to be affected by either the mercury concentration or the water matrix.

It should be pointed out that although ICP-MS is an ideal characterization technique for the analysis of trace metal concentrations in water samples, it is really sensitive to matrix interferences for samples with high salinity content [27]. The PIM-based separation allows the preconcentration of these metal ions from saline samples into a 10^{-3} M cysteine solution, which can be analyzed by ICP-MS free of matrix effects and thus, solving the problems associated with interferences from saline samples.

Table 3.12. Metal ion concentrations in the receiving phase of the PIM- system. Feed phase: 100 ml of water samples spiked with 5 µg L^{-1} of Hg and 100 µg L^{-1} of Cd(II), Ni(II) and Pb(II). Receiving phase: 5 mL of 10^{-3} M cysteine.

	[Metal] receiving phase ± SD (µg L^{-1})			
	Cd(II)	Ni(II)	Pb(II)	Hg(II)
Sea wàter	1548 ± 5	151 ± 17	731 ± 220	74 ± 4
Groundwater	1410 ± 15	141 ± 28	602 ± 90	98 ± 1

3.3.5 Conclusions

The composition of a PIM incorporating TOMATS as a carrier has been optimized to ensure the maximum transport of Hg(II). The influence of the different components of the membrane, such as the kind of polymer or the nature of the plasticizer, has been studied. It has been found that Hg(II) extraction is possible independently of the polymer used (i.e., CTA or PVC), but its transport is only possible with CTA-based membranes incorporating a plasticizer. Plasticizers with different characteristics (i.e.: dielectric constant, viscosity, density) have been tested and best results in terms of Hg(II) transport efficiency have been obtained when using the plasticizer NPOE. When the receiving phase contained 10^{-3} M cysteine no interference of the feed solution (i.e., water sample) matrix, including its metal ion composition, affected the Hg(II) TE. The PIM studied also allowed the transport of Cd(II), Ni(II) and Pb(II) into the receiving solution together with Hg(II). These results show that the PIM studied can be viewed as a promising preconcentration tool to facilitate the detection of toxic metals at trace levels in natural waters.

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Chapter 3

SECTION 4

Development of new binding phases for the DGT technique based on an ionic liquids for Hg(II) determination

3.4.1 Abstract

Determining bioavailable trace concentrations of mercury (Hg) in water is still a challenging analytical task. In this study, we report a novel method for determining Hg in natural waters using newly developed sorbents. Silicon dioxide at a nanoparticle range (Si-np) and cellulose powder at a microparticle range (Cel-p), both modified with the ionic liquid trioctylmethylammonium thiosalicylate (TOMATS), have been tested as sorbents (sorb-TOMATS) for Hg(II) extraction. These novel sorb-TOMATS materials were characterized, and parameters affecting the extraction were examined. A similar Hg(II) extraction efficiency (97%) and extraction capacity (9 mg Hg/g) was obtained for both sorb-TOMATS, while only a 25% of metal was extracted using non-impregnated materials. Moreover, these sorb-TOMATS were effectively embedded in agarose gel and were tested as a novel binding phase for the Diffusive Gradients in Thin Films (DGT) technique. Research showed Si(np)-TOMATS sorbent to be a useful tool as a binding phase in the DGT technique for Hg(II) measurements, since it also allowed the elution of the extracted Hg(II). This new binding phase showed satisfactory linear correlation between the accumulated Hg(II) mass and deployment time, which is in agreement with the DGT principle. In sum, this novel sorbent is a good binding phase for Hg(II) determination, and seems to be a suitable option for its monitoring in natural waters when integrated in the DGT design.

3.4.2 Background and aims

Mercury (Hg) is a toxic heavy metal [1] found in the environment due to both natural and anthropogenic sources, and that it is globally distributed [2], with the aquatic environment acting as a sink for atmospheric Hg. Due to its high toxicity, Hg has been classified in the European Water Framework Directive (2008/105/EC) as one of the "33 Priority Pollutants"[3]. The detection of inorganic Hg(II) is of paramount importance given that, besides its toxicity, it can also be transformed to methylmercury, and be biomagnified through the aquatic trophic chain [4]. Therefore, the development of analytical methodologies to both facilitate mercury detection and monitoring in the aquatic environment is of great importance [5].

In previous studies, we have developed detection systems for Hg(II) based on the ionic liquid (IL) trioctylmethylammonium thiosalicylate (TOMATS) [6–8]. We have shown that this IL exhibits a high affinity towards Hg(II) both in solvent extraction and when incorporating the IL in a polymer inclusion membrane (PIM). The use of a PIM made of 50% (weight fraction) cellulose triacetate (CTA) as the polymer and 50% TOMATS allowed the effective extraction of Hg(II) in natural waters as different as ground water, river water and sea water with no water matrix effects. Moreover, when the good extraction performance of PIMs was combined with the direct analysis of the loaded membrane, by means of Energy Dispersive X-ray Fluorescence (EDXRF), it was possible to determine Hg(II) in water samples at low µg L^{-1} levels. In addition, a similar PIM, but with a plasticizer incorporated, allowed Hg(II) preconcentration to a receiving phase consisting of 10^{-3} M cysteine solution. This methodology enabled the preconcentrated Hg(II) to be measured using inductively coupled plasma-atomic emission spectroscopy (ICP-AES) or –mass spectrometry (ICP-MS) instruments, which require the use of aqueous samples. This methodology exhibited a good linear correlation between the initial metal concentration present in different kinds of water samples, and metal preconcentrated in the receiving phase. Besides, it was found that no biofilm grew significantly on the PIM surface in early deployment times in the field.

The use of PIM with TOMATS was also investigated as a binding agent in the Diffusive Gradient in Thin films (DGT) technique for total Hg (THg) determination in natural waters [7]. The DGT technique was developed for the in situ determination of kinetically labile metal species in aquatic systems by Davison and Zhang [9], and it has been successfully evaluated for Hg species in natural waters [10–14]. The principle of the DGT technique is based on the diffusion of the dissolved species through a membrane-diffusive layer and its accumulation in a resin embedded in a gel (binding phase). These layers are separated from the source solution by a filter membrane, and assembled into a plastic device with an opening that only allows the exposition of the filter to the deployment solution [15]. The time weighted average concentration of metal in the solution can be calculated according to Fick’s law.

The DGT technique has been applied in a wide range of natural waters [16], and nowadays it is possible to sample any labile species if a suitable binding agent is embedded into the receiving phase gel [15]. Hg species are usually measured using selective resins including thiol groups [17–19]. Among them, 3-mercaptopropyl-functionalized silica gel resin is one of the most widely used [12,13,20,21].

Even though binding agents used in the DGT technique are generally employed in the form of resins, in our early study, different designs for the DGT-PIM devices were mounted and tested, including polyacrylamide gel, in some of them, as the diffusive layer. Despite the good Hg(II) extraction exhibited, it was found that Hg(II) collected in PIM-DGT devices did not follow Fick's law of diffusion, thereby limiting the range of application of the DGT technique. However, it seems appropriate to advance in this research by studying new possibilities to entrap the IL in a binding phase to act as a perfect planar sink.

Mercury can be absorbed by activated carbons thanks to the functional groups that largely determine the surface properties of the sorbent [22]. Interestingly, the IL TOMATS has been successfully used to impregnate activated carbon, obtained from palm shell, and used for Hg(II) removal from highly concentrated synthetic aqueous solution through a batch adsorption technique [23]. Hg(II) removal efficiency with modified activated carbon was higher than that obtained with the virgin material, showing the abilities of the impregnated sorbent to interact with Hg(II). Moreover, iron oxide nanoparticles (NPs) have also been efficiently impregnated with TOMATS to be used as a new sorbent to extract cadmium [24].

In the present study, we have reported the preparation and characterization of new impregnated sorbents (cellulose powder and silicon dioxide nanoparticles) with TOMATS for Hg(II) extraction. The possibility of incorporating the new sorbents into an agarose gel has also been investigated, as well as the elution of the extracted metal. Finally, new DGT devices incorporating silicon dioxide NPs impregnated with TOMATS, embedded in agarose gel, and including an agarose diffusive layer, have been prepared and evaluated.

3.4.3 Materials and methods

Reagents and solutions

The IL TOMATS was prepared from the commercial reagent trioctylmethylammonium chloride (Aliquat 336) (Sigma-Aldrich, UEA) and sodium thiosalicylate (TCI, Japan) as described in [7,8].

Two different materials were used for the impregnation of sorbents with TOMATS (sorb-TOMATS): silicon dioxide at nanoparticle size (10-20 nm) (Si(np)), and cellulose powder at microparticle range (20 µm) (Cel(p)), both from Aldrich Chemistry (USA). Ethanol (Panreac, Spain) was used to dissolve the IL for the preparation of sorb-TOMATS.

Agarose (Fisher BioReagents, Belgium) was used to prepare the in-house manufactured DGT gels, for both diffusive and binding gels. Skived teflon of 0.5 and 1 mm thickness were used as spacers between glass plates for gels and sorbent gel, respectively. Moreover, a microwave and an oven at 80ºC, were employed for heating the solution.

L-Cysteine (Cys) (MercK KGaA, Germany) was used for elution studies

Simulated natural water (SNW) with a composition of 2 mM $NaHCO_3$, 1 mM $CaCl•H_2O$, 0.5 mM Na_2SO_4 was prepared by the dilution of sodium nitrate, sodium bicarbonate, calcium chloride hexahydrate (Panreac, Spain) and sodium sulphate (Merck KGaG, Germany) in Milli-Q water (18.2 MΩ cm). Mercury working solutions were prepared daily from a 1000 mg L^{-1} ± 2 mg L^{-1} mercury standard for ICP in HNO_3 12% w/w (Fluka, Switzerland) in aqueous solution of sodium nitrate (Panreac, Spain). Sodium nitrate (Panreac, Spain) was diluted in Milli-Q water and used as aqueous solution for the DGT studies.

Instruments

Sorbent spectrums were recorded using an Agilent Cary 630 FTIR spectrometer. The scanning electron microscopy (SEM) observations of sorbents were made using a field emission SEM with a field emission electron source (FE-SEM) Zeiss, model DSM 960 (Germany). The samples were placed on a stub and coated with carbon (model K950 turbo evaporator, Emitech Germany). Digital images were collected and processed by ESPRIT 1.9 BRUKER program. Energy dispersive X-ray (EDX) analysis was obtained using the detector xFLASH 5010 (Bruker) and processed by ESPRIT 1.9 BRUKER –AXS Microanalysis GmbH (Germany) program.

An inductively coupled plasma optical emission spectrometer (Agilent Technologies, Agilent 5100 Vertical Dual View ICP-OES) was used for measuring Hg content in aqueous samples in a concentration range of 25 – 2000 µg L^{-1} (λ= 253.652 nm). When working with lower concentrations (0.1 – 25 µg Hg L^{-1}), an inductively coupled plasma mass spectrometry (ICP-MS) system (Agilent Technologies, Agilent 7500c ICP-MS) was employed. The isotope ^{202}Hg was selected because it is the most abundant. To avoid the possibility of interferences during sample analyses, standards were always prepared with the corresponding matrix.

Preparation of impregnated sorbents (sorb-TOMATS) and extraction experiments

Sorbent impregnation was carried out following a similar procedure for impregnation of palm shell activated carbon [23]. Briefly, 0.3 g of TOMATS were dissolved in 50 mL of ethanol. Afterwards, 1 g of Si(np) or Cel(p) were added to the mixture and stirred under orbital agitation (150 rpm) for 48 hours at room temperature. Then, the solution was allowed to evaporate.

To evaluate the efficiency of the prepared sorb-TOMATS, Hg(II) extraction experiments were performed using 0.1g of each sorbent, and placed in contact with 25 mL of 500 µg L^{-1} Hg in SNW under orbital stirring for 24 h. Prior to the analysis, the aqueous solution was filtered by cellulose acetate syringe filters (0.45 µm pore size, 25mm diameter) and the extraction efficiency (EE) was calculated using Eq. 3.6:

$$EE\ (\%) = \frac{[Hg]_i - [Hg]_f}{[Hg]_i}\ x\ 100 \qquad \text{(Equation 3.6)}$$

Where $[Hg]_i$ denotes mercury concentration in the aqueous solution at the beginning of the experiment and $[Hg]_f$ denotes mercury concentration in the aqueous solution at the end of the experiment.

The extraction capacity was studied for both impregnated sorbents using 0.01g of each sorbent in 25 mL 1 mg L^{-1} Hg(II) in SNW. The capacity of each sorbent was calculated following Eq. 3.7:

$Capacity = \frac{M_i - M_f}{M_s}$ Equation 3.7

Incorporation of Sorb-TOMATS into agarose gel and extraction and elution experiments

Agarose gel incorporating sorb-TOMATS (1.5% w/w agarose, 1.5% w/w sorb-TOMATS) were prepared following the basis of a procedure explained by Zhang and Davison [25]. The appropriate amount of agarose and the same amount of sorb-TOMATS were diluted in the required volume of warm Milli-Q water (80ºC). The solution was heated in a boiling water bath, and stirred until agarose was completely dissolved. The hot solution was immediately pipetted and assembled between two preheated glass plates separated by 0.5 mm Teflon spacers, and was left to cool down at room temperature until reaching its gelling temperature. Gels were cut in disks and stored in 0.03 M $NaNO_3$ solution until its use.

To evaluate if the sorbents were also effective in terms of Hg(II) extraction, despite being in a gel matrix, some experiments were done using a disk of gel incorporating sorb-TOMATS and using the same extraction conditions as described previously. In this case, however, it was not necessary to filter the solution.

Similarly, the elution of the loaded sorbent gels was investigated by soaking loaded disks in 5 mL of cysteine. Different cysteine solutions as well as agitation performance were investigated. Elution efficiency was evaluated using Eq. 3.8:

$$Elution\ (\%) = \frac{[Hg]_e}{[Hg]_i} x \frac{1}{V_r} x100 \qquad (Eq. 3.8)$$

Where *$[Hg]_i$* denotes mercury concentration in the initial feed, *$[Hg]_e$* refers to mercury concentration in the eluent after the procedure and *V_r* is the volume of the eluent solution.

Si(np)-TOMATS DGT preparation and assembly

Diffusive gels of agarose were prepared following the procedure previously described by Zhang and Davison [25] but using a Teflon spacer of 1 mm.

The assembling of the DGT was carried out using a plastic DGT solution deployment moulding (3.14 cm_2 window) (DGT Research Ltd., UK), which was the structure responsible for enclosing the following layers (from bottom to top): Si(np)-TOMATS sorbent gel, diffusive agarose gel and WhatmanTM 0.45 µm nylon membrane filter.

DGT units were assembled 2 days before deployment and stored at 4°C in the refrigerator in zip plastic bags containing some drops of 0.03M $NaNO_3$. For experimental proposes, DGT devices were immersed in 5 L of 0.1 $NaNO_3$ (5 and 10 µg Hg L^{-1}) or groundwater (5 µg Hg L^{-1}), suspended by a nylon string, in a stirred aqueous solution. DGT units were taken at different deployment times (3, 7, 24, 48 and 72 h). In addition, 5 mL of solution were taken at each DGT deployment time. DGT units were dismantled and resin gels were introduced in 5 mL of 10^{-3}M cysteine in vortex agitation for 30 seconds. Subsequently, the aqueous solutions were analysed by ICP-MS.

3.4.4 Results and discussion

Sorb-TOMATS materials

Characterization

The ability of TOMATS to absorb on the surface of Si(np) and Cel(P) for the formation of sorb-TOMATS was confirmed by FT-IR. The FT-IR spectra of both sorbents before and after impregnation are shown in Figure 3.19. Characteristic bands in 2900-2800 cm^{-1} and 1300-1400 cm^{-1} are related to the long hydrocarbon chains of TOMATS [24]. For Si(np), Figure 1b, bands at 950 and 1090 cm^{-1} could be attributed mainly to the vibration of Si-OH and Si-O-Si groups [26]. Unfortunately, the absorption band of the thiol group near 2500 cm^{-1} was not observed, probably because of the low intensity of this band, as previously found [27].

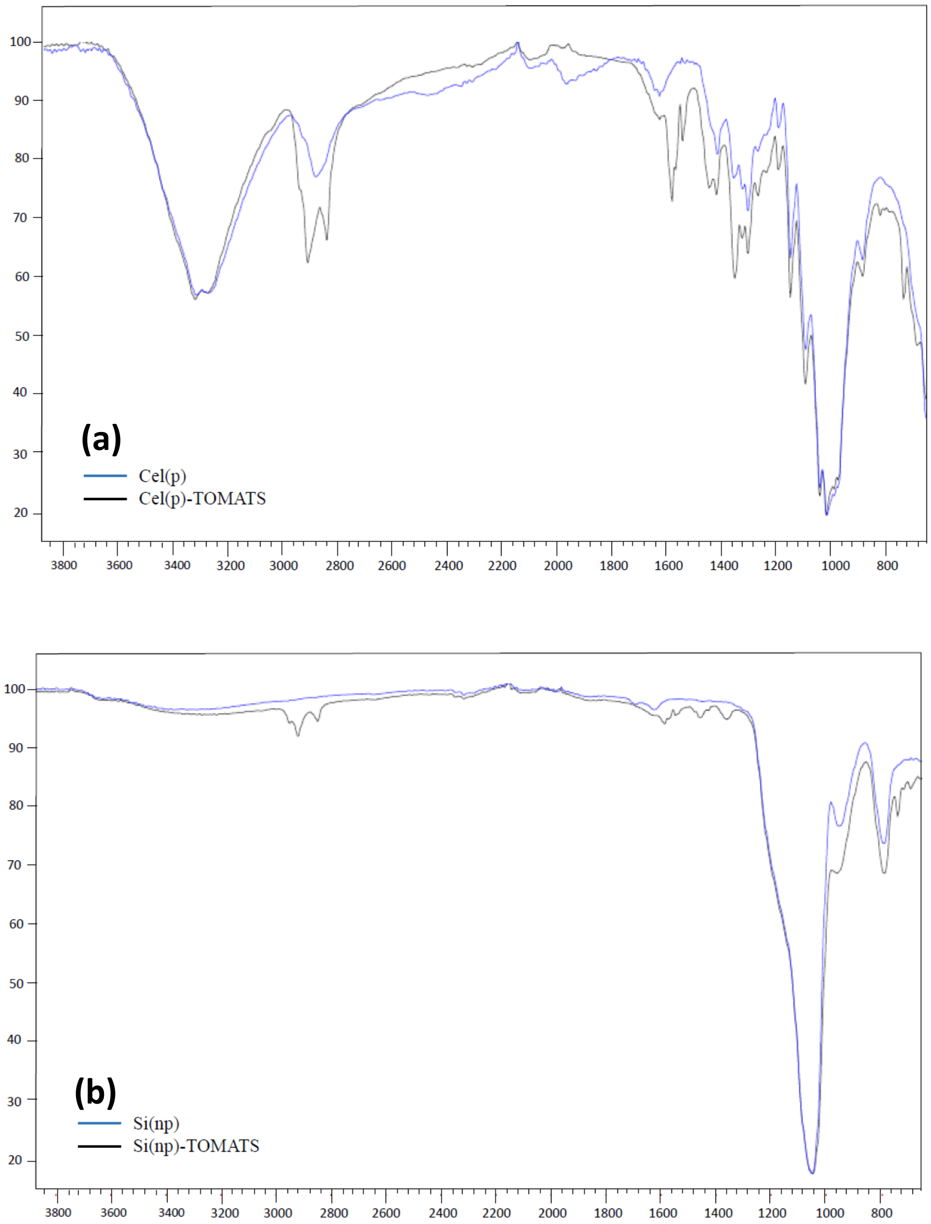

Figure 3.19. FT-IR spectra of (a) Cel(p) and (b) Si(np) sorbents before and after TOMATS impregnation.

The sorbent characteristics before and after impregnation were also investigated. Fig. 3.20 shows SEM images of the virgin materials ((a) for SiO2 and (d) for cellulose), and modified ones ((b,c) for Si(np)-TOMATS and (e,f) for Cel(p)-TOMATS). In the case of SiO2, the original material is seen to be formed by different-sized particles, the most abundant being nanoparticles. However, after impregnation with TOMATS, only particles at a micron scale were obtained, probably due to aggregation facilitated by the IL. This aggregation also appeared in the case of the cellulose sorbent. However, in both cases, the surface of the impregnated sorbents appears smooth and uniform, with no IL droplets, showing good material compatibility

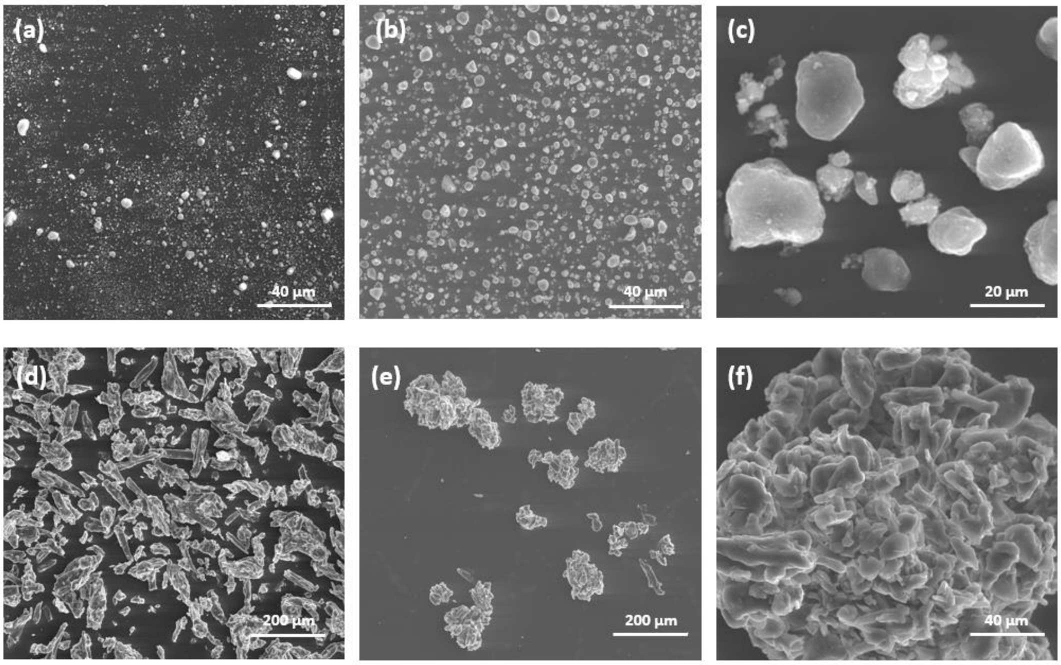

Figure 3.20. SEM images corresponding to (a) SiO2 nanoparticles, (b,c) Si(np)-TOMATS, (d) cellulose powder, (e,f) Cel(p)-TOMATS.

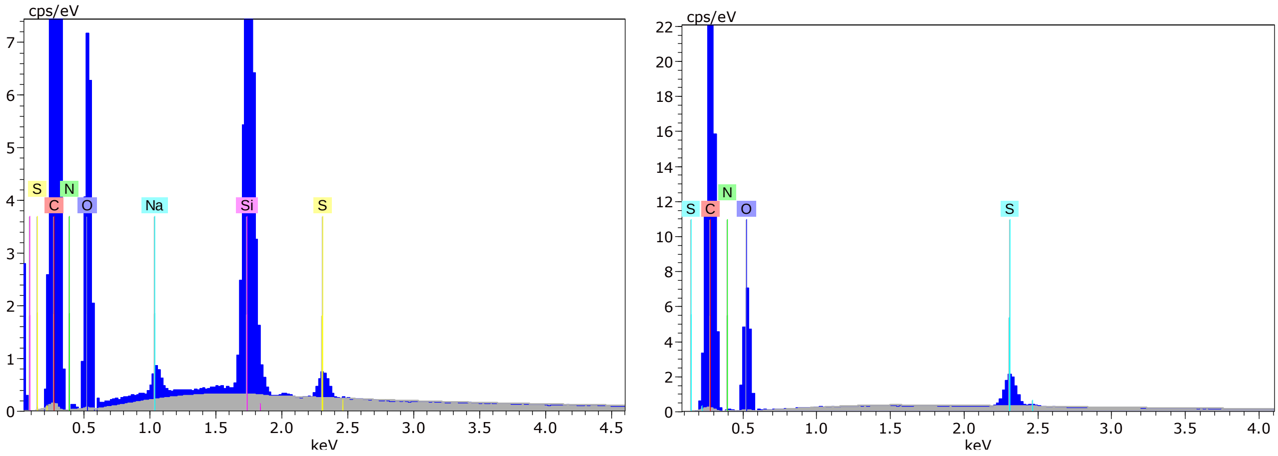

Figure 3.21. EDX spectra of Si(np)-TOMATS (a) and Cel(p)-TOMATS (b) sorbents.

The presence of characteristic elements was investigated in terms of the EDX analysis for both sorb-TOMATS (see Fig. 3.21). As expected, besides elements of the former sorbent, the characteristic peak of S atom (near 2.2 KeV), only present in the IL, appeared in both impregnated sorbents.

Extraction efficiency

Si(np) has been previously reported for the adsorption of U(VI) from aqueous solutions in batch and fixed-bed systems [28], and studied for Cd(II) removal from aqueous solutions combined with heat-inactivated Aspergillus ustus [26]. Thus, we first investigated Hg(II) extraction efficiency for both sorbents before and after impregnation in order to evaluate the necessity to impregnate them with TOMATS. Results are presented in Figure 3.22 As can be seen, Hg(II) extraction with both sorb-TOMATS was very effective and fast, since after only 10 min of contact, about 90% of initial Hg(II) was extracted. Similar results were obtained by Ismaiel et al. (2013), who reported that the binding of Hg with TOMATS is high during the initial stages when a high availability of free TOMATS exists on the modified activated carbon surface, and that, later, coordinate interaction between TOMATS and Hg ions occurs at equilibrium [23]. Concerning sorbents without TOMATS, Hg(II) extraction barely achieved 25% of efficiency after 40 minutes of contact time. Thus, further experiments were performed with impregnated sorbents.

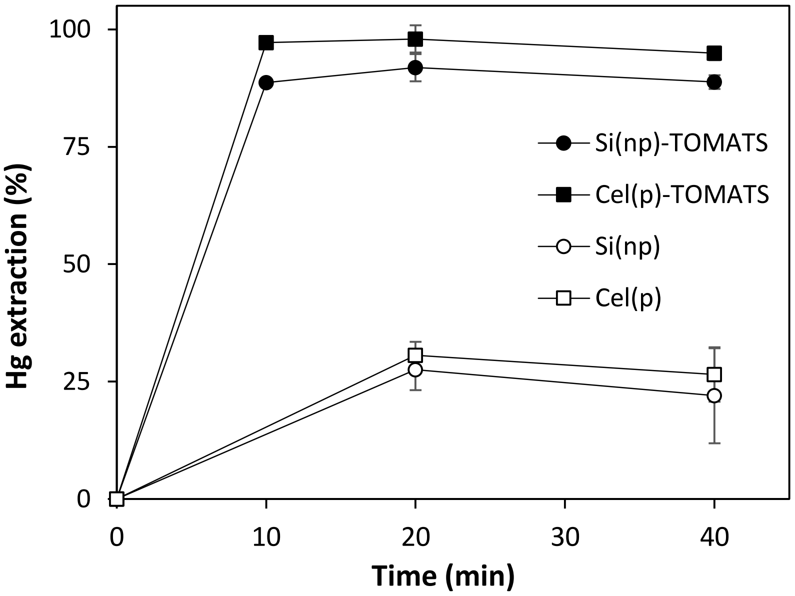

Figure 3.22. Effect of contact time on Hg(II) extraction efficiency by 0.1g of sorbent or sorb-TOMATS in 25 mL of SNW 500 µg L^{-1}, orbital stirring (n=2).

Furthermore, it was found that the extraction capacity of both sorb-TOMATS was very similar: 9.4 ± 0.2 mg Hg/g for Si(np) – TOMATS and 9.7 ± 0.2 mg Hg/g in the case of Cel(p) – TOMATS. The fact that both the kinetics of extraction and capacity of the sorbent are independent on the former material characteristics are in concordance with the fact that TOMATS is uniquely responsible for Hg(II) extraction, and that both sorbents, despite their different chemical nature, are useful for being impregnated by the IL.

Sorb-TOMATS gel

With the aim of using sorb-TOMATS as the binding phase in the DGT technique, we first evaluated the possibility of incorporating the sorbents into a polyacrylamide gel, which has been used in many studies concerning Hg monitoring [12-15]. The gel solution was prepared as described in [10]. However, after solution casting and a long period in the oven, the solution was still liquid and a gel could not be obtained. Consequently, agarose was tested. The procedure for agarose gel preparation did not entail the use of the oven. Instead, the cast solution was left to cool down at room temperature (gelling temperature is 36ºC or below) and it turned out to be a more appropriate procedure for embedding the sorb-TOMATS in a gel. Following this procedure, both Si(np) – TOMATS and Cel(p) – TOMATS gels could be obtained. To evaluate if sorbents incorporated in the gel were still efficient in terms of Hg(II) extraction, some experiments were run using both a gel disk (which contained 0.004 g of sorbent) and also the corresponding amount of loose particles, for comparison purposes (see Fig. 3.23). As can be observed, the use of a smaller amount of sorb-TOMATS required, on the one hand, longer contact times to achieve the extraction of > 90% Hg(II). On the other, similar efficiency values (around 90%) were achieved after 8 h of contact time, when comparing the EE of sorb-TOMATS and the same sorbent but in the gel matrix.

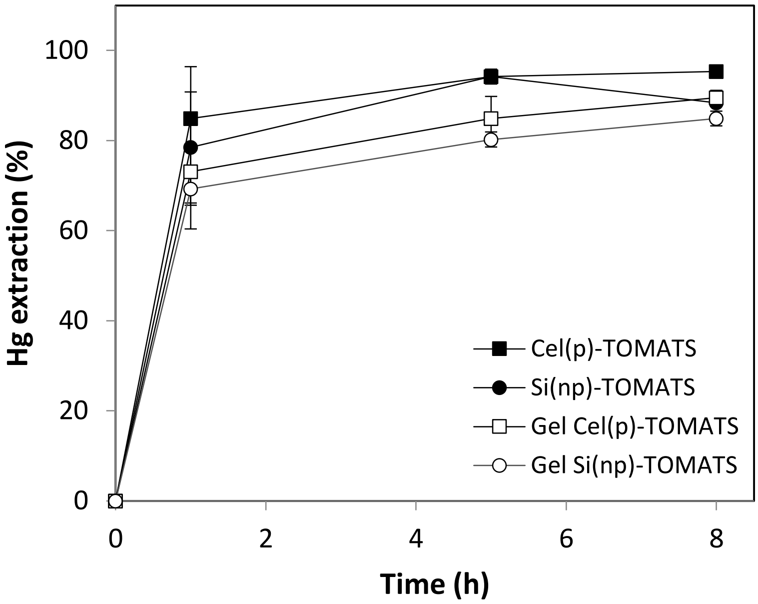

Figure 3.23. Effect of contact time on Hg(II) extraction efficiency by 0.04 g of sorb-TOMATS (loose particles or incorporated in agarose gel) in 25 mL of SNW 500 µg L^{-1}, orbital stirring (n=2).

Elution studies were also performed using loaded sorb-TOMATS gels. It should be borne in mind that generally only a fraction of the bound metal is eluted. This fraction is known as the elution factor (*f*) and is required to calculate the mass of diffuse ions when working with the DGT technique [29].This factor has been calculated in this work by using Eq. 3.9:

$$f = \frac{[Hg^{2+}]e}{[Hg^{2+}]i - [Hg^{2+}]f} \qquad (Eq.3.9)$$

Where *[Hg]$_e$* means de Hg(II) concentration in the eluent solution, *V$_e$* means the volume of the eluent solution, *[Hg]$_i$* means the initial concentration in the bulk solution and *V$_i$* means its volume.

Since cysteine solutions enabled Hg recovery from membranes incorporating TOMATS [8], a solution of 5 mL of 10^{-3} M cysteine was investigated as a means of recovering Hg from the sorbent gels (orbital agitation, 15 h, n=2). It was found that elution efficiency was 61±10 % for Cel(p) – TOMATS gel while this value increased up to 72±4% for Si(np) – TOMATS gel. Using this latter material, we tested the effect of agitation on the elution process. Results in terms of elution efficiency (%) are represented in Figure 3.24. Within the error limits, all the elution processes tested for Hg(II) had a value of around 70% (elution factor, *f*, of 0.7). Moreover, no significant differences ($p < 0.05$) were obtained when comparing the elution procedures. Also, a 0.01 M cysteine solution was tested, using vortex agitation, to check whether increasing the concentration could enhance the recovery of the metal. However, a similar value of elution efficiency (70 ± 18) was obtained. Therefore, the elution process was fixed using 5 mL 10^{-3} M cysteine solution in vortex agitation (30 seconds).

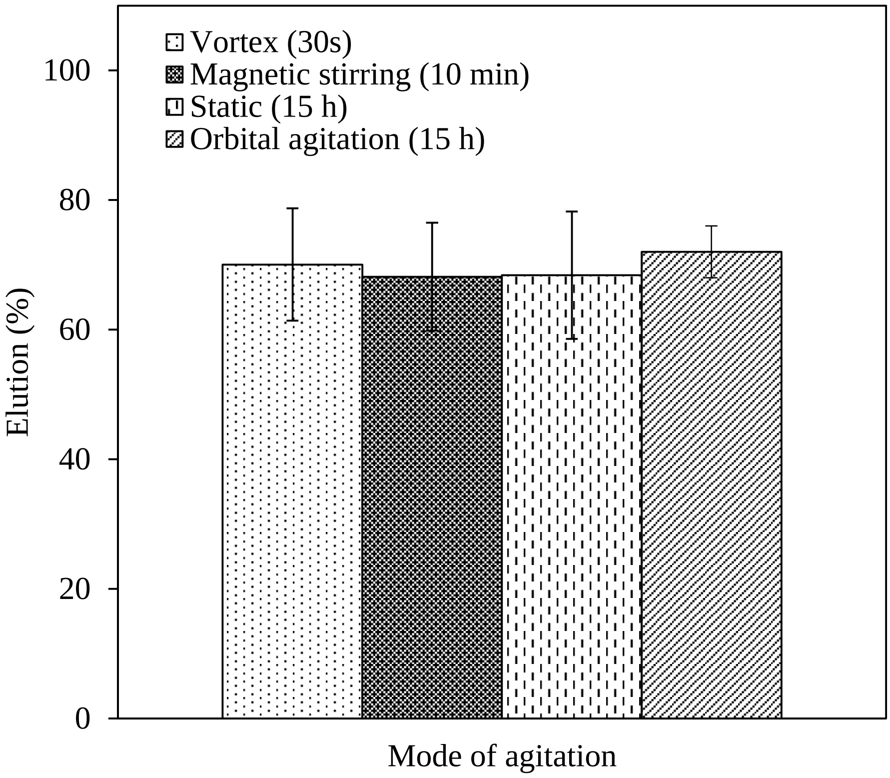

Figure 3.24. Effect of agitation on Hg(II) elution efficiency of loaded Si(np)-TOMATS gels using 5 mL 10^{-3} M cysteine solution.

Taking into account the good performance of Si(np)-TOMATS incorporated in agarose gel for both the extraction and elution experiments, this sorbent was chosen to be incorporated in DGT devices.

DGT incorporating Si(np)-TOMATS

Uptake rate

DGT devices were calibrated with two different Hg(II) concentrations, 5 and 10 µg L^{-1} in 0.1M $NaNO_3$. Results obtained are presented in Figure 3.25. As shown, Si(np)-TOMATS worked successfully as a binding layer in DGT, accumulating Hg(II) in proportion to exposure time independently of the initial metal concentration in the bulk solution. These results are in accordance with Fick's law of diffusion, which states that the diffusion flux is proportional to the concentration gradient. The measured mass of accumulated metal, *M*, provides a flux, *J*, directly from the area of the exposed gel, *A*, and the deployment time, *t*, as follows:

$$J = \frac{M}{At} \qquad (Eq. 3.10)$$

Assuming that the free metal ion concentration at the interface between the resin and diffusive layer is negligible, then the in situ flux is given by equation Eq. 3.11:

$$J = \frac{CD}{\Delta g} \qquad (Eq. 3.11)$$

Thus, the mean concentration in solution during the deployment time is obtained by combining eqs. 3.10 and 3.11 into 3.12.

$$C = \frac{M\Delta g}{AtD} \qquad (Eq. 3.12)$$

To take into account the variable Hg concentration (*C*) in the sampling solution during the experiment caused by the adsorption of Hg(II) on the container walls, the mass of accumulated Hg(II) in the resin layer was normalized by the solution concentration of Hg(II) (aliquots of 5 mL were withdrawn at each sampling time).

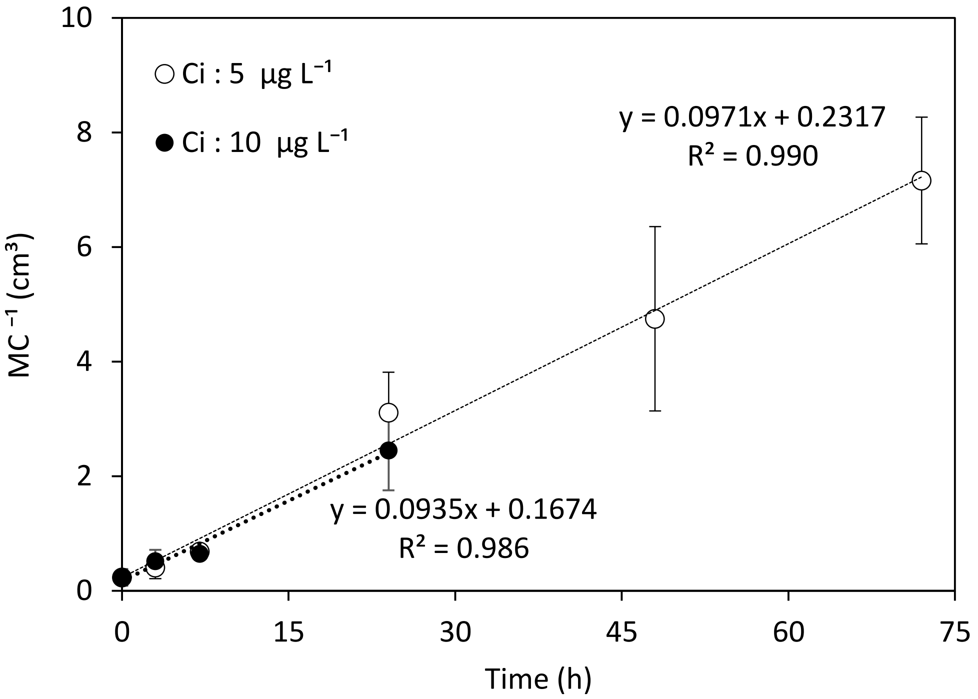

Figure 3.25. Kinetics experimental studies of two different initial Hg concentrations. Mass of Hg accumulated in the resin gel (M) normalized by the Hg concentration in the aqueous solution at each time set.

Calculation of D

It is possible to calculate the diffusion coefficient (*D*) of Hg(II) in the diffusive layer according to Equation 3.13 which relates the deployment time and the mass of metal accumulated by the DGT units (normalized for Hg concentration in solution)

$$s = \frac{DA}{\Delta g} \qquad (Eq. 3.13)$$

Thereby, the diffusion coefficient can be calculated with Equation 3.14.

$$D = \frac{s\Delta g}{A} \qquad (Eq\ 3.14)$$

The *D* of Hg(II) in the diffusive layer for both initial Hg(II) concentrations tested were not significantly different, with values of 9.45 x 10^{-7} cm^2 s^{-1} for 5 µg L^{-1} and 9.10 x 10^{-7} cm^2 s^{-1} for 10 µg L^{-1}. These results are extremely near to the order of magnitude 10^{-6}, which seems to be the most common for Hg(II) [30,31].

Effect of water characteristics

In this research, we also studied the application of in-house manufactured DGTs containing Si(np)-TOMATS as a binding phase with natural groundwater spiked with Hg(II). Results obtained with DGTs (n=3) deployed during 24h in groundwater spiked at 5µg L^{-1} concentration level of Hg(II) were also compared in Figure 3.26 with those obtained in 0.01 M NaNO3 spiked at 5µg L^{-1} level. As it can be seen, the slopes of the curves obtained for both cases presented considerable similarities. Furthermore, a good linearity of the calibration curve was also obtained with groundwater. These results open a wide range of possibilities for developing DGT passive sampler devices using SiO_2 nanoparticles impregnated with TOMATS.

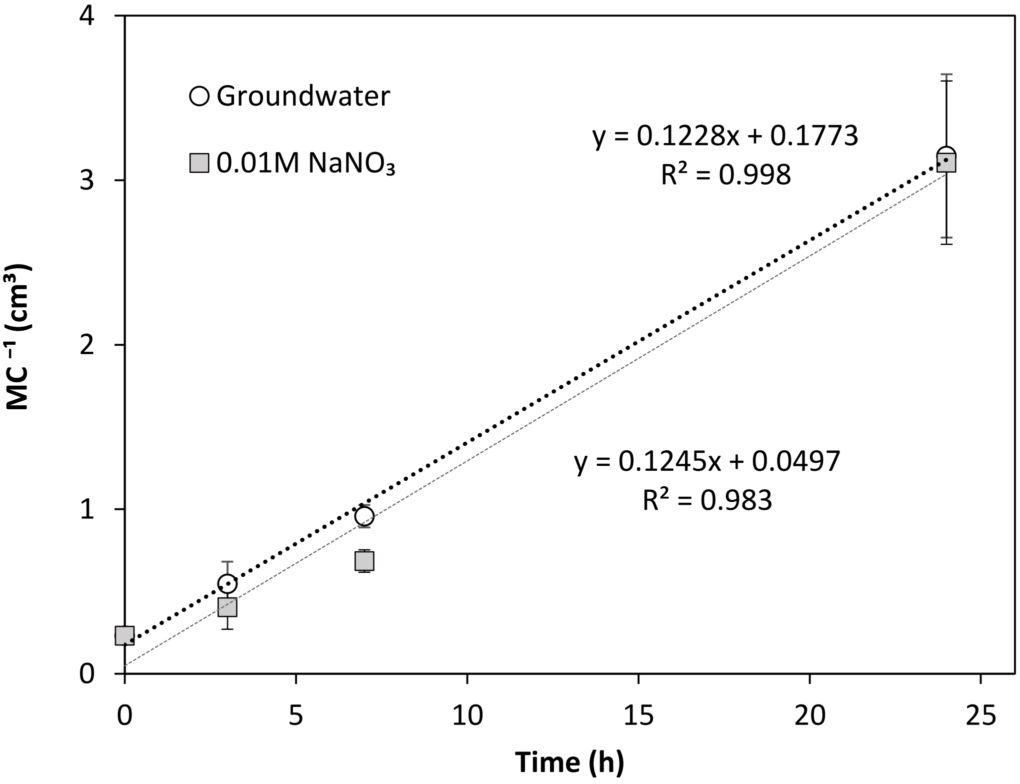

Figure 3.26. Kinetics experimental studies of 0.01M $NaNO_3$ and its application in groundwater. Mass of Hg accumulated in the resin gel (M) normalized by the Hg concentration in the aqueous solution at each time set.

3.4.5 Conclusions

Si(np) and Cel(p) were effectively impregnated with the IL TOMATS and embedded in agarose gel. Both gels allowed Hg(II) extraction from aqueous samples, but elution with 10^{-3} M cysteine solution was more efficient for Si(np) functionalized with TOMATS.

Si(np)-TOMATS sorbent was successfully used as a binding phase in the DGT technique for Hg(II) measurements. The new binding phase showed satisfactory linear correlation between the accumulated Hg(II) mass and deployment time, which is in agreement with the DGT principle. In addition, Si(np)-TOMATS performance for Hg(II) evaluation was not affected when deployed in spiked samples of natural water. This easy handling and cost-effective binding phase appear to be an interesting choice for Hg(II) determination and a useful tool for mercury monitoring in natural waters.

3.4.6 References

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Chapter 4

General discussion and future perspectives

As mentioned in the introduction of this thesis, the presence of mercury in aquatic systems is of concern due to its widespread occurrence and the high toxicity of its compounds. Hence, it is of paramount importance to progress towards simple and robust analytical methodologies to facilitate the measurement of this metal in environmental waters. Considering both the low concentration of the metal as well as the complexity of water samples, these analytical methodologies should comprise a separation/preconcentration step prior to determination with common analytical instrumentation.

Seeking a global solution for mercury monitoring, we have focused in this thesis on the use of ionic liquids (ILs) as extractants for Hg(II) species. An IL is a salt in which the ions are poorly coordinated, resulting in compounds being liquid below 100°C, or even at room temperature (room temperature ionic liquids, RTILs). They are formed by an organic cation (which contains N or P atoms) and an organic or inorganic anion, and their use as extracting agents can be manipulated by a proper selection of the anion, which is supposed to bind to the species of interest. Although ILs can be synthetized, and some of them are even commercialized, in this thesis we have evaluated the possibility of obtaining the ILs of interest by a simple anion-exchange reaction using the commercial IL Aliquat 336 (trioctylmethylammonium chloride) as a source of the cation, and the sodium salts of the reagents thiosalicylate and salicylate as the source of anions to obtain the ILs, trioctylmethylammonium thiosalicylate (TOMATS) and trioctylmehtylammonium salicylate (TOMAS), respectively. These ILs have been chosen to test their ability to coordinate with Hg(II) and to form the basis of innovative analytical methodologies to facilitate Hg(II) detection. It is worth mentioning that TOMATS, bearing a thiol group, was previously described in the literature as exhibiting a high ability to extract Hg(II) [1]. However, this study was conducted using a commercial product (a task-specific ionic liquid). In the case of TOMAS, this IL has not been previously investigated as a possible extractant for Hg(II).

From our studies, we have shown that the preparation of ILs by exchanging the chloride anion present in Aliquat 336 by either thiosalicylate or salicylate anions resulted in the satisfactory production of the expected reagents, which was confirmed by elemental analysis, proton nuclear magnetic resonance, and FT-IR. In Fig. 4.1 an image of the ILs obtained is shown.

Figure 4.1. Image of the ILs produced in the laboratory. Left: TOMATS; Right: TOMAS.

The efficiency of TOMATS and TOMAS in extracting Hg(II) was first investigated in solvent extraction experiments. As shown in Fig. 4.2, after 5 hours of contact time, 95% of Hg(II) was extracted by TOMATS, whereas 51% of Hg(II) extraction was achieved with TOMAS.

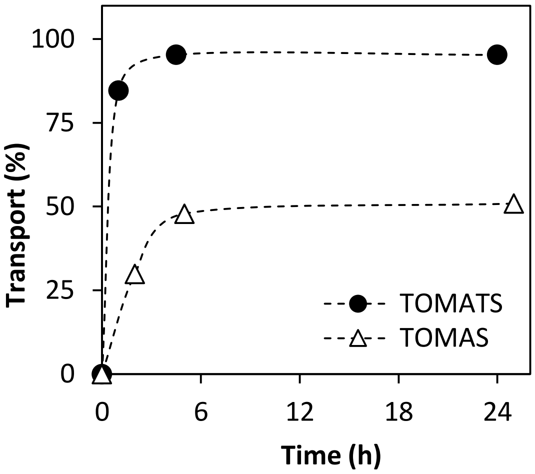

Figure 4.2. Kinetics of Hg(II) extraction efficiency for both ILs. Aqueous solution: 3 mL 1000 µg L^{-1} Hg(II) in 0.01M $NaNO_3$; Organic phase: 3 mL 10^{-4} M IL in CH_2Cl_2.

Hence, even though Hg(II) extraction was more favourable using TOMATS, as expected, TOMAS, bearing a hydroxyl group instead of a thiol group, was also able to interact with Hg(II) thanks to the chelating effect of the carboxylate group [2].

The solvent extraction technique possesses some drawbacks, such as high organic solvent consumption, the possible leaching of both IL and organic solvent to the aqueous phase, with the associated environmental risk [3], and the impossibility of performing extraction and retro-extraction in one single step. In this thesis we have investigated different routes for immobilizing ILs, such as polymeric membranes or solid sorbents, in order to handle ILs easily and take advantage of their good performance as extracting agents.

Polymer inclusion membranes

Polymer inclusion membranes containing both TOMATS and TOMAS have been successfully prepared by the solvent casting method using CTA as the polymer. The membranes resulting from this study have been characterized, and in all the cases, the membranes formed were dense, with no pores in their structure, homogeneous, flexible and mechanically stable.

To test whether the inclusion of the ILs in a polymeric matrix could hamper their extraction abilities, a study using a PIM with a composition of 50% CTA + 50% IL (% in mass) was performed in contact with a solution of 1000 $\mu g\ L^{-1}$ Hg(II) in simulated natural water. It was found that the extraction efficiency profile followed the same trend as that observed in solvent extraction. However, the kinetics was slower, since PIM with TOMATS needed more than 8 h to quantitatively extract the metal. In the case of TOMAS, the PIM extracted about 55% Hg(II) in 6 h.

Taking into account the great efficiency of the PIM containing TOMATS, we investigated the use of a PIM, with a fixed composition of 50% CTA + 50% TOMATS, to act as a sorbent to preconcentrate Hg(II) from natural waters followed by X-ray fluorescence analysis. One of the main advantages of using XRF instruments is that there is no need to elute the extracted metal, since a solid, in this case the PIM, can be analysed directly. The new analytical methodology resulting from this thesis has proven to be very efficient in determining low concentrations of metal in a great variety of natural waters, such as ground water, tap water, river water and sea water. The fact that the signal of the EDXRF spectra of Hg(II) collected in the PIM did not differ significantly in the different types of waters highlights the great applicability of this methodology. On the one hand, these results revealed that the presence of major ions commonly found in natural waters did not disturb the efficiency of the system; and, on the other, they showed that TOMATS is able to extract Hg(II) regardless of the species of the metal (which may be anionic, such as in the case of sea water, or neutral or cationic for other waters). It is important to highlight that the extraction process was done without any previous treatment (i.e. filtration or pH modification, for example), showing the simplicity of the methodology. Furthermore, it was demonstrated that by using 500 mL of sample volume and a 1 cm^2 piece of PIM, a LOD of 0.2 μg L^{-1} was found, thereby making it possible to determine Hg in drinking waters at the permissible level of 1 μg L^{-1}.

An important advancement resulting from this thesis is that we have shown, for the first time, that not only are PIMs efficient as a preconcentration tool but they are also a suitable medium to permit the preservation and storage of metal for long periods of time without any alteration in their concentration. This fact is of great relevance in the case of Hg, since the volatile characteristics of this metal makes sample preservation a difficult task. Hence, extracting the metal in a PIM will allow detection to be made when possible, without affecting measurement accuracy.

Considering the great efficiency of the PIM containing TOMATS for Hg(II) extraction, we have also investigated in this thesis whether this membrane could be used not only for extraction but also to transport the metal from the water to a receiving phase. This would enable the recovery of the metal in an aqueous solution, which is the requirement when analysis is conducted with more common analytical instrumentation found in laboratories, such as ICP-OES or ICP-MS.

Therefore, it was necessary to investigate the elution of the extracted metal. Both elution and transport experiments were carried out with membranes containing the two ILs investigated in this thesis, TOMATS and TOMAS. Complexing agents such as EDTA and cysteine were tested as possible reagents to recover the mercury extracted from the 50% CTA + 50% IL PIMs. The fact that EDTA only could strip Hg from a PIM containing TOMAS showed that the complex formed by TOMAS and Hg ions was probably weaker than the complex formed with TOMATS. Cysteine solutions, on the contrary, were suitable for the recovery of Hg ions from PIMs containing either TOMATS or TOMAS.

To perform transport experiments, PIMs were placed in a special device that contained 5 mL of receiving solution and in contact with 100 mL of natural water containing low amounts of Hg(II). Transport through a PIM comprises several steps: (i) extraction of the metal at the interface aqueous solution-membrane; (ii) diffusion of the extracted compound (Hg-TOMATS) through the membrane; (iii) release of the metal to the receiving solution. In this thesis we have demonstrated that the composition of the PIM is of paramount importance to obtain efficient transport systems. Although a PIM made of 50% CTA + 50% TOMATS was very effective for extracting Hg(II), when this PIM was used as a transport system, with a 10^{-3}M cysteine solution as a receiving phase, only 38% of Hg(II) was transported. In the case of a PIM of the same composition but with TOMAS, transport was even lower (only 9%). Consequently, we investigated the effect of the addition of a plasticizer in the formulation of the PIM as well as the use of the polymer PVC for membranes prepared with TOMATS. Membranes consisting of 50% PVC + 50% TOMATS were prepared to compare with PIMs with CTA, while the effect of the plastizicer was investigated for both polymers, and the plastizicer NPOE was used for PIMs with a composition of 50% polymer + 30% TOMATS + 20% NPOE. Our results demonstrated that Hg(II) extraction was >90% for all the membranes tested, but that only the 50% CTA + 30% TOMATS + 20% NPOE PIM allowed good transport efficiency (84±7%). Surprisingly, PIMs made of PVC did not allow the recovery of the metal to the cysteine solutions. The morphology of PVC membrane was investigated and SEM images showed that the inner structure of the membrane was inhomogeneous, unlike CTA. Hence, this polymer was chosen to perform studies on the role of the plasticizer. Different membranes were prepared with a fixed composition of plasticizer, and transport studies were conducted for each membrane. Moreover, hydrophilicity was evaluated in terms of contact angle. It was found that the addition of a plasticizer increased the hydrophilic character of the membrane, but that this value was not related to the efficiency of the membrane. For example, PIMs with the plastizicer FPNPE exhibited a contact angle value similar to NPOE, but the extraction efficiency of the former was only about 30%. Among all the plasticizers studied, we found that the best membranes were those with plasticizers as different as NPOE (viscosity= 11.1 cP, dielectric constant= 24) and DBS (viscosity= 9.5 cP, dielectric constant= 4.5). Taking all these results into account, it can be said that although a plasticizer is required in a CTA-based PIM to obtain efficient Hg(II) transport, it would appear that a main parameter (dielectric constant, viscosity, functional group, length of aliphatic chain, etc.) governing this behaviour does not exist. Thus, the optimization of the PIM components has to be done experimentally.

Once the composition of the PIM was optimized and fixed at 50% CTA + 30% TOMATS + 20% NPOE, we evaluated its performance as a system for the preconcentration of Hg(II) in natural waters. Our results have demonstrated that this simple PIM device is a powerful tool to preconcentrate Hg(II) ions in a 10^{-3}M cysteine solution in a wide range of metal concentration. Moreover, this PIM system is widely applicable to natural waters since we have demonstrated that Hg(II) was effectively detected in river water, ground water, sea water, hot spring water and mineral water.

The effect of the presence of other metals was also investigated since TOMATS is able to interact with other metallic species that might hamper the extraction efficiency of Hg(II) ions. Experiments done using the PIM-device in contact with natural waters containing Cd(II), Ni(II) and Pb(II) have shown that these metals can also be transported through the PIM to a 0.1 M EDTA solution, but with different efficiencies (Cd(II)>Pb(II)>Ni(II)). These results are of great interest when designing a preconcentration system for heavy metals in natural waters. However, despite the coordination of TOMATS with these metal ions, when Hg(II) was also present in the sample, and a 10^{-3}M cysteine solution was used as the receiving phase, the PIM system effectively preconcentrated Hg(II) without being affected by the presence of the other metals.

In this thesis, we have also explored for the first time possible biofouling growth on the PIM surface when deployed in natural waters for a long period of time. This study is of interest for future applications of the PIM system as a passive sampler for mercury monitoring. We have shown that PIMs deployed for 7 days in a controlled water reservoir did not show significant biofilm growth. After one month of deployment, some diatoms appeared on the surface membrane but, taking into account that 7 days is a common time period used in passive sampling studies, this PIM-system appears to be a promising tool for the design of suitable passive sampler devices for freshwater environments.

To sum up, as a result of the investigations conducted in the framework of this thesis, two novel analytical methodologies based on a PIM have emerged: one that combines PIM extraction and EDXRF analysis (PIM-EDXRF), and another entailing a PIM-device and the subsequent analysis of the receiving solution by ICP-MS (PIM-device-ICP-MS). Some relevant parameters of both methodologies are shown in Table 4.1 for comparison purposes.

Table 4.1. Comparison between the two preconcentration methods based on a PIM with TOMATS developed in this thesis.

Analytical feature	PIM-EDXRF	PIM-device-ICP-MS
PIM composition	50% CTA + 50% TOMATS	50% CTA + 30% TOMATS + 20% NPOE
PIM surface (cm^2)	0.79	1.5
Sample volume (mL)	500	100
Elution	not required	5 mL 10^{-3}M cysteine
LOD (μg L^{-1})	0.2	0.29
LOQ (μg L^{-1})	0.67	0.74
R^2 (range)	>0.99 (0.6 -10 μg L^{-1})	>0.99 (1 - 10 μg L^{-1})
Recovery from mineral water	90% (1 μg L^{-1})	116% (5 μg L^{-1})

As explained before, the main difference between both systems was the elution step, which was not necessary for the PIM-EDXRF methodology, but achieved by means of 5 mL of 10^{-3}M cysteine when an ICP-MS or ICP-OES was used. Despite this, and together with differences concerning the PIM surface and sample volume, it is important to highlight that both methodologies achieved similar LOD and LOQ values and in concordance with the limits established for drinking water. Moreover, linearity throughout the concentration range achieved values greater than 0.99 and recovery values of around 100% for both systems, showing the good performance of these innovative analytical methodologies for Hg(II) determination from aqueous samples.

Impregnated sorbents

One of the most common passive sampling methodologies is the DGT technique, which is based on the diffusion of the dissolved species through a membrane-diffusive layer and their accumulation in a resin incorporated in a gel. Due to the high affinity of

TOMATS towards Hg(II), in this thesis we have investigated the possible use of TOMATS as a binding phase in the DGT technique. Since it was not possible to directly incorporate TOMATS into agarose or polyacrylamide gels, probably due to an inappropriate compatibility between reagents, we investigated the possibility of using TOMATS impregnated into inert sorbents to be incorporated in DGT gels.

Two different sorbents with different characteristics were tested. The inorganic sorbent silicon dioxide, at a nanoparticle size (10-20 nm) (Si(np)), and the microparticle range (20 µm) of the organic sorbent cellulose powder (Cel(P)). The impregnation of both sorbents was easily conducted by placing the material in contact with a solution of TOMATS in ethanol, followed by its subsequent evaporation. The materials obtained (sorb-TOMATS) were characterized and used for Hg(II) extraction experiments. It was shown that both sorb-TOMATS efficiently extracted Hg(II) from a simulated natural water sample, irrespective of the former material. It is important to highlight that this efficiency was not altered when the impregnated sorbents were embedded in agarose gel.

The possible elution of the extracted Hg(II) was also investigated. It was found that 30 s agitation of loaded Si(np)-TOMATS gel in 5 mL 10^{-3}M cysteine solution allowed 70% recovery of the metal.

Based on these encouraging results, these impregnated sorbents were tested as binding phases in the DGT technique. The in-house manufactured DGT devices consisted of 0.04 g Si(np)-TOMATS embedded in agarose gel as the binding agent (thickness of 0.5 mm), 1 mm agarose gel as the diffusive layer, and a 1 mm thick membrane filter with a 0.45-µm pore size, to protect the diffusive gel.

A passive sampler calibration under controlled laboratory conditions is usually conducted prior to field application and thus, we performed different experiments to better study the possibility of using these new binding gels in the DGT technique. The uptake rate of the devices was studied using 5 L of 0.01M $NaNO_3$ spiked at two different Hg(II) concentrations, 5 and 10 µg L^{-1}. The slopes of the calibration curves were similar, demonstrating that the uptake rate was not affected by the initial metal concentration. The diffusive coefficient (D) was also calculated and values of the same order of magnitude were obtained ($9.099 \bullet 10^{-7}$ and $9.449 \bullet 10^{-7}$ for 5 and 10 μg L^{-1}, respectively). Similar results were obtained when Hg(II) was in groundwater instead of a controlled saline solution. This revealed that the water chemical characteristics did not affect the performance of the DGT device incorporating the new binding phase.

Future perspectives

Future applications of the studies carried out in this thesis will include, for example, the use of PIMs as sorbents for mercury preservation, which would be of great importance due to the problems related to Hg(II) sample stability. Studies performed in remote places, with a lack of adequate laboratory instruments, would benefit from the use of a PIM for in-situ extraction of the metal present in the water (no matter what the water composition may be) as a medium in which to preserve mercury until the appropriate analysis could be done.

Moreover, considering the simplicity of the extraction procedure, together with the good performance exhibited by PIMs with TOMATS, it would be of great interest to extend the study of these membranes to the extraction of other metals. Taking into account that EDXRF allows multielemental determination, the use of PIMs could be viewed as an all-in-one solution for the extraction-preservation-analysis steps, required in many analyses.

The possible biofouling growth on the PIM surface should be studied in greater depth over longer periods of time, and some modifications of the membrane should be studied to verify whether transport efficiency is affected by biofouling. We believe that the PIM-based device here designed can play an important role in future studies on Hg monitoring in aquatic environments as varied as rivers or harbours.

The use of impregnated sorbents could be further exploited as a means of preconcentrating Hg(II) in micro-columns that can easily be incorporated in on-line measurements by ICP.

For DGT devices incorporating the impregnated sorbents as a binding phase, it would be very interesting to evaluate the effect of other parameters found in environmental conditions, such as the presence of DOM, flow rate, or sunlight. In addition, since biofouling is the main drawback when dealing with passive sampling techniques, it would be of real interest to study its growth onto this new DGT type.

Finally, comparison under the same conditions as the new tools developed in this thesis for Hg(II) monitoring (the DGT unit and the PIM-device), would be of a great interest to establish the possible advantages or drawbacks of each one, and thus provide a framework of application for each system.

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Chapter 5

Conclusions

The general main conclusions extracted from the research conducted in this thesis are summarized as follows:

- The ILs TOMATS and TOMAS have been successfully prepared by an anion exchange reaction using the commercial reagent Aliquat 336 and the anions thiosalicylate and salicylate anions, respectively.

- Both ILs TOMATS and TOMAS can be efficiently incorporated in a polymer inclusion membrane when the CTA is used as the polymer. Resulting membranes are dense, homogeneous and mechanically stable, and can extract Hg(II) from aqueous solutions, with PIMs with TOMATS being more efficient than PIMs with TOMAS.

- A reliable and simple analytical methodology for the determination of Hg(II) at low µg L^{-1} levels from different types of natural waters has been developed based on the extraction of the metal by a PIM, with a composition of 50% CTA + 50% TOMATS, in combination with EDXRF analysis. Moreover, it has been found that this PIM was useful for preserving the metal extracted for a long period of time with no alteration in its concentration.

- It has been demonstrated that the addition of the plasticizers NPOE and DBS (20% w/w) to a PIM made up of CTA (50% w/w) as the polymer, and TOMATS (30% w/w) as the carrier, allowed the effective transport of Hg(II) from a natural water to a 10^{-3}M cysteine solution, used as a receiving phase.

- The use of a device containing a PIM with a composition of 50% CTA + 30% TOMATS + 20% NPOE has enabled the effective preconcentration of Hg(II) from different types of natural waters (*i.e.* ground water, river water and sea water) in the cysteine solution, with no significant effect on the water matrix, and thus, facilitating its determination. Even though other toxic metals such as Pb(II), Cd(II) and, to a lesser extent Ni(II), were also transported through the PIM, the effectivity of the system towards Hg(II) was not affected.

- The mentioned PIM was not colonized by biofilm after a week of deployment. However, longer deployment times allowed the biofilm to grow on the surface of the membrane.

- New modified sorbents have been successfully prepared by the impregnation of TOMATS onto both cellulose and silicon dioxide particles, exhibiting a high efficiency for the extraction of Hg(II) in a very short contact time. Moreover, when these sorbents were incorporated in an agarose gel matrix the extraction efficiency was not affected.

- Silicon dioxide nanoparticles impregnated with TOMATS have been successfully used as a novel binding for the DGT technique using agarose gel.