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Non-polar contaminants in estuarine waters: new extraction methods and passive sampling choices

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A thesis submitted for the international degree of Philosophiae Doctor in Environmental Contamination and Toxicology

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ESKER ONAK

Nestor eta Maitaneri, lan hau burutzeko aukera emateagatik eta urte hauetan zehar eskainitako laguntzagatik eskerrik asko. Beti egon zarete prest nirekin elkar lanean jarduteko. In the same way, I am really grateful to Albrecht and all the colleagues from the UFZ. It was a great experience to work in your team.

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Guztioi bihotz bihotzez eskerrik asko.

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ABBREVIATIONS AND ACRONYMS

2,4'-DDD	o,p'-dichlorodiphenyldichloroetane
2,4'-DDE	o,p'-dichlorodiphenyldichloroethylene
2,4'-DDT	o,p'-dichlorodiphenyltrichloroethane
4,4'-DDD	p,p'-dichlorodiphenyldichloroetane
4,4'-DDE	p,p'-dichlorodiphenyldichloroethylene
4,4'-DDT	p,p'-dichlorodiphenyltrichloroethane
4nOP	4-nonylphenol
4tOP	4-tert-octylphenol
AcN	acetonitrile
ADBI	4-acetyl-1,1-dimethyl-6-*tert*-butylindane (celestolide®)
AHMI	6-acetyl-1,1,2,3,3,5-hexamethylindane (phantolide®)
AHTN	7-acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphthalene (tonalide®)
ANOVA	analysis of variance
ATII	5-acetyl-1,1,2,6-tetramethyl-3-isopropylindane (traseolide®)
BBP	benzyl-2-butyl phtalate
BTEX	benzene, toluene, ethylbenzene and xylenes
CCD	central composite design
CE	Capillary electrophoresis
cHex	cyclohexane
CIS	cooled injection system
Chlorp	Chlorpyrifos (Pestanal®)
Chlorf	Chlorfenvinphos
C_{TWA}	Time-weighted average concentration
CPE	cloud point extraction

DDT	dichlorodiphenyltrichloroethane (general acronym to design all the family)
DEHP	bis(2-ethylhexyl)phthalate
DI	direct immersion
DLLME	dispersive liquid-liquid microextraction
DOM	dissolved organic matter
DOP	di-octyl phthalate
DPMI	6,7-dihydro-1,1,2,3,3-pentamethyl-4(5*H*)-indanone (cashmeran®)
EDA	effect-directed analysis
EG	Ethylen glycol
EPA	Environmental Protection Agency
EQS	Environmental Quality Standard
FUSB	focussed ultrasonic cup booster
GC	gas chromatography
GC-MS	gas chromatography-mass spectrometry
HCH	hexachlorocyclohexane
HDPE	high density polyethylene
Hex	*n*-Hexane
HF	hollow fiber
HHCB	1,3,4,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-(*γ*)-2-benzopyrane (galaxolide®)
HPLC	high performance liquid chromatography
HS	headspace
ID	capillary tube (32 mm length, 0.0111” ID, 0.0300” OD, accuracy 1%)
K_{ow}	octanol–water distribution coefficient
LC	liquid chromatography
LC-MS/MS	liquid chromatography tandem mass spectrometry
LD	liquid desorption

LDPE	low-density polyethylene
LLE	liquid-liquid extraction
LOD	limit of detection
LPME	liquid phase microextraction
LVI	large volume injector
LVI-PTV-GC-MS	large volume injection programmable temperature varorizer gas chromatography mass spectrometry
MA	1-*tert*-butyl-2-methoxy-4-methyl-3,5-dinitrobenzene (musk ambrette)
MASE	membrane-assisted solvent extraction
MDL	method detection limit
MEPS	microextraction in packed sorbents
MESCO	membrane-enclosed sorptive coating device
MESI	membrane extraction with sorbent interface
MIP	molecular imprinted polymers
MK	4-aceto-3,5-dimethyl-2,6-dinitro-*tert*-butylbenzene (musk ketone)
MLPME	membrane liquid phase microextraction
MLR	multiple linear regression
MM	1,1,3,3,5-pentamethyl-4,6-dinitroindane (musk moskene)
MMLLE	microporous membrane liquid–liquid extraction
MS	mass spectrometry
MX	2,4,6-trinitro-1,3-dimethyl-5-*tert*-butylbenzene (musk xylene)
OD	capillary tube (32 mm length, 0.0111” ID, 0.0300” OD, accuracy 1%)
PA	polyamide
PAH	polycyclic aromatic hydrocarbon
PBDE	polybrominated diphenyl ether
PC	polycarbonate
PCB	polychlorinated byphenyl

PCP	personal-care product
PDB	polyethylene diffusion bag
PDMS	polidimethylsiloxane
PE	polyethylene
PES	polyethersulfone
PET	polyethylene terphthalate
POCIS	polar organic chemical integrative sampler
POM	polyoxymethylene
POP	persistent organic pollutants
PP	polypropylene
PRC	performance reference compounds
PS	passive sampling
PS	polystyrene
PTFE	polytetrafluoroethylene
PTV	programmable temperature vaporiser
PUF	polyurethane foam
RAM	restricted access material
REACH	registration, evaluation, restriction and authorisation of chemicals
RSD	relative standard deviation
SB	stir-bars (or Twisters)
SBSE	stir bar sorptive extraction
SCX	strong cation exchanger
SDME	single-drop microextraction
SIM	selected ion monitoring
SLM	supported liquid membrane
SPE	solid phase extraction
SPME	solid phase micro-extraction
SPME-MC	solid phase microextraction-micellar desorption

SPMD	semi-permeable membrane devices
SR	silicone rod
ST	silicone tubes
SVOC	semi-volatile organic compounds
t_{cis}	cryo-focusing hold time
T_{cis}	cryo-focusing temperature
TD	thermal desorption
TD-GC-MS	thermal desorption-gas chromatography-mass spectrometry
TDU	thermal desorption unit
TOC	total organic carbon
T_{TDU}	thermal desorption temperature
t_{vent}	vent time
TWA	time-weighted average. See C_{TWA}
USB	ultrasound bath
v_{flow}	vent flow
v_{inj}	injection speed
v_{press}	vent presuure
VOC	volatile organic compounds
WBL	water boundary layer
WFD	Water Framework Directive
WWTP	waste water treatment plant

Resumen

La gran cantidad de micro-contaminantes orgánicos antropogénicos que se liberan al medio ambiente debido a las actividades diarias del ser humano, han llevado a numerosos países a renovar y actualizar la legislación sobre la presencia de contaminantes orgánicos en agua. La persistencia de estos contaminantes en entornos acuáticos interfiere en procesos naturales y por tanto, es necesaria una monitorización continua de los mismos, tal y como se recoge en la legislación internacional sobre medio ambiente. Sin embargo, la determinación precisa de contaminantes orgánicos a niveles traza o inferior ($ng \cdot L^{-1}$), utilizando métodos de extracción que sean amigables en el laboratorio y con el medio ambiente, es todavía un reto analítico. Por ello, el principal objetivo del presente trabajo consiste en el desarrollo de métodos analíticos adecuados para la determinación de contaminantes orgánicos emergentes y prioritarios en muestras de agua ambientales, principalmente en aguas de estuario y en efluentes de depuradora. Los contaminantes que han sido seleccionados han sido básicamente no polares y sobre los que no hay ninguna norma que regule su estatus ecotoxicológico (fragancias musk sintéticas, lindano y derivados, pesticidas organoclorados y organofosforados, alquilfenoles y ftalatos).

En este contexto, en lo que hace referencia al análisis de contaminantes orgánicos en muestras de agua a niveles traza, hay una necesidad creciente de desarrollar métodos analíticos que permitan la determinación rápida, sensible y simultánea de una amplia variedad de contaminantes. Por lo tanto, a pesar de que la idoneidad de diversas técnicas de preconcentración como pueden ser SPE, SPME y/o SBSE han sido ampliamente aceptadas en los trabajos científicos de las últimas décadas, resulta patente el interés en buscar nuevas alternativas. Por otro lado, atendiendo a los requisitos de las nuevas directivas y regulaciones que conciernen a la protección de los ecosistemas acuáticos, se ha visto la necesidad de desarrollar nuevas estrategias de muestreo que hagan compatible la verificación de los estándares analíticos con los costes necesarios. En

este sentido se ha estudiado las posibilidades que ofrecen los sistemas de muestreo pasivo.

Como consecuencia, en esta tesis se han seguido dos líneas principales de investigación:

1. En primer lugar, hemos desarrollado y validado una serie de métodos analíticos respetuosos con el medio ambiente, sensibles y de bajo coste, que permitan el análisis de varios micro-contaminantes orgánicos no polares en muestras de agua.
2. En una segunda etapa, hemos desarrollado y aplicado procedimientos de muestreo pasivo para la misma serie de contaminantes previamente mencionados, en aguas de estuario y efluentes de depuradora.

En lo que respecta a la primera línea de investigación, cabe resaltar los logros que se describen a continuación.

Se ha desarrollado un método analítico novedoso que ha permitido la monitorización de diez fragancias sintéticas en muestras de estuario y de efluentes de depuradora. Para ello, se ha desarrollado un método de extracción basado en una membrana no porosa denominado MASE. El análisis de los extractos se ha llevado a cabo mediante cromatografía de gases espectrometría de masas utilizando inyección de grandes volúmenes (LVI-PTV-GC-MS). Con el objetivo de alcanzar los límites de detección marcados por las distintas regulaciones, se han optimizado todos los parámetros que pudieran afectar tanto en la etapa de la extracción como en el análisis. De esta manera, se pudieron detectar dichos compuestos a niveles de $ng \cdot L^{-1}$ tanto en agua de estuario como en efluente de depuradora.

Por otro lado, hemos utilizado barras de silicona y barras agitadoras de polidimetilsiloxano para la microextracción basada en la absorción de compuestos orgánicos no polares mencionados en efluentes de depuradora y en estuarios. El análisis se ha realizado en todos los casos mediante GC-MS, siendo necesaria una etapa previa de desorción química y desorción térmica, para las barras de silicona y barras agitadoras de

polidimetilsiloxano, respectivamente. Los métodos optimizados resultaron idóneos para la determinación de los compuestos objetivo en aguas residuales y en agua de estuario a niveles de $ng \cdot L^{-1}$.

Actualmente, la diversidad de materiales con distintas características físico-químicas existentes en el mercado, permiten desarrollar nuevos métodos de extracción y preconcentración de los analitos. Así, en este trabajo se ha estudiado la idoneidad de cuatro materiales poliméricos comunes y de bajo coste (polipropileno, poliamida, polietersulfona y raffia) como materiales de sorción de compuestos presentes en matrices acuosas. Para estudiar su viabilidad, se optimizaron todos los parámetros que pudieran afectar en la etapa de extracción. Los extractos obtenidos mediante la desorción química de dichos materiales se analizaron mediante GC-MS. Algunos materiales, como la polietersulfona o la raffia, proporcionaron resultados satisfactorios para la determinación de los compuestos orgánicos previamente mencionados no sólo en aguas de estuario sino también en aguas residuales. Estos resultados permitieron que dichos materiales también fueran considerados como materiales potenciales en los sistemas de muestreo pasivo.

De este modo, la segunda línea de trabajo se centró en el desarrollo y la aplicación de estrategias de muestreo pasivo para la determinación de compuestos orgánicos en agua. Para ello, se ha trabajado en varias fases, destacando los hitos que se resumen a continuación.

Inicialmente, se diseñó y realizó el montaje de un dispositivo experimental en el laboratorio para la calibración de muestreadores pasivos. Este dispositivo de calibración consistió en un sistema acuático dinámico que garantizaba condiciones hidrodinámicas constantes y reproducibles para exponer los muestreadores pasivos durante largos periodos de tiempo (semanas). El dispositivo estaba compuesto por varias bombas, tanques de agua y un carrusel metálico girado por un rotor donde se colocaban los muestreadores pasivos (ver Figura 1). En lo que respecta a los muestreadores pasivos, la acumulación en tres materiales poliméricos (PDMS en barras agitadoras, PES en formato tubo y láminas de POM) fue calibrada para cada analito objeto de estudio. Estos

materiales podían encontrarse dispuestos en dos modalidades: polímeros expuestos sin protección o polímeros protegidos mediante una membrana (muestreadores MESCO).

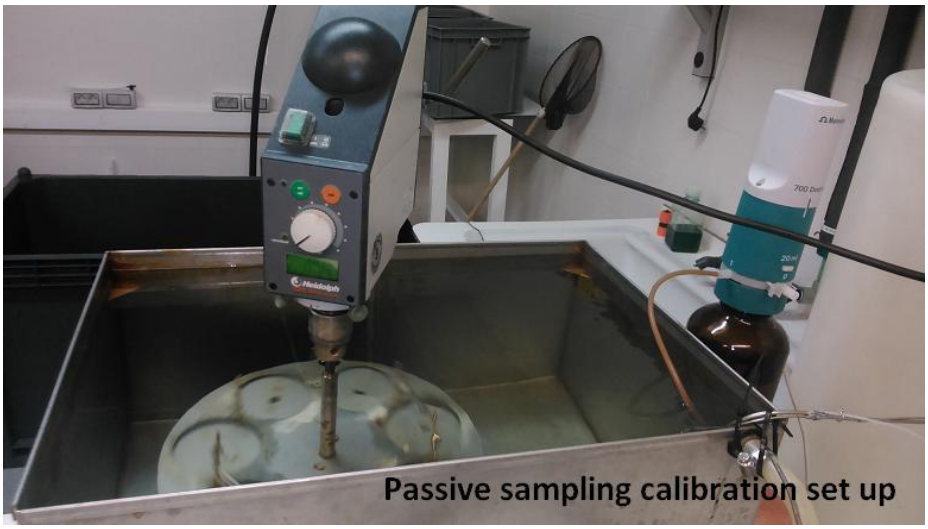

Figura 1. Dispositivo experimental empleado para la calibración de muestreadores pasivos.

Tras estudiar la aplicabilidad de los materiales poliméricos como muestreadores pasivos en agua dulce, se procedió a estudiar la viabilidad de estos sistemas en entornos más salinos, como son los estuarios y la costa marina. La media-alta salinidad del agua de estuario puede afectar a la cinética de la acumulación de contaminantes en muestreadores pasivos, por lo que es necesario estudiar la estabilidad de los valores de la velocidad de muestreo (Rs) en zonas intermareales como es el caso de los estuarios. Para ello, se implementó un nuevo dispositivo experimental análogo al anteriormente descrito, en la Estación Marina de Plentzia. Utilizando el agua de mar de la que se disponía en esta Estación, se trabajó a distintos niveles de salinidad con los muestreadores pasivos.

Las dos fases de investigación nos ha permitido llevar a cabo el análisis de un amplio espectro de contaminantes orgánicos y la monitorización efectiva de muchos de ellos en puntos de muestreo clave, como son los efluentes de depuradora o las zonas de estuario. Estas técnicas de muestreo pasivo desarrolladas permiten ser optimistas con respecto al cumplimiento de los requerimientos normativos actuales para la conservación de los ecosistemas acuáticos. La aplicabilidad de estos sistemas puede ser extendido a posteriores trabajos de investigación en los que se requiera monitorizar compuestos orgánicos no-polares como los estudiados en este trabajo. La experiencia adquirida en desarrollar sistemas de muestreo pasivo ha permitido extender su uso a otro tipo de compuestos. En este sentido, actualmente se está trabajando en el desarrollo de muestreadores pasivos para la determinación de compuestos farmacéuticos en aguas medioambientales, compuestos que son frecuentes en el entorno y que requieren una monitorización constante.

1. ESTUARINE CONTAMINANTS AT A GLANCE

1.1 The issue of pollution of water ecosystems

Water is the most valuable good for all living organisms and it plays a key role in the development of life itself. In fact, water is essential for the proper functioning of all ecosystems. Nature can accommodate the quality of water through a fair number of processes such as leaching of organic matter and nutrients, weathering of bedrock material or atmospheric deposition processes. However, in the last decades the disruption of many human activities in these weak equilibria is being severely threatened (Sipes, 2010). During the last century, human population has experienced an exponential industrial and technological progress, and as a result of this huge growth, the demand of water has increased at exceeding rates, not only for domestic consumption but also for a variety of economic activities. The steady development, in terms of human settlements, use of resources, infrastructural development, agricultural activities to name a few, is closely related to the increase of the quantity of residues and discharges that are released continuously into the environment, and mostly disposed into water. Unfortunately, and due to the lack of environmental awareness during the first half of the last century, this increasing amount of residues has caused a huge impact on environmental compartments, especially regarding the damage of aquatic ecosystems and organisms (Meffe and de Bustamante, 2014).

The contamination of water is also influenced by the increasing production and use of different chemical substances from the 1950s. In fact, many chemicals, produced by chemical industry, are continuously released into the environment, and they present a hazard to the aquatic environment causing chronic toxicity in aquatic organisms, accumulation of pollutants and loss of populations and biodiversity (Brack et al., 2016; Malaj et al., 2014). Unfortunately, a tiny fraction of this universe of chemicals is monitored and regulated. At present, a total of about 14 million chemicals are on the market, while the number of known chemicals is about 50 million (CAS), both increasing (Brack et al., 2016; Brack et al., 2011).

Thankfully, the awareness of water quality and pollution has evolved, and in a large number of studies it is already pointed the links between the presence of chemical contaminants in the environment and the human health (Tiedeken et al., 2017). In this sense, compounds such as organochlorines, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), heavy metals and other organic micro-contaminants to name a few, have been extensively studied and are considered priority pollutants (Roose and Brinkman, 2005). Nevertheless, new unregulated contaminants that have been overlooked for a long period of time are currently emerging, thanks to the development of more sensitive detection and quantification methodologies (Leendert et al., 2015). In most cases, the effects that these unregulated pollutants can have over the environment and the risks they can pose for human health are still unknown, and has become a new challenge for the scientific community and decision makers (Lopez de Alda et al., 2003).

Estuaries and coastal areas are the interface of land and oceans and are characterised by a high dynamism in space and timescales. As such, these environments are the habitat for unique groups of organisms and under the direct influence of many human activities. Therefore, risk assessment approaches find a challenging scenario in these areas since the variety of physical and chemical inputs and biological endpoints is one of the most complex (Amiard-Trinquet & Rainbow, 2009; Newmann, 2002).

All of these facts lead to the necessity of promoting specific laws and regulations in order to prevent the environmental degradation of fresh, transitional and sea waters, soils and sediments and air. Although there are no legal discharge limits for micropollutants, several responses have been promoted by the European Union (EU) in order to protect and fight for the sustainability of surface waters, such as the Drinking Water Directive (Directive 98/83/EC), the Water Framework Directive (WFD) (Directive 2000/60/EC), or the Marine Strategy Framework Directive (MSFD) (Directive 2008/56/EC). Together with these directives, initiatives such as the Commission for the Protection of

the Marine Environment of the North-East Atlantic (OSPAR), the Baltic Marine Environment Protection Commission (HELCOM) or REACH Regulation (Regulation EC nº 1907/2006) proposed environmental objectives in the field of water policy to identify priority pollutants (Decision number 2455/2001/EC) and to set environmental quality standards for water (Directive 2008/105/EC), pinpointing the risks derived from the exposure to these contaminants. Afterwards, in order to meet the protection of the aquatic ecosystems and the human health, the amended environmental regulations (Directive 2013/39/EU) recommended the monitoring of 45 substances included in a list of the Priority Substances (Barbosa et al., 2016; Lepom et al., 2009). Thereafter, in 2015, the watch list of substances for European Union-wide monitoring was recently amended (Decision 2015/495/EU), including previously considered pharmaceuticals, antibiotics, hormones, pesticides, a UV filter (2-ethylhexyl-4-methoxycinnamate) and an antioxidant (2,6-di-tert-butyl-4-methylphenol) commonly used as food additive (Barbosa et al., 2016).

In the case of the USA regulations, the Environmental Protection Agency (EPA) has set 129 chemicals as the priority pollutants (EPA, 2014). In addition to this, other initiatives have been promoted at a worldwide scale, such as The Stockholm Convention on Persistent Organic Pollutants, which was adopted in 2001 and entered into force in 2004. It is a global treaty whose purpose is to safeguard human health and the environment from harmful substances that accumulate in the environment and affect the well-being of humans and wildlife[1]. As the number of potentially hazardous chemicals grows and the difficulties to handle the effect of mixtures or the biological endpoints are taken into account, the need to prioritize the target compounds or to develop holistic assessment methodologies becomes a must as it has been pointed in the most recent literature (Diamond et al. 2011; Sturla et al., 2014).

[1] www.pops.int (last retrieved November 2016)

1.2 Priority and emerging pollutants

As important as the identification of the pollutants present in the aquatic media and the assessment of the risks linked to a certain exposure level, it is the identification of the most likely sources. Apart from the classical industrial activities, in the case of estuaries and coastal areas under the stress of urban activities, one of the most important sources is associated to the management of urban residues and the effluents of waste water treatment plants (WWTP). The focus on these sources is especially remarkable in developed countries, where most of the urban sewages are collected and treated in centralized plants, and the products that show a growing concern are those related to the human well-being (pharmaceuticals, cosmetics, personal care products, etc.). It is a fact that most of the conventional WWTPs are not designed to completely eliminate organic compounds at low concentrations. In this context, the non-degradable or partially removed compounds in WWTPs are likely to be detected in surface water (see Figure 1.1) (Barbosa et al., 2016).

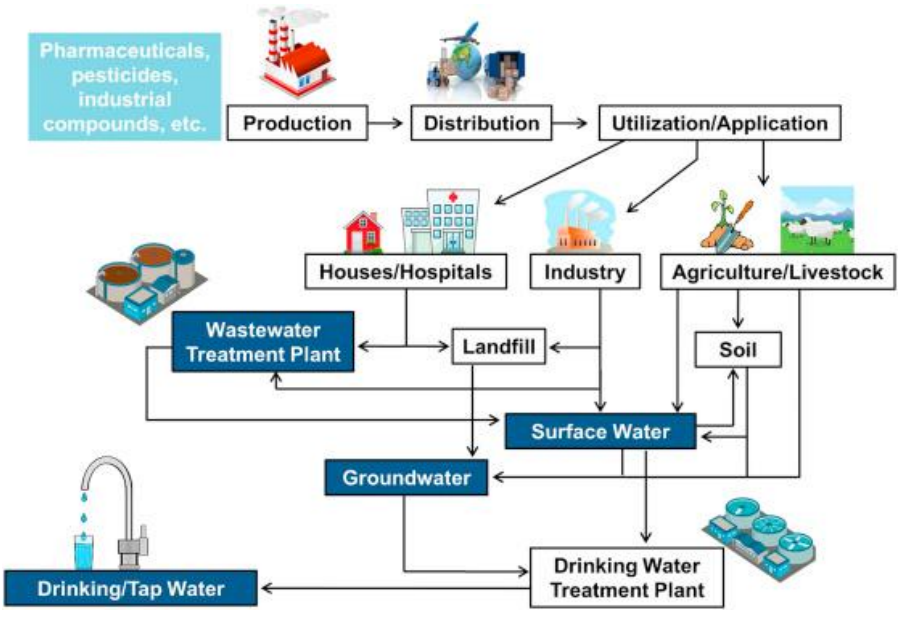

Figure 1.1. Representative sources and routes of pollutants in the environment (Barbosa et al., 2016)

From the chemical point of view, the concern on potential pollutants is split between the persistent organic pollutants (POPs) and the polar and labile emerging contaminants. In the former case, the major risks come from a particular combination of physical and chemical properties such as: (i) the high stability (they remain intact for long periods of time in the environment); (ii) the high transportability and distribution (they are prone to long range transport and distribution in the environment); (iii) the high bioaccumulation potential in the fatty tissue of living organisms (they are often found at higher concentration levels in the food chain); and, finally, (iv) their potential toxicity to both humans and wildlife. On the contrary, many contaminants included among the emerging ones are polar and chemically labile, which means that their solubility in water is much higher, though their half-live is much shorter. In this case, when the point source is the effluent of a WWTP, the risks come from the continuous chronic exposure to non-lethal levels.

In the case of the POPs, twelve of them (i.e., the so-called "dirty dozen") are recognized chemicals able to promote adverse effects on humans and the ecosystem: polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDD), polychlorinated dibenzofurans (PCDF), dichlorodiphenyltrichloroethane (DDT), hexachlorobenzene, mirex, toxaphene, aldrin, dieldrin, endrin, chlordane and heptachlor. As long as new compounds were found hazardous for the ecosystems and human health they were included in the list. Currently, there are 26 chemicals listed under global control (Hung et al., 2016) including compounds such as the lindanes to name a few (Nadal et al., 2015).

Although the initial environmental concern was focused towards the well-known contaminants, the development of more sensitive analytical instrumentation and methodologies allowed the identification of new organic contaminants, especially during the last decades. Due to the lack of common chemical descriptors to identify these

compounds, the large variety of uses and effects, the lack of specific regulation regarding the monitoring or their toxicity, and the real or perceived adverse effects, these compounds have been included within the general name of emerging contaminants (ECs) or contaminants of emerging concern. Among ECs many organic flame retardants (OFRs), perfluorinated compounds (PFCs) and currently used pesticides are included. Besides these well-known chemicals, there are many other chemicals for which there are no reliable analytical methods and data to draw conclusions about their risk, such as pharmaceuticals and personal care products (PCPPs) (antimicrobials and antibiotics, synthetic hormones, polycyclic musks, etc.) and still unknown chemicals such as many transformation products and nanomaterials as well (Diamond, 2011, Barbosa, 2016). In fact, the latest update of the NORMAN network[2] (February 2016) includes more than 1000 compounds under this general name and among them we can find anthropogenic compounds such as pesticides, industrial compounds, pharmaceuticals, personal care products, steroid hormones, drugs of abuse among others, together with their metabolites and by-products, which can be even more hazardous (Barbosa et al., 2016; Pal et al., 2010). ECs are not necessarily newly developed compounds; they may have been present in the environment for long time but their presence and implication for the environment's integrity are only recently recognised (Daughton, 2004).

Leaving aside the use of more sophisticated approaches such as non-target analysis, it is rather difficult to tackle the analysis of many of both types of contaminants in a simple way, and this fact demands the use of extraction techniques to split the complexity of the sample and of the subsequent analysis. Consequently, the pivotal point of this work is oriented towards the non-polar emerging contaminants usually found at the effluents of WWTP and in many estuaries and harbours of the Basque Country (Montero et al., 2013; Zorita et al. 2015).

[2] www.normandata.eu (last retrieved November 2016)

The environmental quality status of the estuaries and coastal areas of Basque Country has been thoroughly studied by the surveillance programs carried out by AZTI-Tecnalia (Solaun et al., 2015a; Solaun et al., 2015b) and many researches performed by scientists of the UPV/EHU (Bizarro et al., 2014; Cajaraville et al., 2016). As it can be concluded from many of those works, the quality indicators of the most contaminated area (i.e. the estuary of Bilbao) show a steady improvement. However, there is a neat demand for complementary studies regarding either the distribution of emerging contaminants or the development of bioanalytical tools to assess the effect of certain contaminants typically found in the effluents of WWTPs or in harbours.

According to the experience of our research group in the analysis of significant contaminants in aquatic media, we identified the need to develop improved analytical methods for the analysis of the following families of contaminants.

1.2.1 Synthetic musk fragrances

Synthetic musk fragrances are cyclic compounds of the family of personal-care products (PCPs) which are widely used as additives in the fabrication of fragrances and cosmetics, and also in laundry detergents, fabric softeners, household cleaning products and air fresheners among others. These fragrances, which were synthesized to replace expensive natural musk fragrances, include a broad range of compounds that can be divided in four main groups according to their chemical structure: nitro, polycyclic, macrocyclic and alicyclic (Vallecillos et al., 2015).

Most commonly found nitro-musks are musk ambrette (MA), musk ketone (MK), musk moskene (MM) and musk xylene (MX). They are alkylated nitrobenzenes, which produce hazardous by-products for the environment by means of the reduction of nitro functional groups to amino groups (Gros et al., 2008; Rimkus et al., 1999). The formation

of these amino group based metabolites, considered toxic for humans and environment, is the main reason why the use of nitro musk fragrances in consumer products was limited by the EU, and nowadays they are only present in some perfumes (Bester, 2009; Vallecillos et al., 2015).

Nowadays, polycyclic musks are the most widely used additives. They consist of a group of highly alkylated tetralin or indane substitutes which have been introduced onto the fragrance market due to their better properties, such as higher resistance to light and alkali. The most representative compounds are galaxolide (HHCB) and tonalide (AHTN), accounting 95% of the use of polycyclic musks. For this reason, both compounds have been included on the EPA's high production list[3] and the use of AHTN in the cosmetic industry has been regulated through European Directive 2008/42/EC (Vallecillos et al., 2015). Other polycyclic musks are traseolide (ATII), celestolide (ADBI), phantolide (AHMI) and cashmeran (DPMI) (Regueiro et al., 2009). The risk assessment of these compounds is in ongoing debate because of their endocrine disrupting activity. Nevertheless, no regulations have been published yet in terms of the use of synthetic musks in non-cosmetic products (Sommer, 2004).

Despite their high cost of synthesis, macrocyclic musks appear to be the future alternative of the previous odorous musks, since they present similar properties to those natural products, seem to smell more intensive and are more easily degradable when they reach to the environment. Due to their recent use as well as their low persistence in the environment, there is no account about the presence of these compounds in environmental waters. They consist of 15- or 17-membered ring systems that can be found in nature or synthesized.

[3] https://www.epa.gov/chemical-research/prioritization-high-production-volume-chemicals-under-chemical-assessment-and (last retrieved Nov. 2016)

On the other hand, alicyclic fragrances, also known as linear musks (e.g., helvetolide), are considered the latest generation of synthetic musk fragrances. Although they are still used in PCPs to a very limited degree (Arbulu et al., 2011), they have great advantages compared to classical musks, due to their biodegradable properties and low cost of manufacture compared to macrocyclic musks.

On account of their widespread use over decades, polycyclic musks and nitro musks can be found everywhere in the world and, due to their lipophilic characteristics and slow biodegradation, they can accumulate in sediments, sludge (Vallecillos et al., 2012), surface water (López-Nogueroles et al., 2011) and fish from contaminated rivers and estuaries (Subedi et al., 2011).

1.2.2 Alkylphenols

Alkylphenol ethoxylates are non-ionic surfactants that have been widely used as detergents and dispersing agents in industrial, commercial or household applications (Soares et al., 2008). The degradation of these compounds in the environment or in WWTPs produces compounds that are considered even more toxic and persistent, the alkylphenols, such as nonylphenols and octylphenols, which have been also used in the production of plasticizers and detergents (Dévier et al., 2013).

These compounds are considered endocrine disruptors because they may have negative effect in human reproduction even at low concentrations (Beyer et al., 2012). Alkylphenols are listed as POPs by the WFD and EQS values are also found for these compounds. For instance, the annual average and the maximum allowable concentration for 4-nonylphenol (4nOP) in seawater and surface waters are 0.3 $\mu g \cdot L^{-1}$ and 2 $\mu g \cdot L^{-1}$ respectively. In the case of 4-tert-octylphenol (4tOP), the annual average is 0.01 $\mu g \cdot L^{-1}$ in seawater and in the case of surface water 0.1 $\mu g \cdot L^{-1}$ (Salgueiro-González et al., 2013).

1.2.3 Organophosphorous compounds

During the last decades, the use of organophosphorous compounds as insecticides and pesticides has been widely adopted for crop protection in agriculture, and also in urban and forestry sectors, because of their high efficacy and easy availability (especially in developing countries) (Xu et al., 2012). This family of compounds includes chlorpyrifos (Chlorp), chlorfenvinphos (Chlorf), atrazine, malathion, parathion and diazinon, to name a few. Their relative solubility in water favors their entering to aquatic environments through surface runoff, sprays and soil leachates (Tse et al., 2004). In fact, some studies have recently reported concentrations for Chlorp and Chlorf in groundwater samples from Spain above the EQS of 0.1 $\mu g \cdot L^{-1}$ set for Chlorf in surface waters (Jurado et al., 2012). Moreover, many experimental studies have described the persistency of organophosphorous compounds in the aquatic environment (Rivadeneira et al., 2013) as well as their neurotoxic effects (Al-Badrany and Mohammad, 2007; Szatkowska et al., 2012). Taking into account all these facts, they are proposed as candidates in several monitoring works.

1.2.4 Organochlorine pesticides

Pesticides are chemical substances widely used against plant pests and diseases. However, the use of organochlorine pesticides (OCPs) in agriculture requires a great care and control because of their biocide activity and the risk they represent for human and environmental health (Villaverde et al., 2016). In fact, some laboratory and epidemiological assays suggested that certain OCPs are associated with carcinogenesis, immunotoxicity, neurotoxicity and endocrine disruption, among others (Saoudi et al., 2014). Owing to the mentioned effects and the capacity of these compounds to disperse, to bioaccumulate and to biomagnificate via the food chain, OCPs are listed as POPs (Kuranchie-Mensah et al., 2012). Although their use was drastically diminished, some dichlorodiphenyl trichloroethanes and hexachlorocyclohexanes are still detected in soil and water samples, mainly due to their persistence and bioaccumulation (Jiawei et al., 2008; Montuori et al., 2016). Consequently, their continuous monitoring is highly

recommended by several regulatory programmes as they are listed as POPs by the EU (Directive 2008/105/EC, Directive 2008/32/EC) and the US EPA.).

1.2.5 Phthalates

Phthalates (esters of phthalic acid) are mainly used in plasticizers, as they increase the flexibility, transparency, durability, and longevity of polymeric materials (Heudorf et al., 2007). In addition, they can be found in a wide range of industrial, household and consumer products. As they are not chemically bound to plastics, they can be released from consumer products to the environment during manufacturing, product use and after product disposal, leading to human exposure (Roslev et al., 2007). Besides, they can be dumped into the environment during plastics fabrication and incineration of household waste (Colón et al., 2000; Net et al., 2015).

In the present work we focus our interest on three phthalates: bis-(2-ethylhexyl) phthalate (DEHP), benzyl butyl phthalate (BBP) and di-n-octyl phthalate (DOP). BBP is considered as a substance suspected to produce endocrine alterations, whereas DOP takes part in the list of high toxicity pollutants published by EU. It has to be mentioned that the biodegradation, photodegradation and anaerobic degradation of phthalates hamper their persistence for long time in the environment (Rudel and Perovich, 2009). At the moment, some phthalates are restricted in the EU since 1999 for several uses, for instance, DEHP, DBP and BBP are completely forbidden in all children's toys (Directive 2005/84/EC).

1.3 The challenges for the environmental analytical chemistry

The analytical rush for the better, the faster, the further or the smaller has spun out our means to unexpected limits. Consequently, we are currently able to identify and to quantify what it was simply unknown some decades ago (Milman, 2015; Bletsou et al. 2015). As a consequence of constantly rising environmental quality criteria requirements,

there is a clear need to develop sensitive and robust analytical methods able to provide reliable chemical information about the nature of chemical pollutants and their concentration in the environment (Kot et al., 2000). In this framework, we can outline some key challenges in environmental analysis: (i) the identification and quantification of potential contaminants at trace levels not only in water, but in any environmental compartment, in living organisms, or even in humans; (ii) the compliance of the analytical methods according to the regulations; and (iii) the development of bioanalytical tools to aid the decision-making protocols.

In recent years, the evolution of accurate mass high resolution mass spectrometry has triggered a new trend in analytical data processing towards non-target analytical methods (Baduel et al., 2015). In this framework, the advances achieved in terms of sensitivity are mainly due to the development of hyphenated chromatography – mass spectrometry techniques, which are the analytical techniques chosen in most of the research works dealing with the determination of organic contaminants in environmental matrices (Farré et al., 2012; Wille et al., 2012). In parallel to the outstanding improvements of chromatographic resolution, detection sensitivity and specificity, there has been a great effort to develop efficient methods to extract and enrich trace contaminants from complex matrices, such as, soil, sediment, sludge and wastewater (Albero et al., 2012; Matamoros et al., 2012; Nurmi and Pellinen, 2011; Terzic and Ahel, 2011; Zuloaga et al., 2012). However, shortening of the analytical methods, increasing the automation of the analytical processes, reducing the volumes involved in the whole analytical assays and overcoming the negative effects of matrix components are still the main assignments when new methods are envisaged (Iparraguirre et al., 2014; Li et al., 2015; López-Blanco et al., 2016).

As mentioned before, the ecotoxicological effects observed in many aquatic environments are the consequence of exposures to complex mixtures of chemicals. Since

the comprehensive analysis of all the potential toxic candidates is and endless task, new tools are required to reduce this complexity and to limit the analysis only to those chemicals that might cause any adverse effect. Effect-directed analysis (EDA) is a bioanalytical strategy to meet this challenge (Brack et al., 2016). The basic idea of EDA is to unravel these complex mixtures of chemicals in environmental matrixes, thus separation techniques play a major role. After extraction and clean-up, chromatographic separation techniques are primarily applied in EDA. Preparative separation or fractionation, aims to sequentially reduce the complexity of a sample to yield fractions that can be subjected to biological and chemical analysis and thus, provide information that helps to characterise and identify the chemicals of concern (Brack et al., 2011). EDA has successfully been applied to evaluate endocrine disruptive effects in several water systems, such as WWTPs, rivers, harbour areas, marine sediment, and biota (Weiss et al., 2011).

Finally, let us recall the fundamental role of sample in any analysis regardless of how missed it might look among cooler options as those mentioned above. Assuming that the sample is the genuine proxy holder of the entity we want to measure, in many environmental scenarios we may find a so dynamic and heterogeneous system that makes very difficult grabbing a sample (Bodnar et al., 2013) such as the distribution of contaminants in an estuary, the average loading of a WWTP effluent or the bioavailable fraction of a contaminant in coastal waters. In many of those scenarios we should choose between the collection of many samples along the space and timescales or to integrate smaller samples in any of those two dimensions.

One feasible and promising solution is the use of passive samplers that allows the time integration thanks to the sorptive features of the sampler material (Stuer-Lauridsen, 2005). In fact, a passive sampler is defined as a device containing a sorption medium, where contaminants can pass through it. These devices can be deployed in the water and

left there for a given time frame, with the main aim of collect the contaminants according to their chemical affinities. The accumulated amounts of contaminants along the sampling period, and taking into account the sampling rate (R_s) of the passive sampling device, the time weighted average concentration (C_{TWA}) of a certain analyte can be estimated (Kot et al., 2000; Lohmann et al., 2012).

According to the literature, passive sampling techniques allow the monitoring of a wide range of priority and emerging pollutants (Lohmann and Muir, 2010). Despite current regulation about monitoring of organic contaminants is based on batch or grab sampling (Lohmann et al., 2012), passive sampling techniques have been recommended in the European Commission Guidance Document on surface water chemical monitoring (European Commision, 2009), and in the WFD. This means that PS is recognised as a complementary method to improve the level of confidence in water monitoring data in comparison with conventional spot sampling (Miège et al., 2015).

Finally, the fitness for purpose and affordability of any new analytical method account for its use. Therefore, the development of any new analytical approach should deeply consider the aim of the research and its final application. At this point, specific environmental applications recall the development of appropriate sampling, extraction and analytical methods to get outlined milestones such as: (i) the monitoring of ECs at low concentration levels in environmental samples, (ii) the estimation of the bioavailable fraction of ECs or (iii) the assessment of EC's hazard to the environment.

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Zorita I, Larreta J., Montero N., Rodríguez JG, Franco J., Borja J., Evaluation of the use of bioaccumulation and biological effects tools in caged mussels, within the European Water Framework Directive. Chemistry and Ecology, 2015; 31:432-445.

Zuloaga O, Navarro P, Bizkarguenaga E, Iparraguirre A, Vallejo A, Olivares M, et al. Overview of extraction, clean-up and detection techniques for the determination of organic pollutants in sewage sludge: A review. Analytica Chimica Acta 2012; 736: 7-29.

2. AIMS AND OBJECTIVES

The huge amounts of anthropogenic organic micro-pollutants released to environmental waters due to human daily activities have bequeathed many countries the legacy of organic pollution in water. Their persistence in the aquatic environments disrupts the natural processes and thus, the need of a continuous monitoring of these contaminants is endlessly revealed in several international environmental legislations. However, the accurate determination of organic pollutants at trace levels (i.e., $ng \cdot L^{-1}$) using environmentally friendly methods is still an analytical challenge. Therefore, the principal aim of the present work is the development of several analytical methods required to determine priority and emerging organic pollutants in environmental water samples, mainly, in estuarine and effluent waters from WWTPs, to be applied afterwards for monitoring purposes.

In the framework of the analysis of organic pollutants in water samples at trace level, there is a call for the development of analytical methods that enable a rapid, sensitive and simultaneous determination of a wide variety of pollutants. Though the suitability of several pre-concentration techniques such as SPE, SPME or SBSE has been largely established in the literature, the search of new analytical methods is a matter of further research. On the other hand, attending to the requisites of new directives and regulations concerning the protection of water bodies long term monitoring campaigns are required. Consequently, analytical cost and efforts involved have been boosted. As a consequence, two main objectives have been considered in this PhD Thesis:

1. In the first section, we aimed to develop and validate a set of sensitive, cost-effective and environmentally friendly analytical procedures to allow the analysis of several non-polar organic microcontaminants in environmental water samples.

2. In the second section, we aimed to develop and apply passive sampling strategies to determine some of the previously the previously considered compounds in estuaries and WWTP effluents.

In order to achieve these main goals, several operative objectives were considered. Hence, to accomplish the first main aim (first section), we established the following sub-objectives:

1.1. to develop a novel analytical method approach to monitor ten synthetic musk fragrances in environmental water samples using MASE followed by LVI-PTV-GC-MS. This goal will be achieved after both extraction and analysis optimisation and validation in order to determine the most important conditions that could affect the efficiency of the process.

1.2. to apply silicone rods and stir-bars for sorptive extraction of several persistent and emerging organic compounds (hexachlorocyclohexane compounds, organochlorine and organophosphorous pesticides, polycyclic compounds, octylphenols and phthalates) from aqueous samples followed either by liquid or thermal desorption and analysis by GC-MS. Afterwards, the applicability of the optimised multi-residue methodology was evaluated by analysing the target compounds in real environmental water matrices (i.e., wastewater and estuarine water samples) as well as a basis for a passive sampling method.

1.3. to study the suitability of four commercially available low cost polymeric materials for the extraction of the organic compounds mentioned above in environmental water samples. This main goal will be achieved after the optimisation of the main parameters affecting the extraction procedure and subsequent method validation. The later may be directly affected by the matrix effect and thus, the evaluation of different approaches to correct this effect will be also considered. Finally, the applicability of the optimised

methodology will be assessed by analysing the target compounds in real environmental samples (i.e., wastewater and estuarine water samples).

Regarding to the second objective focused on the development and application of passive sampling strategies to determine organic compounds previously set in the first section, the operative objectives were:

2.1. to calibrate the uptake of three polymers (PDMS in stir-bars, PES tubes or hollow fibres and POM sheets) in two different physical set-ups: free or naked polymers and the membrane enclosed polymers (i.e.,. MESCO samplers).

2.2. to study the influence of the salinity in the affordability of the previously mentioned samplers.

The achievement of these operative objectives would allow the analysis of a broad range of organic microcontaminants and the effective monitoring of many of them in key sites such as the effluents of WWTPs and estuaries according to the requirements of the current regulations.

The accomplishment of these two main objectives will be discussed in the first and second section of this PhD Thesis work.

3. MATERIALS AND METHODS

In this section all the reagents and materials employed in the whole work as well as a brief summary of the developed methods for water analysis are detailed. First of all, cleaning procedures common for all experiments are summarised. Then, reagents and materials employed in different experiments are listed together with their physical properties. Afterwards, the different polymers employed over this work are described and finally, chromatographic methods developed are detailed thoroughly.

3.1 Cleaning procedure

All the laboratory material was carefully cleaned with abundant deionized water (<0.2 $S \cdot cm^{-1}$, Millipore, USA) and without using detergent to avoid possible interferences from those products. The material was sonicated under clean acetone (Q.P., Panreac Química, Spain) for an hour and then rinsed with ultrapure water (<0.057 $S \cdot cm^{-1}$, Milli-Q model, Millipore, USA). After all, the glass material was dried in an oven at 450 °C for approximately 4 hours.

3.2 Reagents and solutions

Among the families that have been thoroughly studied in the present work we can find: musk fragrances, organochlorine pesticides, organophosphorous pesticides, phthalates, and alkylphenols.

3.2.1 Musk fragrances

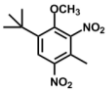
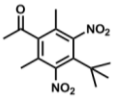
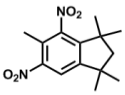
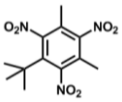
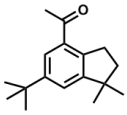
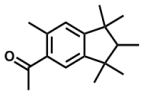
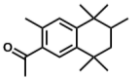
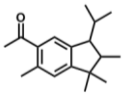
The following standards listed above were employed in the development of the MASE method. The six polycyclic musks: 4-acetyl-1,1-dimethyl-6-*tert*-butylindane (ADBI, celestolide®), 6-acetyl-1,1,2,3,3,5-hexamethylindane (AHMI, phantolide®), 7-acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphthalene (AHTN, tonalide®), 5-acetyl-1,1,2,6-tetramethyl-3-isopropylindane (ATII, traseolide®), 6,7-dihydro-1,1,2,3,3-pentamethyl-4(5H)-indanone (DPMI, cashmeran®) and 1,3,4,7,8-hexahydro-4,6,6,7,8,8-

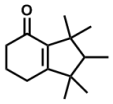
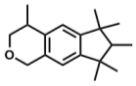
hexamethylcyclopenta-(γ)-2-benzopyrane (HHCB, galaxolide®) were supplied by LGC Standards GmbH (Germany). The four nitro musk fragrances: 1-*tert*-butyl-2-methoxy-4-methyl-3,5-dinitrobenzene (MA, musk ambrette) and 4-aceto-3,5-dimethyl-2,6-dinitro-*tert*-butylbenzene (MK, musk ketone) were obtained from Dr. Ehrenstorfer GmbH (Germany) and 1,1,3,3,5-pentamethyl-4,6-dinitroindane (MM, musk moskene) and 2,4,6-trinitro-1,3-dimethyl-5-*tert*-butylbenzene (MX, musk xylene) from Fluka (Steinkeim, Germany) respectively. The surrogate standards: [$^2H_3$] AHTN and [$^2H_{15}$] musk xylene were purchased from Dr. Ehrenstorfer GmbH (Germany) as 100 mg·L^{-1} in isooctane and acetone respectively. The physicochemical properties and structures of nitro musks and polycyclic musks are detailed in Table 3.1.

Individual stock solutions from each solid standard were dissolved to prepare 1000 µg·g^{-1} in 2-propanol (HPLC-grade, 99.8 %, LabScan, Dublin, Ireland) with the exception of musk xylene and musk moskene, which were supplied in stock solutions of 100 µg·g^{-1} in acetonitrile. All stock solutions were stored in amber vials at -20 °C.

Mixed fresh stock solutions containing 50 µg·g^{-1} of all polycyclic and nitro musks (except musk xylene and musk moskene) were prepared monthly in 2-propanol. Intermediate dilutions at lower concentrations of above mentioned stocks were prepared daily, according to the experimentation.

Table 3.1. Nitro and polycyclic musks with their chemical structures, CAS number, purity, log K_{ow} , vapour pressure and m/z (quantifier, qualifier).

Compound	Structure	CAS No.	Purity (%)	Log K_{ow}	P_v(Pa)	m/z (Q1, Q2)
Nitro Musks						
Musk Ambrette (MA)[1]		83-66-9	99.0	3.7	$3.3\cdot10^{-3}$	253, 268
Musk Ketone (MK)[1]		81-14-1	98.0	4.3	$4\cdot10^{-5}$	279, 294
Musk Mosken (MM)[1]		116-66-5	96.0	5.8	$2.3\cdot10^{-4}$	263, 278
Musk Xylene (MX)[1]		81-15-2	98.0	4.8	$3\cdot10^{-5}$	282, 297
Polycyclic Musks						
Celestolide (ADBI)[2]		13171-00-1	99.8	6.6	$1.92\cdot10^{-2}$	229, 244
Phantolide (AHMI)[2]		15323-35-0	93.1	6.7	$1.96\cdot10^{-2}$	229, 244
Tonalide (AHTN)[2]		1506-02-1	97.9	5.7	$6.08\cdot10^{-2}$	243, 258
Traseolide (ATII)[1]		68140-48-7	83.2	6.3	$9.1\cdot10^{-3}$	215, 258

Cashmeran (DPMI)[2]		33704-61-9	89.5	4.9	5.2	191, 206
Galaxolide (HHCB)[2]		1222-05-5	53.5[1]	5.9	$7.3 \cdot 10^{-2}$	243, 258
[$^2H_{15}$] Musk Xylene (MX)						246, 261
[$^2H_3$] Tonalide (AHTN)						294, 207

[1] Compound corrected with [$^2H_{15}$] MX.
[2] Compound corrected with [$^2H_3$] AHTN.

3.2.2 Priority and emerging pollutants

Several priority and emerging pollutants were studied were included for the optimization of the extraction methods and their application in passive sampling techniques. The characteristics of the compounds studied in this work are summarised in Table 3.2. . The two polycyclic musks AHTN and HHCB were obtained from LGC Standards GmbH (Wesel, Germany). The six organochlorine pesticides: o,p'-dichlorodiphenyldichloroetane (2,4'-DDD), p,p'-dichlorodiphenyldichloroetane (4,4'-DDD), o,p'-dichlorodiphenyldichloroethylene (2,4'-DDE), p,p'-dichlorodiphenyldichloroethylene (4,4'-DDE), o,p'-dichlorodiphenyltrichloro-ethane (2,4'-DDT) and p,p'-dichlorodiphenyltrichloroethane (4,4'-DDT) were supplied by Dr. Ehrenstorfer GmbH (Augsburg, Germany). The three phthalates: benzyl-2-butyl phtalate (BBP), bis(2-ethylhexyl)phthalate (DEHP) and di-octyl phthalate (DOP) and the two alquilphenols: 4-octylphenol (4tOP) and 4-nonylphenol (4nOP) were purchased from Alfa-Aesar GmbH&Co (Karlsruhe, Germany). The two organophosphorous pesticides: Chlorpyrifos (Chlorp, Pestanal®) and Chlorfenvinphos (Chlorf) were provided by Sigma-Aldrich (Seelze, Germany).The deuterated compounds used as surrogate standards were: [$^2H_8$]-4, 4'-DDT and [$^2H_3$]-bis(2-etilhexyl) phthalate ([$^2H_3$]-DEHP) obtained from Sigma-Aldrich (Seelze,

Germany) and [$^{2}H_{15}$]- musk xylene ([$^{2}H_{15}$]-MX) acquired from Dr. Ehrenstorfer GmbH (Augsburg, Germany).

Regarding to the experimentation where these target compounds were involved, individual stock solutions (≈1000 $\mu g \cdot g^{-1}$) from each solid standard were prepared in 2-propanol (HPLC-grade, 99.8%, LabScan, Dublin, Ireland). These solutions were stored in amber vials at -20^{o}C. Mixed fresh solutions with ≈50 $\mu g \cdot g^{-1}$ of each target compound were monthly prepared and lower concentration solutions were daily prepared in accordance with the experiments being carried out.

The solvents, acetonitrile (AcN), *n*-hexane (Hex), dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc), cyclohexane (cHex) (HPLC-grade) and methanol (MeOH) (Anhydrous, HPLC-grade) were supplied by LabScan.

Sodium chloride (NaCl; Merck, Darmstadt, Germany) was used for matrix modification experiments. Humic acids (technical grade) and soluble fiber used to evaluate the matrix effect were supplied by Fluka (Sigma-Aldrich, Germany) and by local pharmacy respectively.

Table 3.2. Priority and emerging pollutants with their chemical structures, formula, CAS number, log K_{ow}, vapour pressure and m/z (quantifier, qualifier).

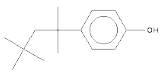
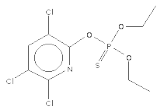
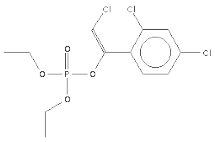
Compound	Structure	Formula	CAS No.	Log K_{ow}	P_v (Pa)	m/z (Q1, Q2)
Alkylphenols						
4tOP *4-tert-Octylphenol*		$C_{14}H_{22}O$	140-66-9	5.28	0.21	135, 107
4nOP *4-Octylphenol*		$C_{14}H_{22}O$	1806-26-4	5.5	$1.31 \cdot 10^{-2}$	107, 135
Organophosphorous compounds						
Chlorpyrifos (Chlorp) *O,O-diethyl O-(3,5,6-trichloro-pyridin-2-yl) phosphorothioate*		$C_9H_{11}Cl_3NO_3PS$	2921-88-2	4.96	$2.71 \cdot 10^{-3}$	197, 199
Chlorfenvinphos (Chlorf) *O,O-Diethyl O-(2-chloro-1-(2',4'-dichlorophenyl)vinyl) phosphate*		$C_{12}H_{14}Cl_3O_4PS$	470-90-6	3.81	$1.00 \cdot 10^{-3}$	267, 269

Table 3.2 cont. Priority and emerging pollutants with their chemical structures, formula, CAS number, log K_{ow}, vapour pressure and m/z (quantifier, qualifier).

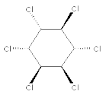
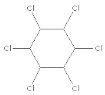
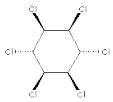
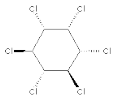
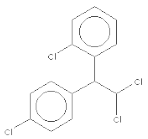
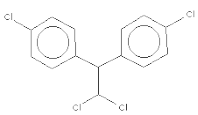
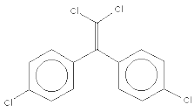
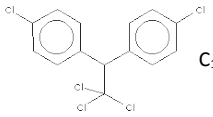
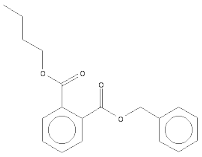
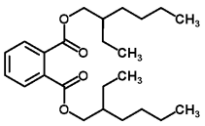
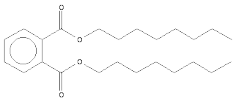
Compound	Structure	Formula	CAS No.	Log K_{ow}	P_v (Pa)	m/z (Q1, Q2)
Organochlorine pesticides						
α -HCH α-hexachlorocyclohexane		$C_6H_6Cl_6$	319-84-6	4.26	$6.00 \cdot 10^{-3}$	181, 183
β -HCH β-hexachlorocyclohexane		$C_6H_6Cl_6$	319-85-7	3.68	$4.80 \cdot 10^{-5}$	181, 183
γ-HCH γ–hexachlorocyclohexane		$C_6H_6Cl_6$	58-89-9	4.26	$5.60 \cdot 10^{-3}$	181, 183
δ -HCH δ-hexachlorocyclohexane		$C_6H_6Cl_6$	319-86-8	3.68	$4.67 \cdot 10^{-3}$	181, 183
2,4'-DDD o,p'-dichlorodiphenyldichloroethane		$C_{14}H_{10}Cl_4$	53-19-0	5.87	$2.59 \cdot 10^{-4}$	235, 237
4,4'-DDD p,p'-dichlorodiphenyldichloroethane		$C_{14}H_{10}Cl_4$	72-54-8	5.87	$1.80 \cdot 10^{-4}$	235, 237
4,4'-DDE *p,p'-dichlorodiphenyl-dichloroethylene*		$C_{14}H_8Cl_4$	72-55-9	6.02	$8.00 \cdot 10^{-4}$	246, 243
4,4'-DDT *p,p'-dichlorodiphenyl-trichloroetane*		$C_{14}H_9Cl_5$	50-29-3	6.79	$2.13 \cdot 10^{-5}$	235, 237

Table 3.2 cont. Priority and emerging pollutants with their chemical structures, formula, CAS number, log K_{ow}, vapour pressure and m/z (quantifier, qualifier).

Compound	Structure	Formula	CAS No.	Log K_{ow}	P_v (Pa)	m/z (Q1, Q2)
Phthalates						
BBP *benzyl-2-butyl phtalate*		$C_{19}H_{20}O_4$	85-68-7	4.80	$1.10 \cdot 10^{-3}$	149, 206
DEHP *bis(2-ethylhexyl)phthalate*		$C_{19}H_{20}O_4$	85-68-7	7.50	$3.40 \cdot 10^{-5}$	149, 167
DOP *di-n-octyl phthalate*		$C_{24}H_{38}O_4$	117-84-0	8.54	$5.96 \cdot 10^{-4}$	149, 279

3.3 Polymeric materials

Several polymers play a key role in the present work. On the one hand, they act as receiving phases for micro-extraction purposes of target compounds in environmental water samples and, besides, as sorptive devices in passive sampling techniques developed. On the other hand, polymeric membranes are used as a support for the liquid phase in MASE and, these membranes were used also as protective layers in passive samplers in the field.

3.3.1 Homemade membranes for MASE

Low density polyethylene (LDPE) was obtained from freezing bags for food (with a membrane thickness of 0.02 mm) and from Garciplast (Barcelona, Spain) (membrane thickness of 0.07-0.095 mm) and polyethylene terphthalate (PET) (membrane thickness of 0.05 mm) was purchased from Goodfellow (England).

Polymeric membrane bags (2.5 cm length and 1 cm i.d. for 200 µL of solvent and 4 cm length and 1 cm i.d for 800 µL of solvent) were tailor-made using a shrink-wrapping device for collecting the organic solvent. After thermally sealing the borders, the overlaying borders were carefully cut in order to minimise the superficial zones where analytes could be absorbed. The homemade membranes were conditioned with *n*-hexane and maintained in clean *n*-hexane before their use in order to minimise the cross-contamination of interfering compounds from the membrane material.

3.3.2 Commercially available polymeric materials

3.3.2.1 PDMS based polymeric material: silicone rod

The commercial silicone elastomer (in flexible rod form of 2.0 mm i.d., 1.23 $g \cdot mL^{-1}$) used for sorptive extraction as well as for passive sampling purposes was purchased from Goodfellow (Huntingdon, England). Silicone rods (SRs) with a length of 1 cm were cut in the laboratory with a sterile sharp blade. After that, the SRs were weighed (≈ 38 mg or nominal volume close to 30 µL) and those with mass differences higher than 3% were discarded. Before the SRs were used, they were sonicated with 1 mL of a mixture of CH_2Cl_2:MeOH (1:1) for 5 min and afterwards, they were cleaned three times using fresh solvent mixture. Finally, the SRs were conditioned at 250 °C for 180 min under a nitrogen stream (ca. 30 $mL \cdot min^{-1}$) and they were kept in closed vials until their use.

3.3.2.2 PDMS based polymeric material: stir-bars

The PDMS stir-bars (SB) (so called Twister®, supplied by Gerstel, Mülheim an der Ruhr, Germany) were used both for quantitative extractions and as passive samplers in this work. Among the different sizes available, 20 mm length and 0.5 mm film thickness size stir bars were chosen for this work. This larger size provides higher sensitivity because of its higher preconcentration capacity in comparison to the other size commercially available, i.e., 10 mm length SB. Prior to their use, conditioning of SB is highly recommended in order to avoid cross contaminations. SB were cleaned and conditioned

according to the technical recommendations provided by the supplier. Firstly, SB were chemically cleaned with AcN:MeOH (1:1, v/v) mixture in an ultrasonic bath for 3 h and then, thermally conditioned under nitrogen stream (ca. 30 mL·min^{-1}) at 290 °C for 3 h in a thermal condition unit (TC2 tube conditioner, Gerstel).

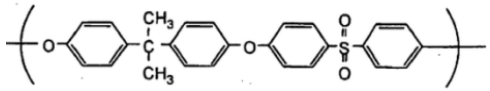
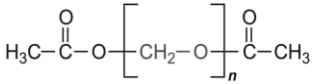
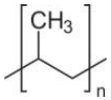
3.3.2.3 Other polymeric materials

Although PDMS based polymeric materials are the most used ones in the literature for the sorption of non-polar organic contaminants, in this work, other polymeric materials were also assessed to be used as sorptive materials for extraction and passive sampling purposes. The characteristics of the commercially low cost polymers used for these purposes are summarised in Table 3.3.

The commercial polyethersulphone (PES) tube used for sorptive extraction was purchased from Membrane GmbH (Germany) respectively. PES tubes (PES_t) with a length of 7.5 cm were cut in the laboratory with a sterile sharp blade. In addition, PES polymer was studied in another format, i.e. PES membranes (PES_m), commonly used as filtering disks (SUPOR® -100, 0.1 µm, 47mm), obtained from PALL Life Sciences (New York, USA). Polyamide (PA), polypropylene (PP) and raffia, were obtained from a local supermarket. PA, PP and raffia were first sealed with the help of a shrink-wrapping device and then, the corresponding boarders were carefully cut to obtain polymers of 1.5 cm length. All the materials were accurately weighed (≈ 40 mg) and those with mass differences higher than 10% were discarded. Finally, polyoxymethylene (POM) sheets (76.2 µm thick) were purchased from CS Hyde Company (Illinois, USA) as an adhesive tape.

Cleaning and conditioning of all the polymers is highly recommended prior to their use as sorptive materials. Thus, all the sorbents except PES and POM were first sonicated three times, for 5 min, using fresh solvent mixture of CH_2Cl_2:MeOH (1:1) (both HPLC-grade, 99.9%, LabScan, Ireland). In the case of POM sheets, prior to their use they

Table 3.3. Characteristics of the polymers

Name	Chemical structure	Supplier	Format	Diameter (mm)	Density ($g \cdot cm^3$)	Max. working temperature (°C)
PA Polyamide	???	Local supplier	Rod	3.0	n.a.	120
PDMS Polydimethyl-syloxane		Goodfellow	Rod	2.0	1.23	250-260
PES Polyether-sulphone		Membrane GmbH	Tube	0.7	1.43	180-220
POM Polyoxy-methylene		CS Hyde Company	Sheet	-	-	150-160
PP Polypropylene		Local supplier	Rod	1.7	0.85	90-120
Rf Raffia		Local supplier	Multiple fibers	2.0	n.a.	120

were precleaned with EtOAc in order to remove completely the adhesive film attached. Then, adhesive free POM sheets were chemically cleaned with pure EtOAc in an ultrasonic bath for 2 hours and then maintained in clean EtOAc for 72 h. After being immersed in solvents, all the materials were dried with a lint-free tissue. Particularly, PP, PA and raffia were additionally thermally conditioned at their maximum working temperature (i.e., 120°C for all of them) for 180 min under a nitrogen stream (ca. 30 mL·min^{-1}). The former step was required in order to remove any possible interference as well as to check the chemical stability of the polymers in contact with different organic solvents (i.e., Hex, EtOAc, MeOH and cHex).

3.4 Chromatographic analysis

3.4.1 GC-MS and LVI-PTV-GC-MS analysis for musk fragrances

The analysis of musk fragrances was performed by means of MASE followed by GC-MS and by LVI-PTV-GC-MS. The MASE extracts obtained after different optimisation steps were analysed in an Agilent 6890N gas chromatograph coupled to an Agilent 5973N mass spectrometer using an Agilent 7683 autosampler. 2 µL were injected in splitless mode at 300 °C in a capillary column HP-5MS (30 m x 0.25 mm, 0.25 µm, Agilent) with hydrogen (AD-1020 Hydrogen Generator, Cinel Strumenti Scientifi, Padova, Italy) as carrier gas at constant flow (1.3 mL·min^{-1}). The following oven temperature program was used for the separation of the target analytes: 60 °C (1 min), temperature increase at 30 °C·min^{-1} to 200 °C, a second increase of 3 °C·min^{-1} up to 240 °C followed by a 30 °C·min^{-1} up to 300 °C, where it was finally held for 3 min. The mass spectrometer worked in the electron impact mode with a potential difference of 70 eV. The temperature of the interface between the chromatograph and the detector was kept at 310 °C while the temperature of the ionization source and quadrupole were maintained at 230 °C and 150 °C, respectively. The measurements were made both in full scan (50 – 502 amu) mode and SIM (selected ion monitoring) mode. Chemstation software (Agilent Technologies) allowed both the control and treatment of the chromatograms obtained.

In order to get best limits of detection, the MASE extracts were analysed by means of LVI-PTV-GC-MS. Hence, LVI of the extracts was carried out using a CIS 4 PTV inlet (Gerstel GmbK & Co, KG, Mülheim an der Ruhr, Germany) consisted of a septumless head and an empty baffled deactivated glass liner kept cool using liquid nitrogen. A 45 µL aliquot of sample extract was injected using a 100 µL syringe operated by a multipurpose sampling device (MPS2 autosampler, Gerstel) at 20 °C while the vent valve was opened for 0.5 min at a flow rate of 75 $mL \cdot min^{-1}$ and a vent pressure of 5 psi. Then, the vent valve was closed for 1.5 min and the temperature of the PTV injection port was increased at 12 $°C \cdot s^{-1}$ to 300 °C and held 2 min at that temperature. Finally, the injector was cleaned at a purge flow of 75 $mL \cdot min^{-1}$ prior to subsequent injections.

Separation and detection were performed in a 6890N gas chromatograph (Agilent Technologies, Avondale, PA, USA) equipped with an Agilent 5975N electron impact ionization mass spectrometer and with a HP-5MS capillary column (30 m × 0.25 mm, 0.25 µm) from Agilent. The oven temperature was programmed from 60 °C (hold 3 min) to 190 °C at 30 $°C \cdot min^{-1}$ and then until 290 °C at 5 $°C \cdot min^{-1}$, which was held for 3 min (total analysis time 30.33 min). Helium (99.9995%, Carburos Metálicos, Barcelona, Spain) was used as carrier gas at a constant flow of 1.3 $mL \cdot min^{-1}$. The transfer line temperature was maintained at 310 °C and the ion source and the quadrupole at 230 °C and 150 °C respectively. Detection was carried out both in the scan (50-525 m/z) and in the selected ion monitoring (SIM) modes simultaneously. To evaluate the mass spectral fragmentation patterns of each compound, a standard mixture (1 $mg \cdot L^{-1}$) was analysed by GC-MS in full-scan mode, and the quantifier ion as well as the qualifier ions were selected in order to get the best chromatographic responses. The m/z values of the fragment ions monitored in the SIM mode are listed in Table 3.1. The first ion was used as quantifier while the second ion was considered as qualifier.

3.4.2 LVI-PTV-GC-MS analysis for priority and emerging pollutants

Separation and detection was performed in a 6890N gas chromatograph (Agilent Technologies, Avondale, PA, USA) equipped with a large volume injection (LVI) system and an Agilent 5975N electron impact ionization mass spectrometer. A 40 µL aliquot of sample extract was injected using a 100 µL syringe in a cooled injection system (CIS) which consisted of a septum-less head and an empty baffled deactivated gas liner cooled with liquid nitrogen. The sample extract was injected at 50 °C while the vent valve was opened for 3 min at a flow rate of 75 mL·min^{-1} and a vent pressure of 2.9 psi. Subsequently, the analytes were focused to the column in splitless mode for 1.5 min while the temperature of the PTV injection port was increased at 12 °C·s^{-1} to 300 °C and held for 5 min. Finally, the inlet was further cleaned at a purge flow of 50 mL·min^{-1} before further injections. Target compounds were separated on a HP-5MS capillary column (30 m × 0.25 mm, 0.25 µm) from Agilent Technologies (Agilent Technologies, PA, USA). The oven temperature was programmed from 60 °C (hold 1 min) to 170 °C at 10 °C min^{-1}, then until 250 °C at 15 °C min^{-1}, and finally until 300 °C at 15 °C min^{-1} (hold 3 min). Hydrogen (AD-1020 Hydrogen Generator, Cinel Strumenti Scientifici, Padova, Italy) was used as carrier gas at a constant flow of 1.3 mL·min^{-1}. The transfer line temperature was maintained at 310 °C and the ion source and the quadrupole at 230 °C and 150 °C respectively. Detection was carried out both in the scan (50-525 m/z) and in the selected ion monitoring (SIM) modes simultaneously. The m/z values of the fragment ions monitored in SIM mode and the retention times are listed in Table 3.2, considering the first ion as quantifier and the rest as qualifiers.

3.4.3 TD-GC-MS analysis for priority and emerging pollutants

The analysis of SBs was performed by means of TD-GC-MS. Thus, SBs were thermally desorbed at a desorption temperature of 300 °C during 10 min using a commercial thermal desorption unit (TDU) (Gerstel) connected to a CIS-4 injector (Gerstel). The TDU unit was equipped with a TDSA autosampler (Gerstel) able to handle

98 positions. All glass tubes containing the stir-bars were placed in a tray that was assembled in the TDSA autosampler. The program of the CIS-4 injector was fixed as follows: a desorption flow of 100 mL·min^{-1}, a vent pressure of 6.6 psi and a cryo-focusing temperature of 0 °C. At a vent time of 0.01 min, the split valve was closed for 1.51 min and the temperature program of the injector was programmed as follows: 12°C·s^{-1} to 300 °C for 3 min.

The TDU was installed in an Agilent 6890 (Agilent Technologies, Palo Alto, CA, USA) gas chromatograph. The chromatographic column used was an HP5-MS (30 m × 0.25 mm, 0.25 µm, Agilent) and the oven temperature was programmed as follows: start at 60 °C (1 min), a temperature increase at 5 °C·min^{-1} to 175 °C (1 min hold), a second increase at 3 °C·min^{-1} to 200 °C (hold 1 min), a third increase at 5 °C·min^{-1} to 240 °C and a last increase at 30 °C·min^{-1} to 300 °C where it was finally held for 5 min. Helium (99.9995%, Carburos Metálicos, Barcelona, Spain) was used as carrier gas at a constant flow of 1.3 L·min^{-1}. The transfer line, ion source and quadrupole analyzer temperatures were maintained at 310, 230 and 150 °C, respectively. Detection was carried out using an Agilent 5975 electron ionization MS system (Agilent Technologies, Palo Alto, CA, USA).

4. NEW SAMPLE PREPARATION TECHNIQUES FOR DETERMINING ORGANIC COMPOUNDS IN WATER

In the main introduction of this work, the regulatory framework focused on the protection of water bodies has been reviewed. As it has been pinpointed, we have emphasized the need of robust analytical methods to assure the reliable analysis of hazardous substances in environmental media. Among the different steps included within the analytical procedure, sample preparation is not only the step where the major source of uncertainties can arise but also where the risk of contamination is the highest. In the recent years there is a clear tendency to develop novel extraction techniques aiming to the simplification of the sample treatments. Among those methods we can include those devoted to the automation and miniaturisation of the extraction step, using low volumes of solvents, or even solventless procedures, in order to pre-concentrate the analytes in the final acceptor phase. These techniques are presented as feasible alternatives to established procedures based on classical liquid-liquid extraction (LLE) or solid-phase extraction (SPE). However, regardless of their novelty, these new methodologies must deal with the same issues when environmental samples must be analyzed: the complexity of most of the matrixes, the presence of uncontrolled interferences, the low concentrations that should be attained (usually at $ng \cdot L^{-1}$ levels), to name a few. Consequently, monitoring programmes demand cost-effective, automated and user-friendly strategies for the analysis of environmental water (Dimpe and Nomngongo, 2016; Moreda-Piñeiro and Moreda-Piñeiro, 2015).

Though a comprehensive description of the analytical extraction techniques can be found in many textbooks and reviews (Etxebarria et al., 2012; Lucena, 2012; Ocaña-González et al., 2016), it is worth summarising the most recent developments and applications of many microextraction techniques to understand both the basis of the workflow and extraction strategies and the background of this particular work.

Nowadays, most of the extraction methods are based on sorptive extraction techniques such as solid phase micro-extraction (SPME) or stir bar sorptive extraction (SBSE), and liquid phase microextraction techniques (LPME). In this chapter the extraction methods developed in the present work are described whereas other related techniques are briefly described.

4.1 Liquid-Liquid Microextraction based techniques

Liquid-phase microextraction (LPME) is a simple liquid-liquid extraction (LLE) which uses only a few mL of acceptor phase in order to pre-concentrate the target analytes. Actually, the principles of LLE and the miniaturized nature of SPME are combined in this technique. LPME procedures can be classified in four main categories: membrane liquid phase microextraction (MLPME), dispersive liquid-liquid microextraction (DLLME), cloud-point extraction (CPE) and single-drop microextraction (SDME) (Kokosa, 2015; Ocaña-González et al., 2016; Sarafraz-Yazdi and Amiri, 2010). The most relevant extraction procedure in the present work is membrane liquid phase microextraction, as it was applied in the development of the extraction method for the determination of musk fragrances in water.

MLPME was introduced years ago as a simple and low-cost alternative to LLE and its development is still ongoing. The extraction takes place between the aqueous sample (donor phase) and the acceptor phase, which is typically a small volume of organic phase. Both phases are separated by a membrane that acts as a selective barrier (Psillakis and Kalogerakis, 2003). The main advantages over conventional LLE are the avoidance of emulsion formation, the lack of the phase separation step and the use of modules with a high surface-area-to-volume ratio (Cordero et al., 2000). There are two main categories depending on the nature of the membrane: (i) those using porous membranes and (ii) those using non-porous membranes.

Porous membranes allow the immobilisation of an organic solvent in the membrane. Based on the nature of the solvent retained in the pores of the membranes two different approaches can be described. The first one, known as supported liquid membrane (SLM) extraction, consists of a three-phase extraction system to extract acidic or basic polar compounds (Yamini et al., 2006). The second is the microporous membrane liquid–liquid extraction (MMLLE), and consists of a two-phase membrane extraction able to extract neutral and/or more hydrophobic organic compounds from aqueous media (see Figure 4.1) (Bedendo et al., 2012).

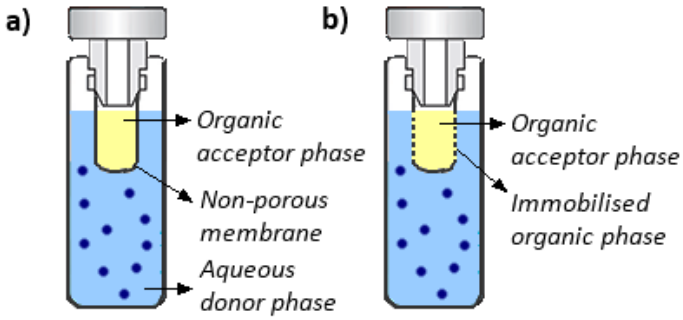


Figure 4.1. Different membrane based extraction techniques. a) Membrane assisted solvent extraction (MASE), b) microporous membrane liquid-liquid extraction (MMLLE).

When non-porous membranes are employed the interaction between the membrane and the organic solvent is negligible. Two different approaches are also distinguished in this case: (i) membrane-assisted solvent extraction (MASE), and (ii) membrane extraction with sorbent interface (MESI).

MASE, consist of a three-phase aqueous–polymeric–organic system, where no organic solvent is deliberately immobilised in the polymeric material. The organic phase placed in the inner side of the membrane acts as the acceptor phase. Polymers usually

used as membrane material in MASE are LDPE, dense polypropylene (PP), PDMS silicone rubbers and asymmetric composite polymeric membranes which are composed of a thin layer of silicone and another layer of polycarbonate (PC) or a relatively thick support layer of porous PP (Salgueiro-González et al., 2015; Schellin and Popp, 2003b; Shi et al., 2012).

By using non-porous polymeric membranes, the analyte extraction rate (permeability) is governed by a solution-diffusion mechanism that highly depends on the analyte solubility and diffusivity into the membrane material. Normally, nonpolar solvents such as heptane, hexane and cyclohexane are used (Hauser and Popp, 2001). After extraction, the organic solvent is usually collected to be analysed by means of chromatographic techniques (Hauser et al., 2004; Schellin and Popp, 2003a; Schellin and Popp, 2005; Schellin and Popp, 2006). MASE has been proven to be a simple, low-cost and virtually solvent-free sample preparation technique which provides a high degree of selectivity and enrichment (Iparraguirre et al., 2014; Shi et al., 2012).

MESI is designed for the analysis of volatile or semi-volatile organic compounds (VOCs and SVOCs) in aqueous and air samples (Kaykhaii et al., 2002; Luo and Pawliszyn, 2000). In this approach, analytes diffuse in an aqueous–polymeric–gaseous system from the aqueous sample through the non-porous polymeric membrane into the gaseous flowing stream on the other side of the membrane.

In general, the transport rate in liquid porous membranes is higher than in non-porous membranes, because of the great diffusivity of species in the liquid medium of the liquid homogeneous membranes. Additionally, in these liquid membranes it is easier to incorporate carriers to increase the permeability of certain species, giving rise to facilitated or coupled transport processes. However, due to solvent leakage out of the liquid membranes, the membrane lifetime is usually longer for polymeric membranes (non-porous) (Etxebarria et al., 2012).

Regarding the development of these sort of extraction procedures it is typically focused to the variables affecting membrane liquid-phase microextraction such as volume of the donor phase, pH of the aqueous solution, addition of inert salts to modify the ionic strength, extraction temperature or extraction time. However there are other variables such as the nature of the membrane or the nature and volume of the extraction solvent that are more specific of these membrane-based extraction procedures (Psillakis and Kalogerakis, 2003).

From a technical point of view, membrane based liquid extraction techniques enable a high degree of flexibility, providing compatibility with common chromatographic techniques such as GC, HPLC or CE coupled to MS. They have been applied to the analysis of several priority and emerging pollutants in water samples (Carasek and Merib, 2015; Płotka-Wasylka et al., 2016a).

The spread of membrane-based microextraction techniques in the analytical sciences is also observed by the increasing number of derived procedures. As mentioned before, the most highlighted alternatives are DLLME, CPE and SDME among others. Briefly, in DLLME, the use of a dispersing agent increases the contact surface to enhance the extraction efficiency (Guan et al., 2016; Spietelun et al., 2014; Yan and Wang, 2013) whereas the use of surfactants in CPE enhances the extraction yield (Altunay et al., 2016; Xie et al., 2010). Finally, SDME can be considered as a basic liquid-liquid microextraction technique which uses a small amount of organic solvents, aqueous solutions or ionic liquids (low-temperature melting salts that form liquids composed entirely of ions) to favour the extraction of the analytes from water samples (Płotka-Wasylka et al., 2016a; Sarafraz-Yazdi and Amiri, 2010).

4.2 Sorptive extraction based techniques

The sample preparation techniques based upon sorptive extraction, such as stir-bar sorptive extraction (SBSE), solid-phase micro-extraction (SPME) and other techniques such as microextraction by packed sorbent (MEPS) (Abdel-Rehim, 2011; Moein et al., 2015; Moein et al., 2014) have proven to be successful and environmentally friendly alternatives to classical LLE and SPE approaches (Dimpe and Nomngongo, 2016; Ocaña-González et al., 2016).

Briefly, in SPME and SBSE the analytes are extracted from the aqueous matrix into a non-miscible liquid or solid polymer, mainly liquid PDMS. PDMS is a very well-known stationary phase in GC, thermally stable in a broad temperature range (−20°C to 320°C) and with remarkable diffusion properties. The major difference between SPME and SBSE relies on the amount of the polymeric phase (i.e., 0.5 mL in SPME fiber versus 24 mL in the smallest stir bar), which determines the extraction efficiency (a schematic representation of a stir bar is shown in Figure 4.2). However, one significant drawback of the increased fiber capacity is the loss of selectivity, since not only the analytes but also most of the interferences are exhaustively extracted (Lord and Pawliszyn, 2000).

In contrast to the big amount of coatings available for SPME fibers (i.e., PDMS, PDMS/divinylbenzene, polyacrilate, etc.), only PDMS coated stir-bars are commercially available so far for non-polar or slightly polar compounds (Iparraguirre et al., 2011). This is one of the main disadvantages of SBSE, since very low recoveries are achieved in the extraction of polar compounds using PDMS as a receiving phase. In order to solve this drawback other alternatives have been developed such as derivatisation or the use of in-house coatings of different polarities (Fumes et al., 2015; Liu et al., 2004). The derivatisation procedure consists of the substitution of functional groups of the polar analytes by means of a derivatisation reagent in order to increase the chromatographic response and to enhance the selectivity of the method (Cacho et al., 2015; Lord and

Pawliszyn, 2000). This procedure can be performed for sorptive extractions such as SBSE or SPME in different modes: in-situ derivatisation (derivatisation reagent is added to the sample), on-polymer derivatisation (the polymer is exposed to the derivatisation reagent) or in-tube derivatisation (derivatisation carried out in the injection port).

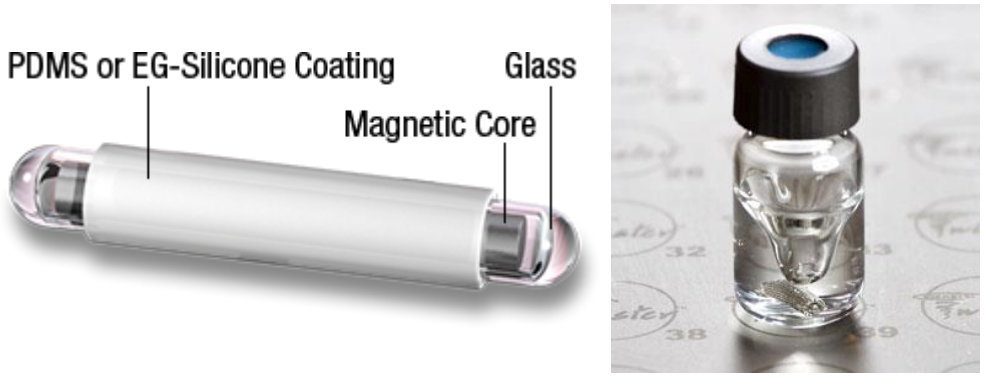


Figure 4.2. Schematic representation of a Stir bar (left) and a extraction vessel were immersion mode is applied (right)

The principles and applications of SPME and SBSE have been reviewed previously (Baltussen et al., 2002; Nogueira, 2012). For a extraction system composed of a stir bar the process is kinetically governed until a steady state is attained, where the extraction efficiency is governed by the distribution or partition coefficient of the target analyte between the PDMS phase and water ($K_{PDMS,w}$) and their respective volumes. The theoretical recovery (R%) of a given SBSE setup can be calculated as represented in Equation 4.1:

$$R = \frac{m_{PDMS}}{m_{w,0}} = \frac{K_{PDMS,w}}{K_{PDMS,w} + \beta} \quad (4.1)$$

where m_{PDMS} and $m_{w,0}$ are the masses of the target analyte in the PDMS and the aqueous phase respectively, and β the phase ratio. The partition coefficient ($K_{PDMS,w}$) is defined as

the ratio of the concentrations of the target analyte between the PDMS phase and the aqueous phase.

The recoveries are higher when low phase-ratios are used and highly non-polar compounds are extracted (Prieto et al., 2010). Recent studies have correlated the $K_{PDMS,w}$ partitioning coefficient with the octanol–water distribution coefficient (K_{ow}) and, although not exactly accurate, K_{ow} gives a good indication of whether and how well a given analyte can be extracted by means of SBSE (David and Sandra, 2007; Kawaguchi et al., 2006).

There are two experimental working regimes according to the sampling time using SBSE: equilibrium and kinetic. In the first case, if long extraction times are assured, the partitioning equilibrium between sample matrix and extraction phase is reached. In the second case a linear uptake is observed at the initial extraction times (Wells, 2003). In the first approach, convection conditions do not affect the amount extracted and the extraction is limited by the partition equilibria between the sample matrix and the sorptive phase. In the second approach, the extraction is interrupted prior to equilibrium and thus, it is necessary to control the convection/agitation and the timing of the extraction, which must be constant in order to guarantee repeatable results (Lord and Pawliszyn, 2000).

SBSE consists of two main steps: extraction and desorption. During the extraction step the stir-bar is put in contact with the solutes by direct immersion (DI-SBSE) or by headspace sampling (HS-SBSE). In the direct immersion mode the stir-bar is added to the aqueous sample under controlled extraction conditions whereas headspace extraction is performed by suspending the coated fibre or stir-bar in the headspace of the vial, which allows a static contact of the polymeric phase with the vapour phase of the aqueous matrix. The DI-SBSE mode is significantly influenced by the sample matrix (Hoh and Mastovska, 2008), whereas the HSSE mode can prevent the matrix effects and allows

modification of the matrix without damaging the polymeric phase (Lord and Pawliszyn, 2000).

Regarding the desorption step, stir bars cannot be directly desorbed in a split/splitless injection port of a gas chromatograph. Hence, the extraction step is followed by a thermal desorption (TD) or liquid desorption (LD) step before analysing by chromatographic techniques. Most applications involve the use of TD, with the advantage of avoiding the use of organic solvents, in order to introduce quantitatively thermally stable volatile and semivolatile analytes to the gas chromatographic system (Baltussen et al., 2002).

TD takes place at temperatures between 150°C and 300°C inside a thermal desorption unit (TDU) (see Figure 4.3) and guarantees the complete introduction of the total amount of extracted analyte into the chromatographic system, improving in this way the sensitivity of the method. A TDU consists of two programmable temperature vaporizers (PTV), where injection is performed in several consecutive steps:

1. First, analytes are isolated at high temperatures (150°C - 300°C) from the stir bar, which is placed in the TDU liner.
2. The analytes desorbed in the warm TDU are continuously transferred to the second TDU (called as CIS injection port), which is maintained at low temperatures (−150°C to 40°C) until the complete desorption of the analytes occurred. The analytes are retained and focused in this part of the injection port before being transferred to the GC column. To this last aim, the temperature of CIS injection port is suddenly risen (150°C - 300°C) and the analytes are introduced to GC column.

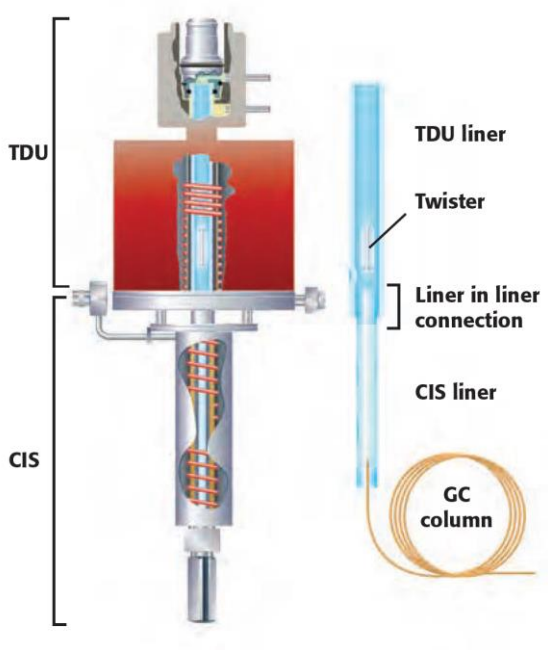


Figure 4.3. Schematic representation of the SBSE desorption step in a TDU coupled to a CIS unit in a GC system (www.gerstel.com).

Liquid desorption (LD) is the alternative desorption mechanism for thermally labile analytes and is generally coupled to LC, CE or even GC if split/splitless or large volume injection (LVI) modes are used. In LD mode, the use of organic stripping solvents (such as acetonitrile, methanol or mixtures with water or aqueous buffers) is required to accomplish the chemical desorption. Due to the polarity of most of the stripping solvents used, LD mode is mainly useful for non-volatile and thermo-labile compounds with an intermediate polarity.

When analytes must be analysed at environmentally relevant values, sensitivity is a must not only during the extraction but also during the analysis. By using thermal desorption step, the analytes trapped in the polymers are completely introduced into the chromatographic system, gaining the sensitivity. However, when liquid desorption

strategy is required, the limits of detection are often worse if split/splitless injection mode is used. In this regard, the use of large volume injection mode in a PTV inlet has gained importance in the last decades as an alternative to the classical split/splitless injection systems in order to gain sensitivity through the analysis (Hoh and Mastovska, 2008). PTV inlets are similar to the classical split/splitless inlets, but with an essential difference based on the sophisticated temperature control function, which implies that they can be rapidly heated or cooled during injection, while the conventional split/splitless inlet works isothermally (see Figure 4.4). Briefly, LVI-PTV injection consists of several steps (Hoh and Mastovska, 2008):

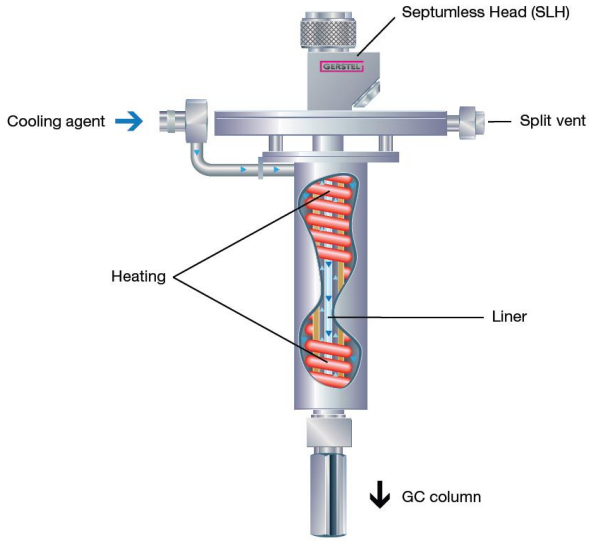


Figure 4.4. Schematic representation LVI-PTV injection system consisting of a septumless head coupled to a CIS-PTV unit in a GC system (www.gerstel.com)

- Sample is introduced at a low temperature (below solvent boiling point) once or using a series of small sample aliquots in the inlet.
- Solvent is eliminated via the split vent, while the higher-boiling analytes are retained in the liner.

- PTV is rapidly heated and the analytes are transferred to the column in the splitless mode. The analytes are focused at the front of the column by keeping the oven temperature below the solvent boiling point.
- After the splitless transfer, the split vent is opened in order to remove the residual solvent vapor and the low-volatile matrix components from the inlet.

Regarding the extraction, several parameters can influence the partition of the analytes between the two phases and thus, the extraction efficiency. Therefore, the most studied variables are extraction time, pH adjustment, addition of an inert salt, addition of an organic modifier, stirring rate, extraction temperature, sample volume and the nature and volume of the polymeric phase (Cacho et al., 2015; Prieto et al., 2010; Vázquez et al., 2008). On the other hand, in the desorption step, desorption time, desorption temperature and cryofocusing temperature are the most studied variables in the TD mode whereas using LD mode, stripping solvent nature, desorption time and desorption volume are the most frequently evaluated variables (Etxebarria et al., 2012).

Apart from the commercially available stir-bars, low cost materials are gaining importance as an alternative to costly SPE fibers or conventional polymers employed used in SBSE. Recently, the trend in sorbent micro-extraction techniques is focused on the search for inexpensive sorbent materials. Due to their low cost per unit, these materials can be disposed after a single use. This fact poses a great advantage as variations in extraction efficiencies due to deterioration of the polymer and memory effects are avoided by using only once each piece of polymer (Prieto et al., 2012).

In this sense, silicon sorbents such as silicon tubes (STs) or silicon rods (SRs) were introduced by Popp et al. as low cost alternative to the stir bars used in SBSE for the extraction of polycyclic aromatic hydrocarbons (Popp et al., 2004) and polychlorinated biphenyl compounds (Montero et al., 2004). Afterwards, SR extraction was applied

principally to the extraction of chlorophenols (Schellin and Popp, 2007), chlorobenzenes (van Pinxteren et al., 2009) some pharmaceuticals (Paschke et al., 2007) and for the simultaneous analysis of nonylphenols, organochlorine and organophosphorous pesticides, synthetic musks and phathalates in water samples (Delgado et al., 2013). An overview of the recent research on the application of polymeric materials for the extraction of organic compounds is summarized in table 4.1.

Nonetheless, due to the poor extraction efficiency of PDMS based polymeric materials for polar compounds; some authors have already proposed new polymeric materials for sorptive purposes including monolithic materials (Huang et al., 2008), molecularly imprinted polymers (MIPs) (Figueiredo et al., 2016; Prieto et al., 2011; Vasapollo et al., 2011), restricted access materials (RAMs) (Fumes et al., 2015; Lambert et al., 2005; Płotka-Wasylka et al., 2016b) or polyurethane foams (PUFs) (Al-Saidi et al., 2016; Portugal et al., 2008). On the other hand, the development of new extraction procedures that integrate the extraction and stirring element in the same device, as in the case of stir bars, has emerged also as a promising research field. In this framework, new approaches such as stir membrane extraction (Alcudia-León et al., 2011; Lucena, 2012), rotating-disk sorptive extraction (Giordano et al., 2011; Richter et al., 2009), stir frit microextraction (Roldán-Pijuán et al., 2012) have been developed and applied to determine polycyclic aromatic hydrocarbons (PAHs), pesticides and BTEX in water samples respectively, obtaining promising results in comparison to well established methods. Despite all these efforts, there is still the need of assessing the suitability of polymeric materials with different properties, which allow extracting simultaneously a wide range of organic contaminants.

Table 4.1. Applications of polymeric materials as extracting agents.

SB

Compound	Matrix	Sample amount	Extraction	Detection	LODs	References
Musks	Vegetables, amended soil	0.5g in 9mL of water	SBSE (0.5 mm film thickness x 20 mm)	TD-GC-MS	0.01-0.8 $\mu g \cdot g^{-1}$	(Aguirre et al., 2014)
Musks	Water	30 mL	SBSE (0.5 mm film thickness x 10 mm)	LD-GC-MS	67-333 $ng \cdot L^{-1}$	(Chase et al., 2012)
Organochlorine Pesticides	Water	100 mL	SBSE (0.5 mm film thickness x 20 mm)	LD-GC-MS/MS	5-57 $ng \cdot L^{-1}$	(Guart et al., 2014)
Organochlorine Pesticides	Seawater	100 mL	SBSE (0.5 mm film thickness x 20 mm)	TD-GC-MS	1-17 $ng \cdot L^{-1}$	(Moreno-González et al., 2013)
Organophosphorous Pesticides	Water	20 mL	DI-SBSE (1.0 mm film thickness x 20 mm)	LD-LC-MS/MS	50-100 $ng \cdot L^{-1}$	(Margoum et al., 2013)
Phtalates	Vegetables	2g in 10 mL of aqueous solution	SBSE (0.5 mm film thickness x 20 mm)	TD-GC-MS	3-21 $ng \cdot L^{-1}$	(Cacho et al., 2012)
Alkylphenols	Vegetables	2g in 10 mL of aqueous solution	SBSE (0.5 mm film thickness x 20 mm)	TD-GC-MS	3-5 $ng \cdot L^{-1}$	(Cacho et al., 2012)
Alkylphenols	Water	10 mL	SBSE (0.5 mm film thickness x 10 mm)	GC-MS derivatization with acetic acid	0.1-3.2 $ng \cdot L^{-1}$	(Nakamura and Daishima, 2004)
Alkylphenols	Water	10 mL	SBSE (0.5 mm film thickness x 10 mm)	TD-GC-MS	0.5-5 $ng \cdot L^{-1}$	(Kawaguchi et al., 2004)

SR

Compound	Matrix	Sample amount	Extraction	Detection	LODs	References
Perfluorinated alkyl substances	Carrot and amended soil	0.5 g	3 pieces of 1 cm length			(Bizkarguenaga et al., 2015)
PCBs, Chlorobenzenes	Water	100 mL	2 mm diameter x 8 cm length rod	TD-GC-MS	0.2-0.6 $ng \cdot L^{-1}$	(Montero et al., 2004)
PAHs	Water	15 mL	1 mm diameter x 1 cm length rod	LC-FLD	0.1-1.2 $ng \cdot L^{-1}$	(Popp et al., 2004)
Pharmaceuticals	Water	480 mL	2 mm diameter x 2 cm length rod	LC-ESI-MS	3000-16000 $ng \cdot L^{-1}$	(Paschke et al., 2007)
Alkylphenols	Water	130 mL	2 mm diameter x 1 cm length rod	LVI-GC-MS derivatization (in-port silylation)	not available	(Cavalheiro et al., 2014)

PES

Compound	Matrix	Sample amount	Extraction	Detection	LODs	References
Alkylphenols	Water	150 mL	5 tubes of 0.7 mm diameter x 1.5 cm length	LC-MS/MS GC-MS derivatization (silylation)	1-11 $ng \cdot L^{-1}$ 8-35 $ng \cdot L^{-1}$	(Ros et al., 2015)
Organochlorine Pesticides	Water	18 mL	0.7 mm diameter x 1 cm length tubes	GC-MS derivatization (silylation)	5-73 $ng \cdot L^{-1}$	(Prieto et al., 2014)
Perfluorinated alkyl substances	Carrot and amended soil	0.5 g	5 tubes of 0.7 mm diameter x 1.9 cm length	LC-MS/MS	0.1-1.8 $ng \cdot g^{-1}$ 0.3-2.9 $ng \cdot g^{-1}$	(Bizkarguenaga et al., 2015)
Benzotriazoles	Water	15 mL	0.7 mm diameter x 5 cm length tubes	LC-QTOF-MS	5-100 $ng \cdot L^{-1}$	(Casado et al., 2013)

4.3 References

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5. MEMBRANE ASSISTED SOLVENT EXTRACTION COUPLED TO LARGE VOLUME INJECTION – GAS CHROMATOGRAPHY–MASS SPECTROMETRY FOR TRACE ANALYSIS OF SYNTHETIC MUSKS IN ENVIRONMENTAL WATER SAMPLES

Personal care products (PCPs) are a newest set of compounds present in water bodies due to their widespread use in daily human life. Though PCPs include a large variety of potential contaminants, in this chapter we will focus on the determination of musk fragrances since some of these compounds have been recently included as candidates for monitoring and regulation in environmental programs [Daughton, 2004] and at low $ng \cdot l^{-1}$ levels

The chosen technical approach to tackle this objective is the application of membrane assisted solvent extraction (MASE) coupled to large volume injection (LVI) in a programmable temperature vaporisation injector (PTV) coupled to gas chromatography-mass spectrometry (GC-MS). In this chapter it is described the optimization and validation of a method to determine 10 synthetic musk fragrances (musks) in surface and wastewater samples.

The development of the method includes the optimization of the chemical extraction parameters and the instrumental ones of the LVI-PTV-GC-MS set-up. Regarding the extraction, the influence of nature and volume of the acceptor phase, the nature of the membrane, the salting out effect, the addition of methanol, the sample volume and the temperature and extraction time were evaluated to optimise the MASE procedure. The performance of this method allows the direct analysis of MASE extracts by means of LVI-PTV-GC-MS eliminating any pre-treatment step and avoiding volatile analyte losses. Concerning the analysis step, several LVI-PTV variables such as cryo-focussing temperature, injection speed and vent time were optimised following an experimental design approach. Finally, the optimised method was applied for the determination of musk fragrances in surface and wastewater samples.

5.1 Experimental

5.1.1 Sampling procedure

Surface water samples and the influent and effluent of two urban WWTPs that collect wastewater from ca. 922.000 inhabitants were analysed in order to test the performance of the method in real environmental waters. The surface water samples from the estuary of Urdaibai (Bay of Biscay, North of Spain) were collected in May 2011. In the case of WWTPs, 24-h flow proportional composite untreated influent (upstream) and final treated effluent (downstream) urban wastewater samples were collected at WWTP of Bakio (Biscay) and at WWTP of Galindo (Barakaldo, Biscay) in May 2011. Samples were collected in pre-cleaned amber bottles and carried to the laboratory in cooled boxes. After collection, samples were filtered through 0.45 μm, stored at 4 °C before treatment and analysed within 48 hours.

5.1.2 Membrane assisted solvent extraction (MASE)

The extraction procedure was performed using homemade LDPE membrane bags: (i) small size (2.5 cm length and 1 cm i.d.) to handle 200 μL of solvent and (ii) large size (4 cm length and 1 cm i.d.) to handle 800 μL of solvent. Both size LDPE membrane bags were prepared using a shrink-wrapping device. After thermally sealing the borders, the overlaying borders were carefully cut in order to minimise the superficial zones where analytes could be absorbed. The membranes were conditioned with Hex and maintained in clean Hex before their use in order to minimise the cross-contamination of interfering compounds from the membrane material.

The extraction was carried out using conventional head-space glass vials. LDPE membranes were attached to a metal funnel and fixed with a Teflon ring (Gerstel, USA). Then, the membranes were filled with 200 μL of Hex and immersed in the water sample, held by a metal funnel which is placed in the bottleneck (see Figure 5.1). Vials were sealed with PTFE septa and aluminium crimp caps.

Figure 5.1. Extraction vessel and metal funnel.

Extraction vials were stirred using a magnetic 15 position stirring hot-plate from Gerstel (USA) at 700 rpm. A temperature controlled bath was used when extractions were performed at 15 °C and 30 °C. Once the extraction step was accomplished, the extracting phase was transferred to a chromatography vial and weighed. In the case of using 800 µL, the extracts were transferred to a 2 mL amber vial and evaporated to dryness at 11 °C under a low stream of nitrogen in a Turbovap LV Evaporator (Zymark, Hopkinton, USA) and the extracts were reconstituted in 200 µL of Hex.

5.2 Results and discussion

5.2.1 Optimisation of the MASE procedure

The MASE procedure is highly sensitive towards the variation of all those variables that might affect the extraction efficiency (Psillakis and Kalogerakis, 2003). Thus, the solvent and its volume in the acceptor phase as well as the nature of the membrane, the salting out effect, the addition of methanol, the sample volume and the temperature and the extraction time were evaluated. The whole optimization procedure was performed using GC-MS analysis.

5.2.1.1 Nature and volume of the acceptor phase

Initially, both the nature and volume of the acceptor phase were evaluated. The boiling point and the polarity of the solvent were considered in order to choose the most adequate solvents. A wide range of solvents with different polarities have been used in the literature in order to extract synthetic musks by LLE Hex, EtOAc, CH_2Cl_2, $CHCl_3$, toluene) (Peck, 2006; Reiner and Kannan, 2011). However, ideal organic solvents for MASE have to meet several conditions such as inertness to the membrane or high solubility for the studied analytes (Hauser and Popp, 2001). Moreover, when large volumes of the extract are needed to be injected in the LVI system, the chosen solvents must be volatile enough to be easily eliminated easily during the vent step. Organic solvents like Hex, EtOAc or $CHCl_3$ fulfil required conditions but, in order to use more environmental-friendly solvents, the use of $CHCl_3$ was discarded. Besides, although MASE uses low solvent volumes (i.e., 800 µL), due to the high volatility of some musk compounds, possible analyte losses can be observed during the MASE extract evaporation step (Silva and Nogueira, 2010). To avoid this last step but to preconcentrate a little bit more, the use of lower solvent volumes such as 200 µL was considered.

Thus, 15 mL of Milli-Q water samples spiked at a concentration level of 10 $\mu g \cdot L^{-1}$ of each compound were extracted using 200 µL and 800 µL of Hex and EtOAc under constant stirring speed (500 rpm), extraction temperature (room temperature) and extraction time (90 min). The results (as chromatographic peak area/sample weight) obtained throughout the assays performed in triplicate are shown in Figure 5.2. On the one hand, Hex provided better extraction yields than EtOAc. The main reason of this difference can be attributed to the permeation of EtOAc through the membrane into aqueous sample due to its high water solubility (8.5 g in 100 g water at 20 °C) (Einsle et al., 2006). As a consequence, the contact and transfer extraction area is reduced, which was reflected both in the little volume of EtOAc recovered after extraction step and in the poor extraction yield. On the other hand, the use of 200 µL of solvent volume provided

higher recoveries since evaporation was avoided and the analyte losses were minimised. Therefore, 200 µL of Hex was chosen as the acceptor organic phase during the optimisation of the rest of the variables.

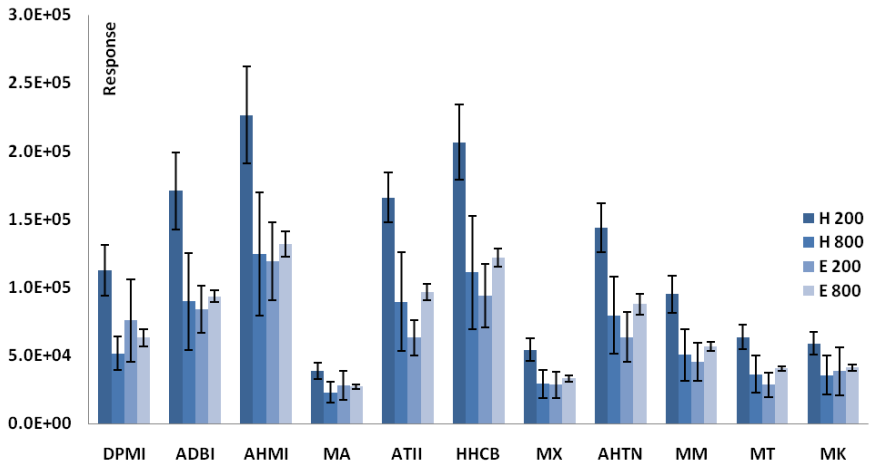

Figure 5.2. Comparison of the chromatographic average responses (n=3, 95 % confidence level) obtained for the different organic solvents (H: Hex and E: EtOAc) and volumes (200 µL and 800 µL).

5.2.1.2 Nature of membrane

Different non-porous membranes were evaluated for the extraction of the target compounds from water samples in order to select the most convenient, i.e. that providing the highest recoveries and lowest losses (Einsle et al., 2006). Although, both porous and non-porous membranes, in this work only non-porous membranes were assessed, since the transference of water to organic solvent through the pores is prevented.

For this purpose, 15 mL of spiked Milli-Q water (10 µg L^{-1}) were extracted using home-made membranes with different materials (LDPE and PET) and thicknesses (0.02 mm and 0.05 mm) during 90 min at room temperature. As it is plotted in Figure 5.3, the selected material and the thickness affect to the response of the extraction (average

response of three replicates). Finally, thin LDPE membranes yielded the best results, surely due to the faster analyte permeation across the membrane. Therefore, thin LDPE membranes were chosen for further experiments.

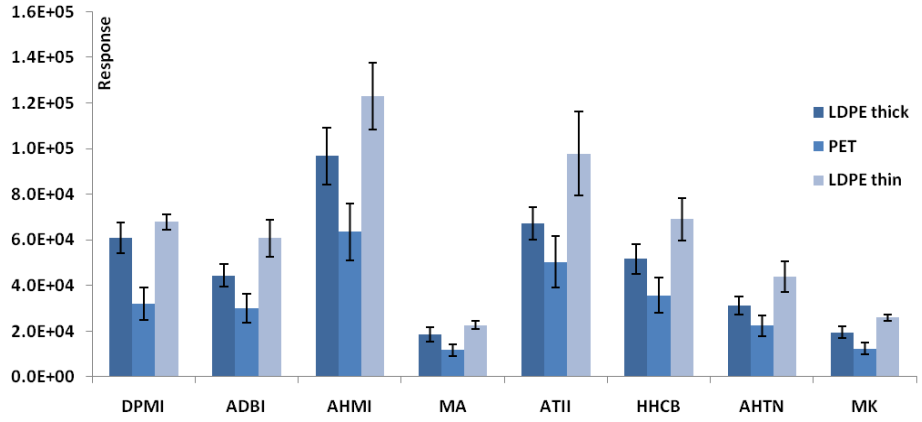

Figure 5.3. Responses (n=3, 95% confidence level) obtained after the extraction with different membrane materials: LDPE thick, PET and LDPE thin.

5.2.1.3 Modifications of the aqueous medium

The characteristics of the aqueous medium (i.e. pH, ionic strength or the addition of organic modifier) are variables to be taken into account in MASE (Prieto et al., 2008). Thus, 15 mL of Milli-Q water at 10 $\mu g \cdot L^{-1}$ concentration level were extracted at previously established conditions (200 µL Hex, 90 min, room temperature and 500 rpm) to study the effects when the aqueous matrix is modified. Firstly, the influence of pH was evaluated at three levels: acidic (pH: 2), neutral (pH: 7) and alkaline (pH: 12). Since the responses were equivalent ($p>0.05$), it was considered unnecessary to adjust the pH of the water samples.

The influence of the addition of an inert salt addition (NaCl) and an organic modifier (MeOH) was studied simultaneously by means of a Central Composite Design (CCD) using Statgraphics (Centurion XV). On the one hand, the addition of salt increases

the ionic strength of aqueous samples, decreasing the solubility of the analytes and improving their transference to the organic acceptor as it has been observed in previous works (Iparraguirre et al., 2011). On the other hand, the addition of organic modifier, can also improve the extraction yields for non-polar compounds since their adsorption in the walls of glassware is avoided, and the solubility of the hydrophobic compounds in the aqueous solution is increased. Thus, the influence of the addition of NaCl and MeOH, were studied within 0 – 20 % and 0 – 10 % ranges respectively.

The responses obtained for the CCD were analysed by means of multiple linear regression (MLR). Similarly to others works found in the literature (García-Jares et al., 2002; Silva and Nogueira, 2010), the addition of MeOH was not significant (p-value > 0.05) whereas the addition of NaCl had a negative effect for all the compounds except for DPMI and MK. As an example, the calculated standardised Pareto charts[1] for the two factors and the interactions for DPMI, MK and HHCB as well as the corresponding response surfaces are shown in Figure 5.4. According to the obtained results, the addition of NaCl and MeOH was not required.

[1] *Standardised Pareto Chart: Statistical graphic provided by the Statgraphics program in order to evaluate the significant standardised effects of the main factors as well as their interactions. The standardised effect is obtained by dividing the estimated effect by its standard error. The vertical line indicates the statistically significant bound at the 95 % confidence level.*

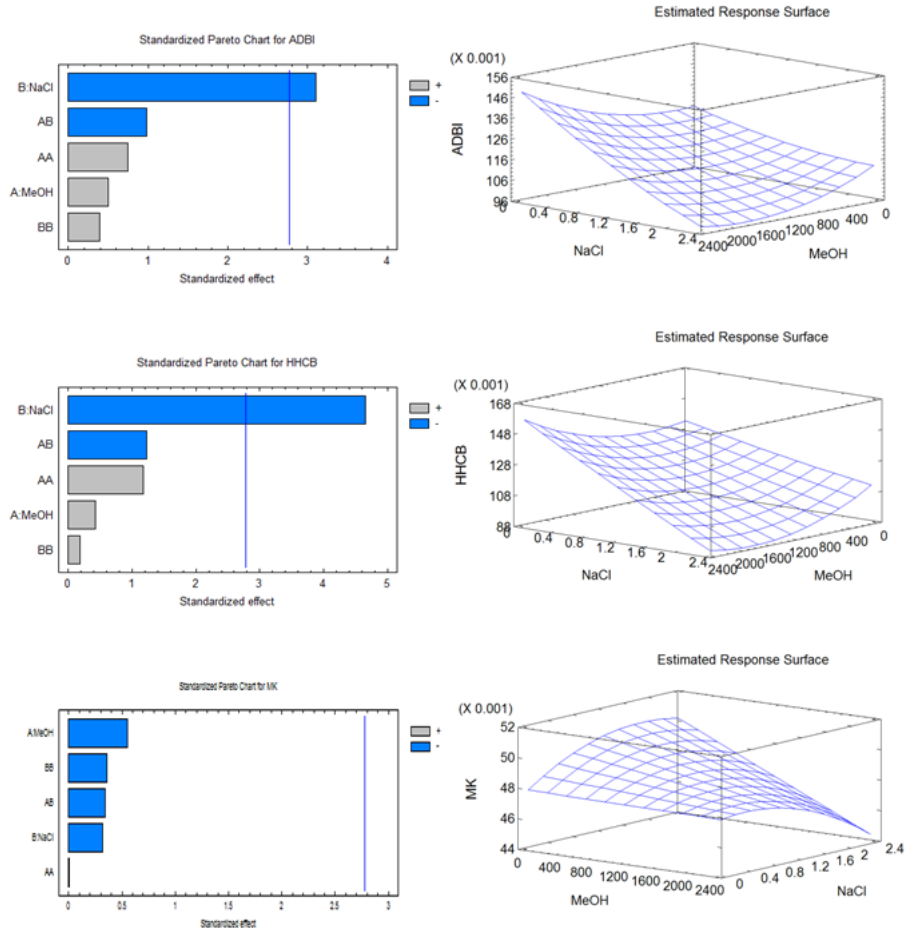

Figure 5.4. Standardised Pareto charts for the main effects and their interactions and the response surface obtained after CCD for three representative musk compounds: a) ADBI b) HHCB and c) MK..

5.2.1.4 Sample volume

Owing to an improvement in the chromatographic responses, the extraction of higher sample volumes (higher mass of analytes) was also studied even if the extraction efficiency may be decreased (Iparraguirre et al., 2011). Three different volumes (i.e. 14 mL, 50 mL and 150 mL) were spiked at the same concentration (10 $\mu g \cdot L^{-1}$) and pre-treated under extraction conditions fixed: (200 µL Hex, 90 min, room temperature and 500 rpm). As it can be observed in Figure 5.5, all the analytes showed increased signals for higher sample volumes. Thus, in order to maximise the chromatographic response yielding better limits of detection, 150 mL sample aliquots were used for further experiments.

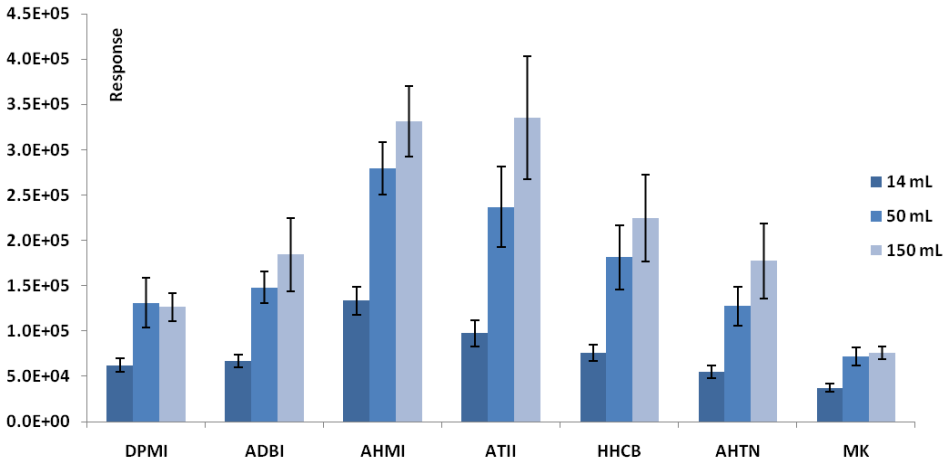

Figure 5.5. a) Chromatographic responses (n=3, 95 % confidence level) obtained for several musk compounds after the extraction of samples of 14 mL, 50 mL and 150 mL.

5.2.1.5 Stirring rate

Once the sample volume was fixed, the stirring rate was optimised. In most of the cases, when a vigorous mixing of the sample is assured, the extraction efficiency can be enhanced due to a decrease of the thickness of the boundary layers. However, too high agitation speeds may increase the formation of bubbles and reduce the extraction

efficiency. Owing to these constraints 150 mL of Milli-Q water spiked at 600 ng·L^{-1} concentration level were extracted at three different stirring rates, 500 rpm, 700 rpm and 900 rpm in triplicate using 200 µL of Hex for 90 min at room temperature. The intermediate and high stirring rates provided higher responses (see Figure 5.6) than the lowest one for all the target compounds, and thus, agitation speed of 700 rpm was fixed for the upcoming experiments.

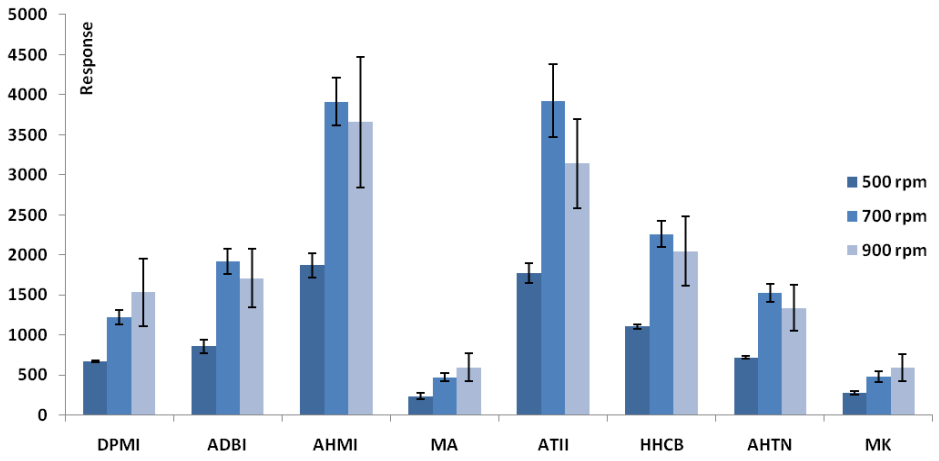

Figure 5.6. Responses (n=3, 95 % confidence level) of synthetic musks achieved with extractions performed at 500, 700 and 900 rpm stirring rates.

5.2.1.6 Time-profile

Finally, the extraction time was studied in order to check at which time equilibrium was reached. The optimal extraction time can take from several minutes to hours depending on the physical and chemical properties of the analytes, the partition coefficient between the volume of the sample and the volume of the organic phase and the experimental conditions (David and Sandra, 2007). In this sense, although at elevated temperatures the extraction equilibrium is reached faster, application of high temperatures can increase the losses of volatile components. In order to fix the extraction

time to assure the equilibrium between both phases, a kinetic experiment from 5 to 720 min was carried out in triplicate at different extraction temperatures (15°C, 22°C and 30°C) and at two concentration levels (600 $ng \cdot L^{-1}$ and 1300 $ng \cdot L^{-1}$) under the previous fixed conditions. In agreement with other authors (Einsle et al., 2006), increasing temperature showed clearly an increase in the response of the studied musks (see Figure 5.7). The equilibrium in these conditions was reached after approximately 240 min (4 hours). Furthermore, very long extractions (i.e. 720 min) showed a decrease in the response due to an evaporation of the organic solvent.

The shape of the kinetic profiles of all the target compounds was comparable, regardless of the temperature or the concentration of the analytes. Therefore, the minimum stirring time to reach the equilibrium was fixed in 4 hours. As expected, the efficiency of the extraction is higher when temperature is increased, but for the routine work it is more convenient to work at room temperature since the reproducibilities were adequate to the purpose of the analysis (see Figure 5.7).

Therefore, for the extraction of 150 mL of water sample, by means of MASE, final extraction conditions were established as it follows: thin LDPE membrane filled with 200 µL of Hex; no addition of NaCl nor MeOH; no variation of pH; stirring speed 700 rpm and extraction time 240 min at room temperature.

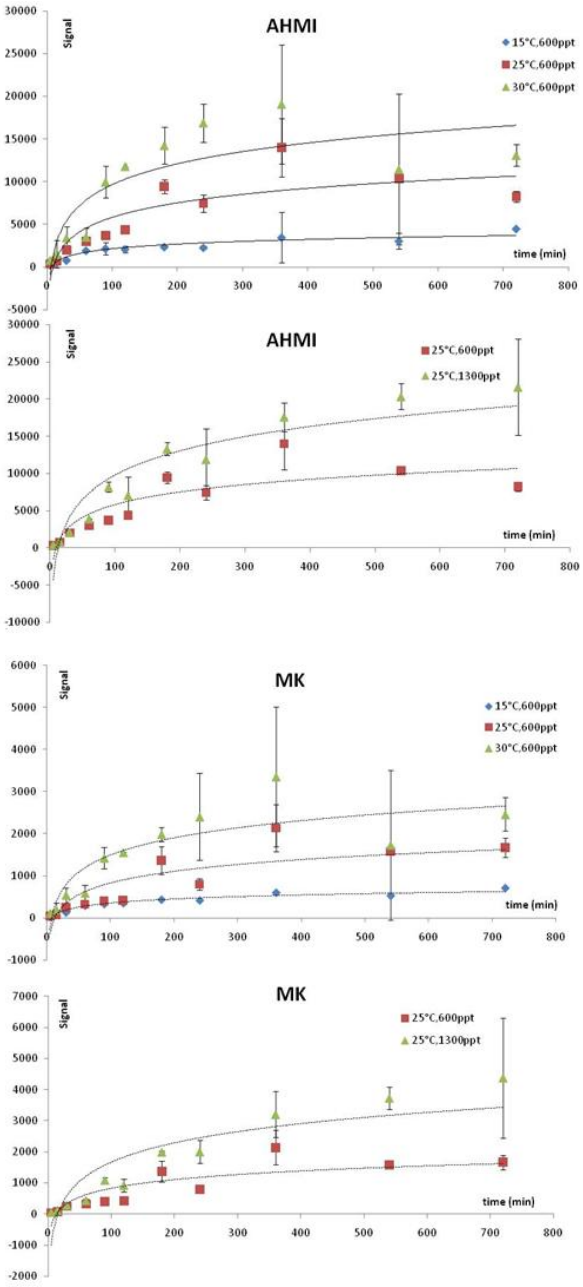

Figure 5.7. Time profile for AHMI and MK at three different temperatures: 15°C, 25°C, 30°C and at two levels of concentration: 600 ng L^{-1} and 1300 ng L^{-1}.

5.2.2 Optimisation of LVI-PTV-GC-MS analysis

In order to fine tune the sensitivity of the method, the optimisation of the LVI-PTV setup should be also considered. During the LVI-PTV injection coupled to gas chromatography, there are several parameters that can affect the efficiency of the analysis, since it results essential to eliminate the solvent during the split vent time while analytes are retained in the CIS-4 unit (as described in section 4.2).

Although the effect of all the instrumental variables might be studied, it is usually better to fix some of them in order to reduce the complexity of the system (Vallejo et al., 2010). In this case, some variables such as vent flow (75·mL min^{-1}), purge flow (75 mL·min^{-1}), splitless time (1.5 min), and injection volume (45 µL) were fixed. An experimental design approach was used to optimize the injection speed (v_{inj}, µL·s^{-1}), cryo-focusing temperature (T_{cis}, °C) and vent time (t_{vent}, min).

A CCD was performed using Statgraphics (Centurion XV) in order to establish the best working conditions for the assessed variables in the following ranges: cryo-focusing temperature (15-70 °C), injection speed (2-6 µL·s^{-1}) and vent time (0.4-5.5 min). The design matrix, involving 18 randomised experiments and the responses (as chromatographic peak areas) are summarised in Table 5.1. The precision of the measurements was estimated from the four replicates of the central point (RSD % values for the all the analytes were between 2 and 4 %).

According to MLR models, injection speed was not significant ($p>0.05$) at the studied range so it was fixed at 6 µL·s^{-1}. The response surfaces were built against the other two variables, as shown in Figure 5.8 for ATII and AHMI. As it can be seen, lower values of vent time (i.e. 0.5 min) provided the best responses for all the analysed target compounds, whereas the T_{cis} optimum values varied from low temperatures (15 °C for

ADBI, AHMI and MA) to medium temperatures (25 °C for AHTN, ATII, HHCB and MK). Hence, it was decided to fix T_{cis} at a consensus value of 20 °C.

*Table 5.1. Central composite design matrix and the responses obtained for the target compounds. A: Cis T, B: Vent Time, C: Injection speed. The replicates of the central point are marked with an *.*

Optimised Variables				***Responses (as chromatographic peak areas)***						
Exp.	A	B	C	ADBI	AHMI	AHTN	ATII	HHCB	MA	MK
1*	42.5	3	3.5	730716	1054000	930785	1777978	1205662	313190	475609
2*	42.5	3	3.5	751578	1080863	943149	1809286	1219162	327407	496188
3	25	4.5	2	1007387	1272695	935532	1756650	1226905	293654	424243
4	25	1.5	5	1463033	1768759	1221477	2383787	1628180	480488	592267
5	42	0.5	3.5	1499694	1827426	1233986	2385578	1658063	510631	611749
6	60	4.5	2	234437	360954	414969	838122	500795	87317	297873
7*	42	3	3.5	771926	1112881	991842	1872195	1247493	338399	519463
8	42	3	1	864906	1169032	894466	1754657	1179110	279277	439336
9	60	1.5	5	568992	895681	932896	1766250	1133142	304302	559267
10	60	1.5	2	685791	1059607	1060147	2041530	1277399	367240	609813
11	42	3	6	770658	1130117	996317	1900617	1287111	217425	538361
12	25	4.5	5	1049472	1321295	944324	1809502	1245862	156027	470290
13	13	3	3.5	1300407	1567218	1086824	2046219	1416719	380046	531333
14	42	5.5	3.5	285118	507098	631329	1212456	801632	N.A	381539
15	72	3	3.5	186987	278604	320742	635059	380908	73947	257694
16	60	4.5	5	174744	275184	346707	655408	420659	36596	259728
17	25	1.5	2	1423548	1717982	1162255	2237615	1558125	245605	579766
18*	42	3	3.5	792105	1145293	974295	1875613	1256414	234567	527515

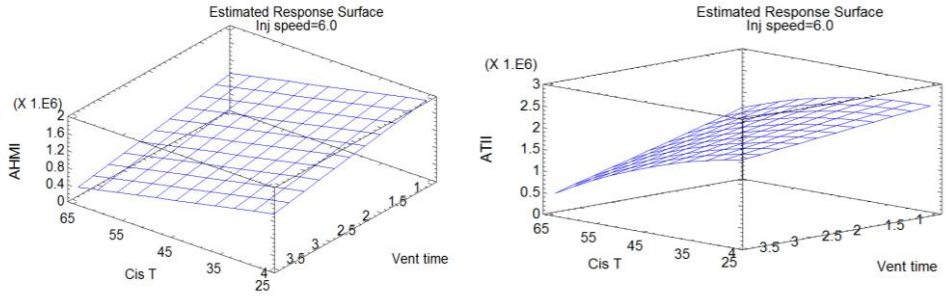

Figure 5.8. Response Surfaces obtained for AHMI and ATII using significant variables parameters (p-value < 0.05): T_{cis} and t_{vent}. Injection speed was fixed at 6 µL·s^{-1}.

Briefly, according to the optimised values, the PTV-LVI values were established as it follows: cryo-focussing temperature is maintained at 20°C in order to inject 45 µL of Hex extract at 6 µL·s^{-1} and the solvent is vented at 75 mL min^{-1} and 5 psi pressure during 0.5 min. Afterwards, the vent valve is closed during 1.5 min while the analytes are quantitatively introduced into the column. After an elapse time of 2 min the vent valve is re-opened and the injector is purged at 75 mL·min^{-1} in order to avoid possible cross-over contamination effects.

5.2.3 Figures of merit

The main features of the optimised MASE-LVI-PTV-GC-MS are summarised in Table 5.3. The calibration curves were built from 10 to 200 ng·L^{-1} in 150 mL of Milli-Q water and also with a set of 6 standards containing concentrations ranged from LOQ to 100 ng·mL^{-1}. [$^2H_3$] AHTN and [$^2H_{15}$] MX were used as surrogates and they were calibrated in order know and correct the recoveries of the target compounds. Linearity was good for all the musks under study obtaining coefficients of determination (r^2) higher than 0.999.

The precision in the chromatographic response was determined in terms of repeatability at low (15 ng·mL^{-1}) and intermediate (75 ng·mL^{-1}) calibration levels for 3 replicates analysed within a day. The RSD % values ranged from 9 to 20 % and from 6 to 9 % for low and high concentration levels, respectively. The precision of the method was also evaluated for spiked Milli-Q water at 100 ng·L^{-1}, obtaining RSD % values between 13 to 22 % for all the target compounds.

Limits of detection (LODs) were calculated as the average signal (n=5) of the blank samples plus three times their standard deviation. LODs were obtained in the very low ng·L^{-1} range, from 3 ng·L^{-1} for ADBI to 8 ng·L^{-1} for ATII. The method detection limits (MDLs) were calculated after spiking effluent WWTP water samples at the corresponding LOD for each analyte following the procedure given by US Environmental Protection Agency (EPA)[2]. The obtained values are in the range of 4 ng·L^{-1} for AHMI and 25 ng·L^{-1} for MX, which are in good concordance with those found in the literature (Arbulu et al., 2011; Basaglia et al., 2011; Gómez et al., 2009; Ramírez et al., 2011; Regueiro et al., 2009; Silva and Nogueira, 2010) (see Table 5.3).

Extraction efficiency and apparent recovery were calculated for spiked Milli-Q water at the 100 ng·L^{-1} concentration level (see Table 5.2). Extraction efficiency was calculated by comparing the spiked concentration with the concentration obtained from external standard calibration. Extraction efficiencies were between 16 % for DPMI and 38 % for AHTN.

[2] US EPA ,'Title 40 of the US Code of Federal Regulations, Part 136, Appendix B,' https://www.gpo.gov/fdsys/granule/CFR-2011-title40-vol23/CFR-2011-title40-vol23-part136-appB/content-detail.html (last retrieved November 2016)

Table 5.2. Main method parameters for the MASE-LVI-PTV-GC-MS procedure.

	PTV-LVI-GC-MS		***MASE-PTV-LVI-GC-MS***					
	Repeatability						***Apparent Recovery*** *(%)*	
Analyte	(RSD %, n=3) (15 ng mL^{-1})	(RSD %, n=3) (75 ng mL^{-1})	***LODs*** *(ng L^{-1}, n=5)*	***MDLs*** *(ng L^{-1}, n=4)*	***Repeatability*** *(%RSD, n=3) (100 ng L^{-1})*	***Extraction Efficiency***[b] *(%)*	*Corrected with surrogate*[c]	*Procedural calibration*[d]
ADBI[f]	9	7	3	4	16	20	81	103
AHMI[f]	9	6	4	4	17	19	79	102
AHTN[f]	2	8	8	24	18	38	80	108
ATII[e]	9	8	3	10	13	19	80	98
DPMI[f]	20	9	5	11	21	16	64	83
HHCB[f]	18	8	4	10	22	20	83	90
MA[e]	13	7	-[a]	7	21	-[a]	59	97
MK[e]	14	8	4	15	21	28	83	96
MM[e]	-[a]	-[a]	3	9	-[a]	41	127	-[a]
MX[e]	-[a]	-[a]	6	25	19	21	87	89

a) No data available
b) Amount extracted to the organic phase during MASE
c) Recovery after correction with the corresponding deuterated analogue
d) Recovery using calibration curve built with spiked Milli-Q
e) Compound corrected with [$^2H_{15}$] MX
f) Compound corrected with [$^2H_3$] AHTN

Table 5.3. Summary of sample type, method, LOD and precision of previous works.

Compound	Matrix	Method	LOD (ng L^{-1})	RSD (%)	Reference
ADBI	MilliQ water	SBSE GC-MS	6	3.7	(Arbulu et al., 2011)
	MilliQ water	SBSE GC-MS	0.06	9.6	(Ramírez et al., 2011)
	Wastewater	LLE GC-MS	7	6	(Gómez et al., 2009)
AHMI	MilliQ water	SBSE GC-MS	1.5	9.4	(Arbulu et al., 2011)
	MilliQ water	SBSE GC-MS	0.02	6.5	(Ramírez et al., 2011)
	Wastewater	LLE GC-MS	17	4	(Gómez et al., 2009)
AHTN	Distilled water	MASE GC-MS	20	6	(Einsle et al., 2006)
	MilliQ water	SBSE GC-MS	1.5	18.6	(Arbulu et al., 2011)
	MilliQ water	SBSE GC-MS	0.03	7.9	(Ramírez et al., 2011)
	Wastewater	LLE GC-MS	16	8	(Gómez et al., 2009)
ATII	MilliQ water	SBSE GC-MS	6	23.5	(Arbulu et al., 2011)
	MilliQ water	SBSE GC-MS	0.05	9.5	(Ramírez et al., 2011)
	Wastewater	LLE GC-MS	18	7	(Gómez et al., 2009)
DPMI	MilliQ water	SBSE GC-MS	6	9.6	(Arbulu et al., 2011)
	MilliQ water	SBSE GC-MS	0.10	10.1	(Ramírez et al., 2011)
HHCB	Distilled water	MASE GC-MS	20	6	(Einsle et al., 2006)
	MilliQ water	SBSE GC-MS	24	23.0	(Arbulu et al., 2011)
	MilliQ water	SBSE GC-MS	0.30	10.3	(Ramírez et al., 2011)
	Tap water	SPME GC-MS	1	0.058	(Basaglia et al., 2011)
	Wastewater	LLE GC-MS	17	7	(Gómez et al., 2009)
MA	MilliQ water	SBSE GC-MS	24	21.2	(Arbulu et al., 2011)
MK	MilliQ water	SBSE GC-MS	6	18.9	(Arbulu et al., 2011)
	MilliQ water	SBSE GC-MS	0.05	6.3	(Ramírez et al., 2011)
	Tap water	SPME GC-MS	0.9	0.044	(Basaglia et al., 2011)
	Wastewater	LLE GC-MS	21	4	(Gómez et al., 2009)
MM	MilliQ water	SBSE GC-MS	24	23.3	(Arbulu et al., 2011)
	MilliQ water	SBSE GC-MS	0.06	4.3	(Ramírez et al., 2011)
MX	MilliQ water	SBSE GC-MS	3	22.6	(Arbulu et al., 2011)
	MilliQ water	SBSE GC-MS	0.05	9.8	(Ramírez et al., 2011)
	Tap water	SPME GC-MS	280	0.007	(Basaglia et al., 2011)
	Wastewater	LLE GC-MS	1	6	(Gómez et al., 2009)

Taking into account the suggested definitions of recoveries and apparent recoveries (Burns et al., 2002), we have used two experimental approaches. In the first one, the apparent recovery was calculated correcting the extraction efficiency with the recovery of the corresponding deuterated analogue added before the extraction. The obtained results were acceptable for all target compounds (around 80%, except for DPMI and MA 60%). It is worth mentioning that in those experiments in which not fresh [$^2H_3$]-AHTN was used, very high recoveries for its analogue AHTN were observed (up to 229 %). [$^2H_3$] AHTN is produced via proton exchange, however, this reaction may be reversed giving the original undeuterated product (Bester, 2005). Therefore, this deuterated compound could introduce interferences. In this sense [$^2H_{15}$]-MX does not undergo any reaction itself, so it can be considered as a better surrogate compared to [$^2H_3$] AHTN. The second approach consisted on the determination of the apparent recoveries by comparing the spiked concentration of the target compound with the concentration obtained from calibration curve built with Milli-Q spiked water samples. In this case, the obtained recoveries were good, even not using deuterated analogues (values between 83 % for DPMI and 108 % for AHTN). Since the recoveries obtained using both approaches are comparable the first one was chosen as faster and easier calibration method for routine analysis.

5.2.4 Evaluation of the matrix effect

The influence of the matrix in real water samples, such as suppression or enhancement of analyte signal in matrix solution, must be considered in order to assure the accuracy of the method. In environmental water samples substantial levels of dissolved organic matter (DOM) (e.g. DOM of 125 mg·L^{-1} can be often found in effluents of WWTPs) can interfere in the extraction of the compounds to the organic solvent, resulting in poorer extraction yields (Einsle et al., 2006).

Among different strategies to solve this drawback several approaches have been suggested in the literature, such as matrix matched calibration, sample dilution, the clean-up of the extracts or the use of deuterated analogues (Jeanneau et al., 2011; Quintana et al., 2007; Rodil et al., 2009), being the last one the most widely accepted. Since high concentration of some musk compounds in WWTPs are expected and thus, the extraction yield may change from sample to sample, the use of matrix matched calibration was initially discarded. Some authors use sample dilution but in order to avoid the loss of sensitivity, the use of isotopically labeled compounds as surrogate standards was evaluated.

Figure 5.9. Extraction vessels containing different concentrations of humic acids.

Preliminary experiments were carried out analysing the matrix effect with synthetic Milli-Q water samples spiked with different amount of humic acids (0, 50, 100, 250, 500 and 1000 $mg \cdot L^{-1}$). The matrix effect was evaluated by comparing responses of analytes in spiked blank water (at 100 $ng \cdot L^{-1}$) with those obtained for target compounds in

presence of humic acids and without any further correction. The assays were performed in triplicate, and to assure the interaction of the target compounds with the synthetic matrix, samples were spiked and stirred for 90 min before performing the extraction (see Figure 5.9). Figure 5.10a shows the significant decrease of extraction efficiency of all target compounds in the presence of high concentration of humic acids. Satisfactory corrections were observed when [$^2H_3$]-AHTN was used for the correction of ADBI, AHMI, AHTN and HHCB, and [$^2H_{15}$]-MX for ATII, DPMI, MA, MK, MM and MX respectively (see Figure 5.10b). The corrected recoveries were within 80-120%, and a more precise way to estimate the concentration can be concluded.

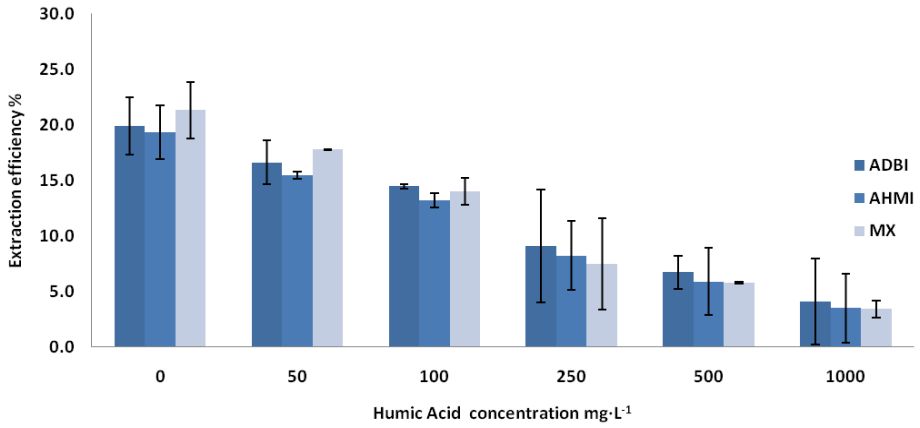

Figure 5.10a. Extraction efficiency (n=3, 95 % confidence level) at different concentrations of humic acids.

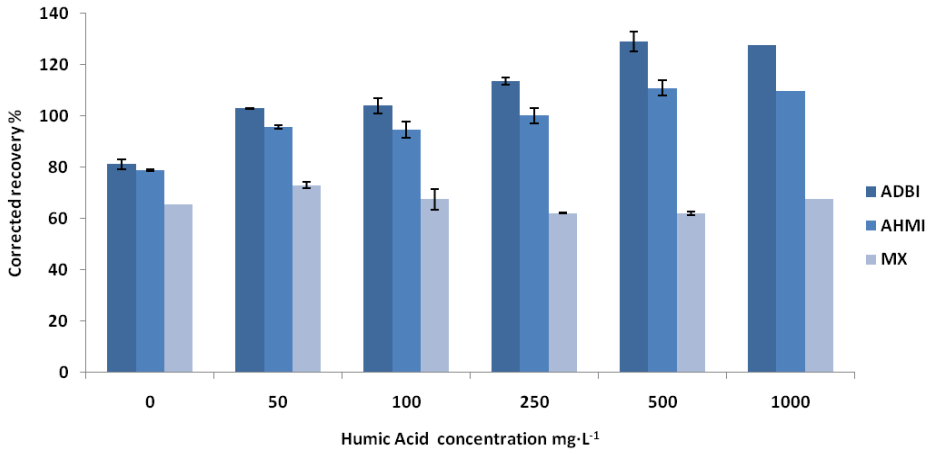

Figure 5.10b. Corrected recoveries (n=3, 95 % confidence level) of ADBI, AHMI and MX using corresponding deuterated analogue (see Table 5.2 for details of deuterated compound).

Matrix effect was also evaluated in real environmental samples such as surface water and wastewater in order to evaluate signal suppression or enhancement due to co-eluting matrix constituents also present in the samples extracts. Thus, three replicates of each type of sample were spiked at 100 ng·L^{-1} of each compound, and labelled surrogates were also added. Extraction efficiency (without correction with surrogates), matrix effect (comparing the obtained concentration with those obtained with spiked Milli-Q water at the same concentration level) and corrected recovery (corrected with surrogates) were evaluated.

Table 5.4. Extraction efficiency, matrix effect and corrected recovery of surface water and WWTP effluent and influent water samples.

Environmental water sample	***Compound***	***Extraction efficiency*** [a] ***(%)***	***Matrix effect*** [b] ***(%)***	***Corrected recovery*** [c] ***(%)***
Surface water (Gernika)	ADBI[e]	39	129	69
	AHMI[e]	37	128	74
	AHTN[e]	38	112	80
	ATII[f]	32	123	64
	DPMI[f]	34	152	106
	HHCB[e]	38	123	70
	MA[f]	32	144	138
WWTP effluent	ADBI[e]	19	48	126
	AHMI[e]	23	57	50
	AHTN[e]	87	75	63
	ATII[f]	12	30	71
	DPMI[f]	3	16	118
	HHCB[e]	385	86	85
	MA[f]	36	100	76
WWTP influent	ADBI[e]	64	164	77
	AHMI[e]	61	156	74
	AHTN[e]	253	284	80
	ATII[f]	36	110	88
	DPMI[f]	103	384	124
	HHCB[e]	420	661	17
	MA[f]	56	170	70
	MX[f]	-	153	-

a) Amount of analyte extracted to acceptor organic phase.

b) Extraction efficiency in real sample / extraction efficiency in Milli-Q water.

c) Recovery after correction with the corresponding deuterated analogue.

e) Corrected with [$^{2}H_{3}$]-AHTN.

f) Corrected with [$^{2}H_{15}$]-MX.

Table 5.4 summarises the results obtained for these assays. Matrix effects were not very remarkable in surface water sample, since both Milli-Q and surface water presented comparable extraction efficiencies. A tolerable enhancement was revealed for DPMI (152 %), but acceptable recoveries were obtained after correcting with labelled compounds (above 65 % for all target compounds).

However, the effect of the sample matrix was more evident in wastewater samples, especially in influent water of WWTPs. In the case of effluent water, a decrease of extraction efficiency was observed for all the target analytes except for HHCB for which recoveries exceeded 100 %. Nevertheless, matrix effect was notably corrected after using deuterated analogues. More attention must be paid to samples corresponding to influent waters of WWTPs. The influence of matrix effect was evidenced in the enhancement of chromatographic responses and thus, recoveries higher than those observed in spiked Milli-Q water. In order to minimise this matrix effect, the results were corrected with [$^2H_3$]-AHTN and [$^2H_{15}$]-MX deuterated analogues.

5.2.5 Application of Membrane assisted solvent extraction to real samples

The developed MASE-LVI-PTV-GC-MS method was applied to real samples in order to check its feasibility in the determination of ten synthetic compounds in four different types of water samples (influent and effluent of WWTPs, estuarine water and drinking water) in triplicate (n=3, 90% of confidence level).

Two synthetic musks were detected in all the samples studied: galaxolide (HHCB) and tonalide (AHTN). HHCB was the main musk found in all the cases and its concentration ranged from 41±7 ng·L^{-1} in surface water of the estuary of Urdaibai to 295±43 ng·L^{-1} in WWTP influent from Galindo, whereas the highest value observed for AHTN was 138±12 ng·L^{-1} in WWTP influent from Galindo. These two musk fragrances are described in the literature as the most commonly detected musk compounds in water

samples(Peck and Hornbuckle, 2004; Pietrogrande and Basaglia, 2007; Reiner et al., 2007; Reiner and Kannan, 2011). Besides these two compounds, ADBI (25±9 $ng \cdot L^{-1}$) and MK (24±7 $ng \cdot L^{-1}$) were also detected in WWTP influent from Galindo. Regarding the concentrations of the target compounds found in the effluents from Galindo, both HHCB (259±54 $ng \cdot L^{-1}$) and AHTN (82±6 $ng \cdot L^{-1}$) were also detected. The latter results can confirm the fact that most of the WWTPs are not efficient enough removing synthetic musks as it has been pointed in the literature (Matamoros and Bayona, 2008; Pietrogrande and Basaglia, 2007; Reemtsma et al., 2006; Reiner et al., 2007; Simonich, 2005).

5.3 Conclusions

In order to provide an environmentally friendly method to quantify synthetic musks in water samples (estuarine, influent and effluent water of WWTPs), a MASE coupled to LVI-PTV-GC-MS has been fully optimised. The use of low extractant volumes allows the direct analysis of the extracts avoiding previous steps in which volatile analytes can be lost. The easy performance makes the developed method interesting for routine analysis in monitoring programs. Furthermore, the combination of MASE and PTV-LVI-GC-MS provides method detection limits of 8-24 $ng \cdot L^{-1}$ which enables detecting analytes at low $ng \cdot L^{-1}$ levels.

The developed method was applied to real water samples and the matrix effect was evaluated. While estuarine samples are not highly affected, the matrix effect observed in wastewater samples can be corrected using deuterated analogues. HHCB and AHTN are the main two synthetic musks observed in most of the analysed samples. This supports that the elimination of these compounds is not effective enough being necessary further monitoring strategies. Although HHCB and AHTN are not included in regulatory monitoring, they are listed since 2011 in the Norman list of emerging substances[3].

[3] http://www.norman-network.com/?q=node/19 (last retrieved November 2016)

5.4 References

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6. PDMS BASED EXTRACTION METHODS

In this chapter it is described the analysis of non-polar contaminants including a number of organic compounds typically found in environmental matrices such as organochlorine and organophosphorous pesticides, phthalates, phenols and personal care products (Green et al, 2003; OSPAR, 2009).

The choice of PDMS as the extracting phase allows a simple procedure based on the absorption of the target contaminants regardless of the final shape of the extracting device. From all the different options, we have used the stir-bars (SBs), since they are commercially available and allow a fast and direct thermal desorption in a thermal desorption unit (TDU) before being analysed in a GC-MS system, and silicone rods (SRs) because they have a larger mass (or volume phase) allowing a higher depletion in the extraction step, though it requires a liquid stripping being injected in the LVI-PTV-GC-MS system. The study of both devices would be also useful to tackle the overall procedure as passive samplers, as it will be detail in chapters 8 and 9.

In this chapter the optimisation of the extraction procedure is described. This optimisation was carried out in three steps: the extraction, and re-extraction when required, the instrumental set-up and the quantification in real samples. In the first two steps different types of experimental designs were used to fine tune most of the key variables. In the case of the quantification the used of deuterated surrogates to assess the recoveries was deeply considered.

Finally, as it was considered in the previous chapter, the main benchmark for this kind of methods is to evaluate its performance in WWTP effluents.

6.1 Experimental

6.1.1 Sampling

In order to test the performance of the extraction methods using PDMS to analyse non-polar organic contaminants in real environmental waters, effluent water of an urban wastewater treatment plant (WWTP) was analysed. Effluent water sample was collected in July 2012 at the metropolitan WWTP of Bilbao, which is the largest WWTP in the Basque Country collecting industrial and urban wastewater from ca. 1 million inhabitants. Samples were collected in pre-cleaned amber glass bottles and carried to the laboratory in cooled boxes (4°C). After collection, samples were filtered through 0.45 µm, stored at 4°C before treatment and analysed within 48 hours.

6.1.2 Silicone Rod Sorptive Extraction

The scheme of the SR extraction procedure is illustrated in Figure 6.1. The pre-concentration of the analytes from water samples was performed using the SRs in 150 mL conventional headspace glass vials. The optimisation of the extraction procedure was performed using 150 mL of ultra-pure water spiked at 100 $ng \cdot L^{-1}$ of each target compound. Deuterated analogues used as surrogates were added prior to the extraction, SRs were immersed in water sample and the vials were sealed with PTFE septa and aluminium crimp caps. The sorption of the target analytes was performed overnight at room temperature and at 900 rpm using a magnetic 15 position stirring plate from Gerstel.

Once the sorption step was over, the SRs were removed and rinsed with ultra-pure water and dried with a clean tissue. The factors affecting liquid desorption (LD) of the sorptive material were also evaluated, i.e., the effect of stripping solvent nature and the effect of sonication mechanism in the desorption step. To accomplish this, the SRs were placed into a 500 µL safe-lock Eppendorf® tube (Eppendorf Ibérica S.L.U., Madrid) filled with 200 µL of an appropriate solvent and assuring the complete immersion of the

SR. The effectiveness of ultrasounds bath (USB, Axtor by Lovango) and focussed ultrasound cup boosters (FUSB, Cup booster BR 3.0, Bandeling, Berlin, Germany) was evaluated. Under optimal conditions, the SR was desorbed with 200 µL of EtOAc for 16 min using ultrasounds bath (2 x 8 min). Finally, the SR was removed and the extract obtained was analysed by means of LVI-PTV-GC-MS (see section 3.4.3 for measurement conditions).

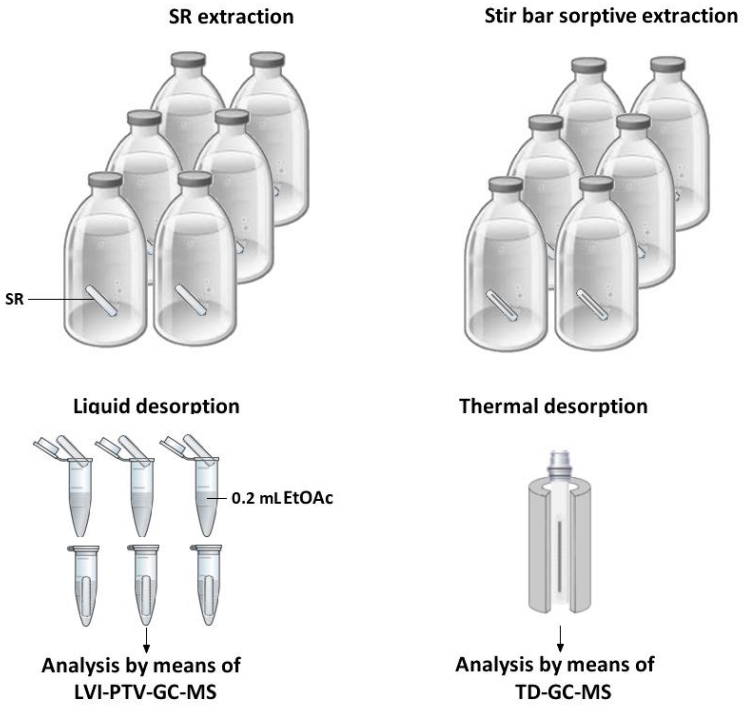

Figure 6.1. Scheme of the SBSE and SR extraction procedures.

6.1.3 Stir bar sorptive extraction

The SBSE process is also shown in Figure 6.1. As in the previous case, the extraction procedure was carried out in 150 mL glass vials using SBs of 20 mm length and 0.5 mm membrane thickness. The glass vials were sealed with PTFE septa and aluminium

crimp caps. Ultrapure water samples spiked at 100 ng·L^{-1} were used for optimisation purposes whereas deuterated analogues used as surrogates were added prior to the extraction to evaluate matrix effect. Under optimum conditions, the extraction was carried out at room temperature (22 °C) overnight at 900 rpm, and neither MeOH nor NaCl were added to modify the extractability of the target compounds. After the extraction, the SBs were removed from the water samples, dried using a lint free tissue, and stored in chromatography vials at -20 °C until the analysis. As it was mentioned, the SBs were directly desorbed and analysed in the TD-GC-MS system (see section 3.4.4 for details on the measurements conditions).

6.2 Results and Discussion

6.2.1 Optimisation of chromatographic methods

Prior to the determination of the optimal parameters for the extraction of water samples, the optimisation of chromatographic methods was carried out by means of design of experiments in order to obtain the highest sensitivity during the analysis step.

6.2.1.1 Optimisation of LVI-PTV-GC-MS

As it was proceeded in the previous chapter, to study the efficiency of LVI some variables were fixed (vent flow: 75 mL·min^{-1}, purge flow: 75 mL·min^{-1}, splitless time: 1.5 min and injection volume: 45 µL) according to a previous work (Vallejo et al., 2010a), and only the injection speed (v_{inj}, µL·s^{-1}), cryo-focussing temperature (T_{cis}, °C) and vent time (t_{vent}, min) were optimised. To achieve the maximisation of the chromatographic peak area of the target compounds (100 ng·L^{-1} in EtOAc) a Central Composite Design (CCD) was carried out using the Statgraphics software (Centurion XV), covering the following factor space: v_{inj} (1 – 6 µL·s^{-1}), T_{cis} (10 – 80 °C) and t_{vent} (0.5 – 5 min). The precision of the measurements was estimated from the four replicates of the central point (RSD %) and most of them were between 3-10% for all target compounds except for 4nOP).

The analysis of the results by means of the analysis of variance (ANOVA) and response surfaces showed that the injection speed was not significant at 95% of confidence level ($p > 0.05$) for any of the studied analytes. This parameter was consequently fixed at an intermediate value, i.e., 3.5 $\mu L \cdot s^{-1}$. T_{cis} and t_{vent} variables showed a significant effect on the chromatographic peak areas, so responses surfaces were built using these two variables. Figure 6.2 illustrates the response surfaces obtained for one analyte of each family. Vent time was significant for most of the analytes studied, getting the highest value at an intermediate value, i.e., 3 min. The cryo-focussing temperature was significant for some pesticides, musk compounds and HCH isomers, for which the highest yields were obtained at low temperatures. Eventually, it was fitted at 50 °C, as a consensus between the best chromatographic signals and N_2 (l) consumption. The values obtained are in good agreement with the optimum values reported for compounds with similar characteristics in works described elsewhere (Bizkarguenaga et al., 2012).

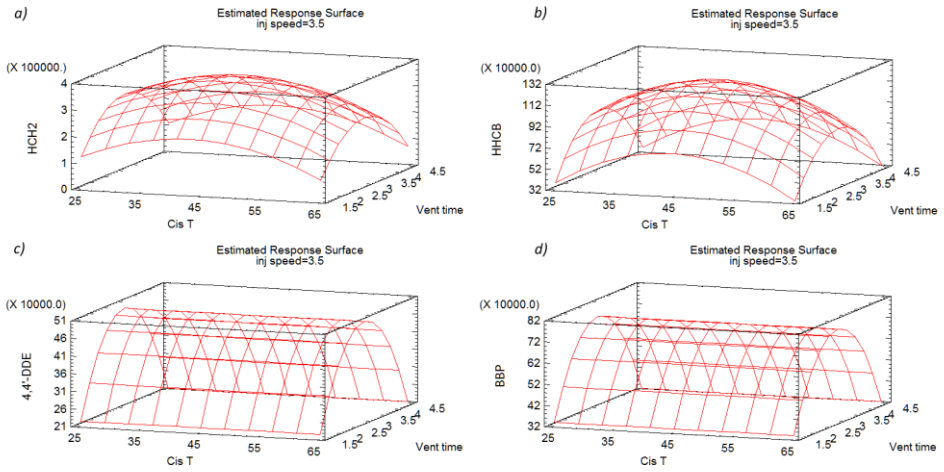

Figure 6.2. Response surface obtained for (a) β-HCH, (b) HHCB, (c) 4,4'-DDE and (d) BBP using significant variables (p-value < 0.05): T_{cis} and t_{vent}. Injection speed was 3.5 $\mu L \cdot s^{-1}$.

6.2.1.2 Optimisation of TD-GC-MS

Owing to the complexity of the instrumental system, the optimisation of TD-GC-MS parameters was conducted in two steps. Initially the variables involved in the cryo-trapping process were analysed while thermal desorption variables were fixed. Then, the parameters affecting thermal desorption were assessed using the previously optimized values of cryo-focussing step.

In both case, the chromatographic peak area of the target compounds was used as the responses in the CCD. Once again, the precision of the measurements was evaluated based on the four replicates of the central point. In the case of the cryo-trapping process the RSDs ranged between 1 and 12% and in the thermal desorption between 2 and 8% respectively (except for DEHP).

In the first step, where parameters related to CIS were optimised, thermal desorption temperature (T_{TDU}, °C) and CIS cryo-focusing hold time (t_{CIS}, min) were fixed at 300 °C and 5 minutes respectively. Then, CIS cryo-focusing temperature (T_{CIS},°C), vent flow (v_{flow}, mL·min^{-1}) and vent pressure (v_{press}, psi) were studied by means of a new CCD in the following ranges: -50°C to 10°C for T_{CIS}, 50 mL·min^{-1} to 100 mL·min^{-1} for v_{flow} and 5.37 psi to 11.40 psi for v_{press}. More in detail, the most significant parameter was the vent pressure, which affected negatively to musk fragrances, DDT related pesticides, 4nOP and phthalates. Then, vent flow affected positively musk fragrances and 4nOP, and in contrast TCIS did not affect significantly any of the compounds under study except phthalates. Therefore, T_{CIS} was set at 0°C, an intermediate v_{press} at 6.6 psi (which corresponds to the half of the head pressure of the column), and a large v_{flow} at 100 mL·min^{-1} as it was not significant or with slight positive effect for some compounds.

Once CIS related parameters were optimised, parameters affecting thermal desorption of stir bars were studied. Hence, desorption temperature was studied in the

range of 190°C to 309°C and CIS cryo-focusing hold time was evaluated in the range of 2.05 min to 11.95 min. According to the results, larger values of cryofocusing time favors the sensitivity for DDT related analytes, Chlorf and BBP. The same happened using larger desorption temperatures for 4nOp, AHTN, organophosphorous pesticides, DDT related pesticides and phthalates. Thus, the best performance of the desorption method was achieved setting up the T_{TDU} and t_{CIS} values at 300 °C and 5 min, respectively.

6.2.2. Optimisation of the liquid desorption of SRs

In contrast to the SBSE method, where stir bars are directly desorbed and analysed in the TD-GC-MS without any pretreatment, SRs required a previous chemical desorption to be analysed by means of LVI-PTV-GC-MS.

MeOH, AcN or aqueous buffers are the most widely used solvents for LD of semi-volatile compounds from SR since a high swelling of the sorptive material is avoided (Prieto et al., 2010). However, the use of non-polar solvents, more suitable for LVI-PTV-GC-MS analysis such as cHex or EtOAc, is also possible because the swelling of the material does not affect their desorption characteristics (Rusina et al., 2007; Silva and Nogueira, 2010; van Pinxteren et al., 2010). Therefore, the efficiency of Hex, cHex and EtOAc as stripping solvents was compared. As shown in Figure 6.3, EtOAc offered the best desorption abilities because it yielded the maximum extraction efficiency, and, at the same time, it showed a lower swelling behaviour, acceptable chromatographic peak shapes and precision (RSD < 15%). Therefore, the SRs were desorbed with EtOAc in the following steps.

Although the LD times at room temperature range often from 15 to 30 min, this time can be reduced by applying external energy sources such as shaking, sonication or increasing the temperature (Prieto et al., 2010). Ultrasound baths are mostly used for LD purposes (Prieto et al., 2010; Silva and Nogueira, 2010), but the use of other systems such as focussed ultrasonic cup boosters (FUSB) can enhance the repeatability of the process

focusing the ultrasound energy to each sample separately in a small ultrasonic bath (<15 mL) (Vallejo et al., 2010b). On this basis, each SR (previously exposed to Milli-Q water samples spiked at 100 ng·L^{-1}) was desorbed three times successively with 200 µL of fresh EtOAc by means of USB during 16 min and FUSB during 15 seconds (Vallejo et al., 2010). Desorption efficiency (%) of USB and FUSB was calculated as the relative amount desorbed in each fraction related to the total amount desorbed after three consecutive extractions.

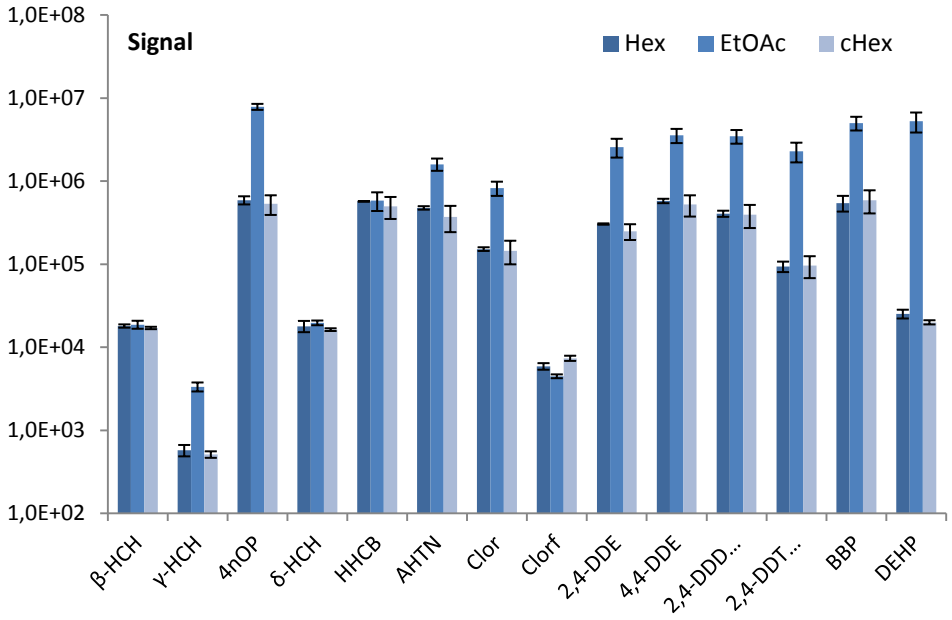

Figure 6.3. Comparison of the chromatographic average responses ((n=3, 95% confidence level) obtained for the different solvents used for liquid desorption of SRs: n-hexane (Hex), ethylacetate (EtOAc) and cyclohexane (cHex).

As shown in Figure 6.4, both sonication approaches were statistically comparable (p-value > 0.05) for all studied compounds in terms of recovery (desorption efficiency: higher than 80% and 70% in the first extraction for USB and FUSB respectively) and

reproducibility (RSD values lower than 20% and 30% for USB and FUSB respectively), which assured a high desorption in a unique step. The fact that USB allows the simultaneous desorption of many samples makes this option more affordable than the FUSB in terms of working time. Therefore, SRs were desorbed with 200 µL of EtOAc, assisted with an ultrasonic bath for 16 min (2 x 8 min) in subsequent experiments.

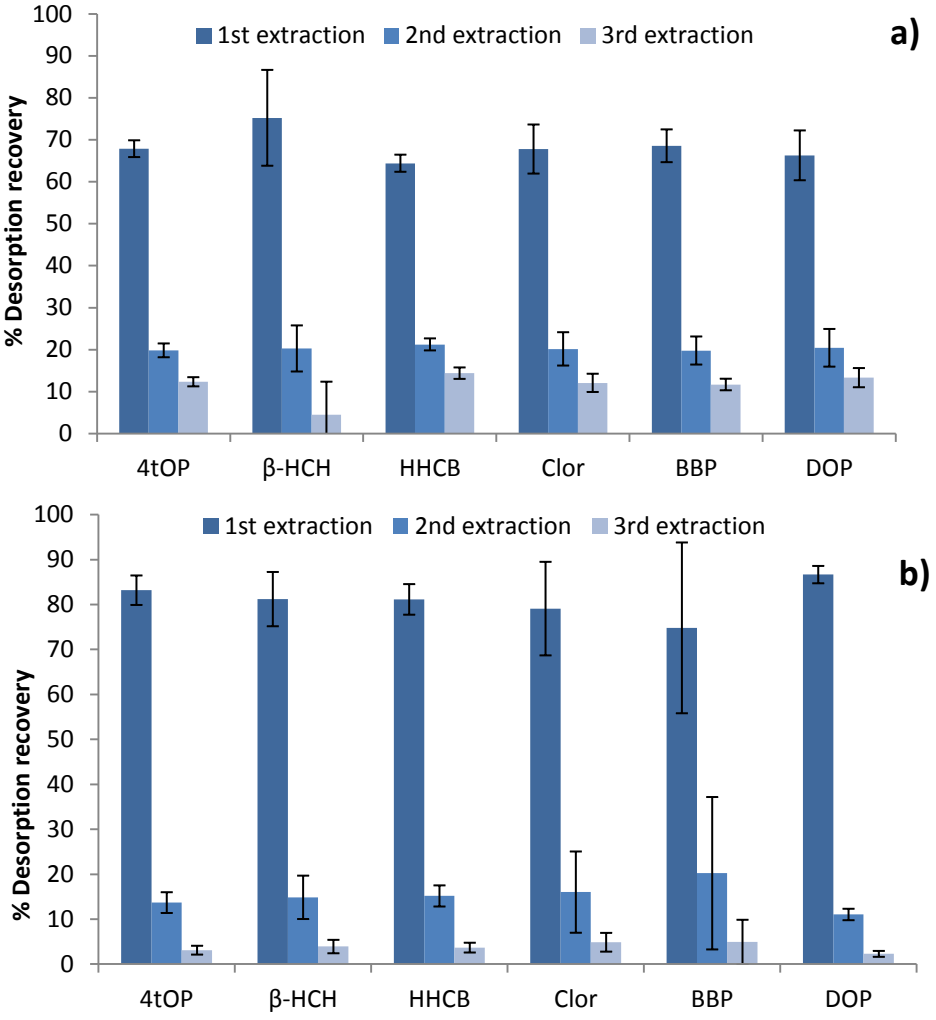

Figure 6.4. SR Desorption recovery % of three successive extractions (n=3, 95% confidence level) obtained by using: (a) focused ultrasound cup booster (FUSB) and (b) ultrasound bath (USB).

6.2.3. Optimisation of SBSE and SR extraction

In order to get the optimum extraction conditions with both extraction techniques (i.e., SBSE and SRSE), several parameters affecting the extraction efficiency were systematically tested: matrix modification by the addition of MeOH and salt, stirring rate and extraction time.

6.2.3.1 Modification of the aqueous phase

As it was also discussed in the previous chapter, the extraction of organic compounds using SR and SB might be affected by the addition of NaCl and MeOH. Consequently, the evaluation of extraction conditions was carried out using the chromatographic conditions optimised beforehand and the optimised desorption conditions for SR and SB.

First of all, the effect of the addition of an inert salt (NaCl) and an organic modifier (MeOH) in SBSE was studied simultaneously by means of a Central Composite Design (CCD) using Statgraphics (Centurion XV). As in the previous case, both variables were studied using fortified ultrapure water fortified at 250 $ng \cdot L^{-1}$ in the range of 0 – 20 % (w/v) and 0 – 20 % (v/v), respectively. The chromatographic peak areas were used as responses variables for the CCD and MLR was used to build the response surfaces. The repeatability of the experiments was estimated from the four replicates of the central point (RSD < 6 % for all the target compounds except DEHP).

Similarly to other works found in the literature (Ochiai et al., 2011; Polo et al., 2005; Quintana et al., 2007; Ramírez et al., 2011), the addition of NaCl in SBSE had a negative effect for all the compounds except for HCH isomers and Chlorf. On the other hand, the addition of MeOH showed in general a negative effect and its effect was not significant (p-level > 0.05) for musks and some DDT related isomers. As can be seen Figure 6.5 shows the calculated standardised Pareto charts for the two main factors and the

interactions for Chlorf and 4,4-DDE and the corresponding response surfaces built with significant variables. Hence, the addition of MeOH and NaCl was discarded in SBSE for further experiments.

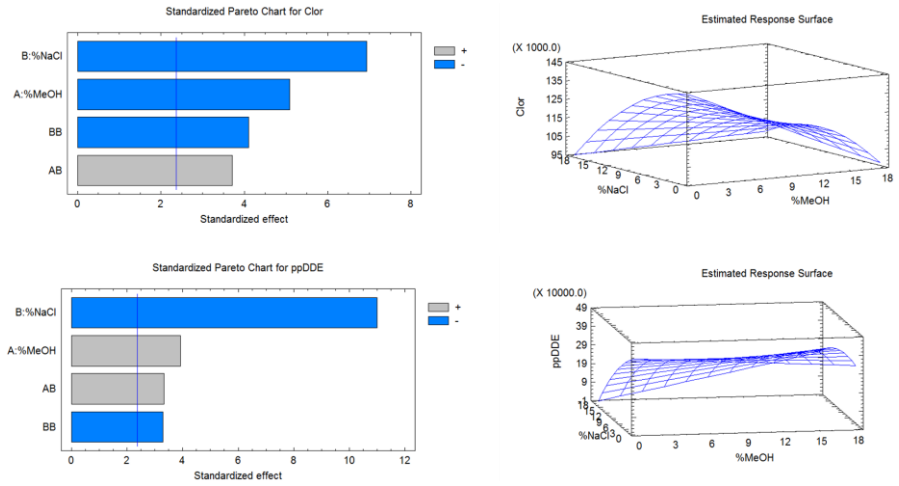

Figure 6.5. Standardised Pareto charts for the main effects and their interactions and the response surfaces obtained after CCD for Chlorf and 4,4'-DDT using significant variables (p<0.05).

Regarding to the SR extraction, based on the results obtained previously for SBSE, the addition of inert salts was not studied. Moreover, it has been suggested that the addition of NaCl reduces the extraction efficiency of non-polar compounds (log K_{ow} > 3.5) due to the increase in the viscosity of the sample, and hence, leading to slower extraction kinetics (David and Sandra, 2007; Prieto et al., 2010). In this sense, in other sorptive methods developed with environmental water samples, NaCl was not added in order to avoid the reduction of the extraction efficiency of organochlorine pesticides (Ochiai et al., 2011), polycyclic musks (Ramírez et al., 2011) and phthalates (Polo et al., 2005). On the other hand, there is still a high controversy about the addition of MeOH, and although its addition was studied for SBSE, its influence was also assessed for SR. The effect of MeOH addition was evaluated by adding MeOH at 0, 5 and 10% into 150 mL ultrapure water

spiked at 250 ng·L^{-1}. Similarly to other works found in the literature (Ochiai et al., 2011; Ramírez et al., 2011), as well as those obtained for SBSE, the increase of MeOH percentage produced a slight decrease in the response that was statistically not significant (p-value > 0.05) for all the target compounds (see Figure 6.6). Therefore, using both extraction methods, the addition of MeOH and NaCl was discarded.

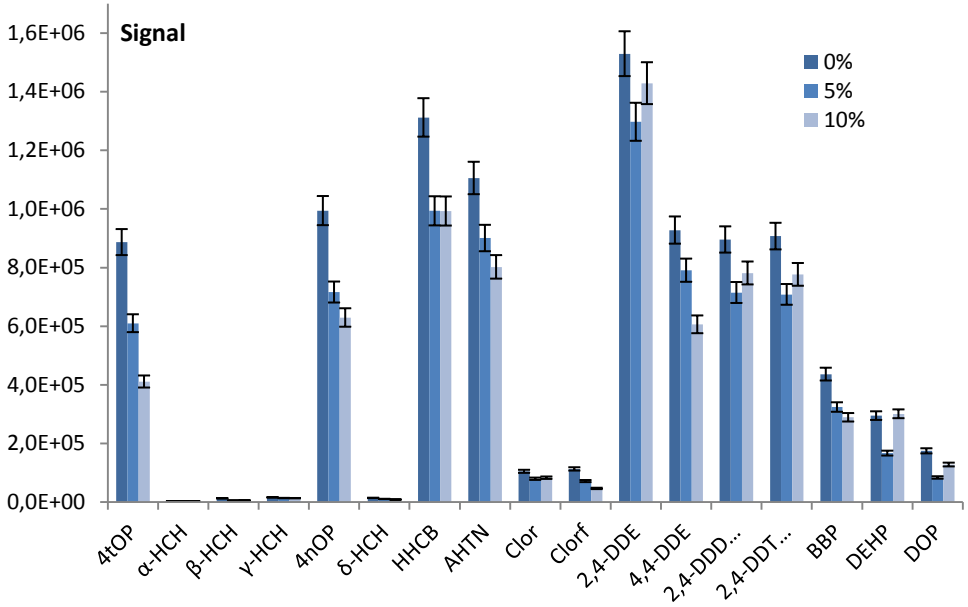

Figure 6.6. Effect of MeOH addition (0%, 5% and 10%) in the extraction of the target compounds using SRs (n=3, 95% confidence level).

6.2.3.2 Stirring rate

The stirring rate is also a variable that can speed up the performance of the extraction. In most cases, when a vigorous mixing of the sample is assured, the extraction efficiency can be enhanced due to a decrease of the thickness of the boundary layer and thus, the diffusion of molecules into the polymer is accelerated. However, too high agitation speeds, can promote bubble formation which can lead to lower extraction efficiencies. Therefore, 150 mL of fortified Milli-Q water (200 ng·L^{-1}) were extracted at

three different stirring rates, 600 rpm, 800 rpm and 900 rpm in triplicate under previously fixed extraction conditions (no addition of MeOH nor NaCl). Since, for almost all the compounds under study (except for HCH isomers and 4tOP, where no differences were observed), high stirring rates resulted in better responses both for SB and SRs (see Figure 6.7) so an agitation speed of 900 rpm was chosen in the upcoming experiments. It should be remarked that higher stirring rates would probably yield even higher recoveries, however working at rates over 900 rpm for SBs and 800 rpm for SRs was not operatively safe due to an ineffective stirring.

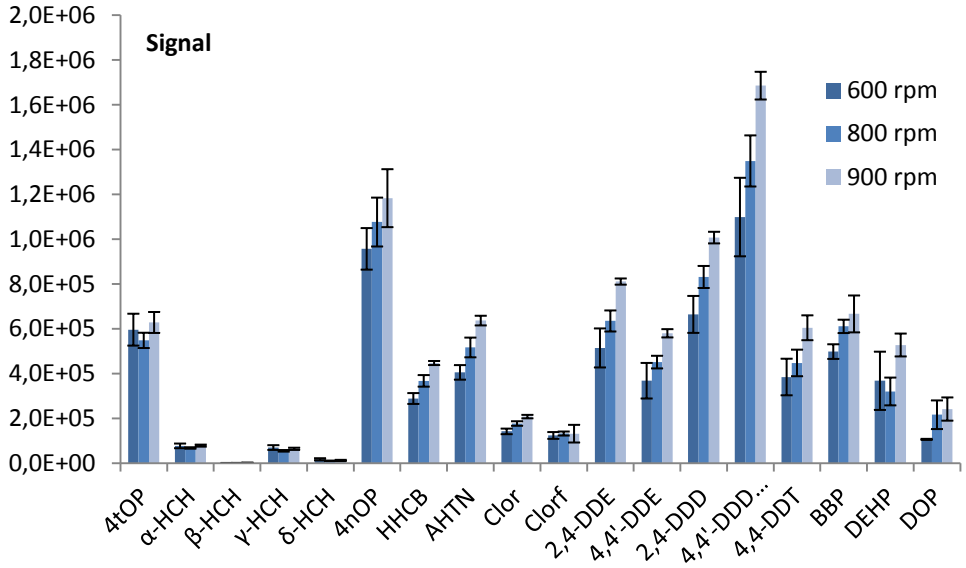

Figure 6.7. Peak areas obtained for assays performed with SBs at different stirring rates using (n=3, 95% confidence level).

6.2.3.3 Extraction time

Another important feature is the time required to reach equilibrium. For that reason, once the extraction variables were fixed, the extraction time profiles were evaluated for both for SR and SB. On the one hand, extraction times ranging from 5 to

1440 min (i.e., 15, 30, 45, 60, 90, 120, 180, 300, 420, 540, 720 and 1440 min) using 150 mL of Milli-Q water spiked at 500 ng·L^{-1} were evaluated in duplicate for SR extraction (see Figure 6.8a). On the other hand, assays using SBs were also performed in duplicate at different extraction times comprised between 5 min and 840 min (i.e., 5, 15, 30, 60, 90, 120, 180, 240, 360, 480, 840 min) using 150 mL of fortified ultrapure water (500 ng·L^{-1}) (Figure 6.8b).

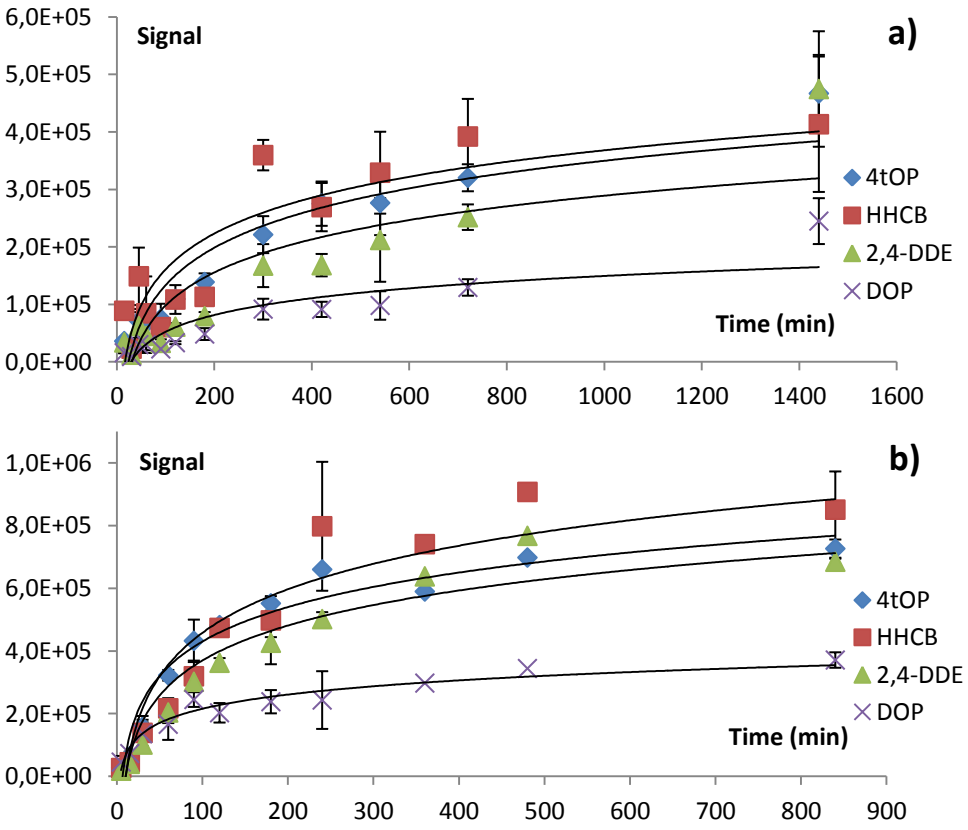

Figure 6.8 Extraction time profiles using SR (a) and SBs (b) for 4tOP, HHCB, 2,4-DDE and DOP. Assays performed under optimised conditions in duplicate (n=2, 95% confidence level).

Both in the SBSE and SR extraction, equilibrium was reached for all compounds at around 420 min (7h) and it was comparable to other works using sorptive extraction techniques (León et al., 2006; Pérez-Carrera et al., 2007; Prieto et al., 2010). Hence, further experiments were conducted overnight to ensure equilibrium conditions.

6.2.4 Figures of merit of SBSE-TD-GC-MS and SR-LVI-PTV-GC-MS

Due to the lack of certified reference materials spiked water samples were used to test the analytical features of the extraction methods developed in this study (see Table 6.1). Procedural calibration curves were performed in the range of 10 to 200 ng·L^{-1} in 150 mL of Milli-Q water, including deuterated analogues used as surrogates which were also calibrated. External calibration curves were built with a set of 6 standards containing concentrations ranged from blanks to 300 ng·L^{-1} and measured by LVI-PTV-GC-MS. In the case of SBs, the external calibration curves were built using 2 μL glass capillary tube (32 mm length, 0.0111" ID, 0.0300" OD, accuracy 1%) filled with all compounds in hexane (9 calibration points covering a concentration range of 0.25 – 150 ng), which were placed in empty desorption tubes. Linearity of external calibration curves was good for both analytical techniques and for all the target compounds. In the case of LVI-PTV-GC-MS the coefficients of determination (r^2) were close to 0.999 for synthetic musks, phthalates, pesticides and octylphenols, and close to 0.995 for HCHs respectively. On the other hand, coefficients of determination (r^2) close to 0.999 were achieved for, HCHs, phthalates, pesticides and octylphenols, and close to 0.997 for synthetic musks by means of TD-GC-MS. The linearity of procedural calibration curves was also adequate obtaining r^2 values between 0.992 and 0.998 using SRs and between 0.989 and 0.999 using SBs respectively.

The reproducibility of the whole extraction methods was evaluated using spiked Milli-Q water at 100 ng·L^{-1}. All the values were between 4 and 9 % for all the analytes extracted using SRs except for DEHP and DOP (> 15%) and between 1 and 11 % for SB assays, except for DEHP and DOP (22 and 23 % respectively). Overall, the RSD values

obtained by means of SBSE-TD-GC-MS were better than those obtained with SR-LD-LVI-PTV-GC-MS.

Extraction efficiency for SB and SR was calculated using 150 mL of Milli-Q water spiked at 100 $ng \cdot L^{-1}$ and comparing this concentration with the concentration obtained using an external calibration procedure. As summarised in Table 6.1, the extraction efficiencies for all the target analytes were significantly lower for SR (between 10 and 33% except for HCH isomers and DOP) in comparison with those obtained using SBs (between 18 and 88 %). The low extraction efficiencies for HCHs, which have Log K_{ow} in the range 3.7 and 4.3, using both techniques point towards that absorption of polar compounds is restricted for PDMS sorptive materials as it is reported in the literature (David and Sandra, 2007). On the other hand, the results obtained are in agreement with the values found in the literature (Arbulu et al., 2011; Ochiai et al., 2011; Tan et al., 2008).

The apparent recoveries were obtained by comparing the spiked concentration of the studied compounds with the concentration obtained from the procedural calibration curve (i.e., built with Milli-Q spiked water samples and submitted to the whole procedure). The apparent recoveries obtained for extractions performed using SR were between 84 and 123% for all the compounds except for DOP (131%). Regarding to the SBSE assays, the apparent recoveries were between 87 and 136 % for all target compounds except for 4tOP and DEHP.

Table 6.1. Main method parameters for the SR-LD-LVI-PTV-GC-MS and MESCO-LD-LVI-PTV-GC-MS procedures.

	SR-LVI-PTV-GC-MS					SBSE-TD-GC-MS				
Analyte	% RSD (n=3; 100ng/L)	LOD (ng/L)	MDL (ng/L, 95 %)	Extraction Efficiency (%)	Apparent Recovery (%)	% RSD (n=3; 100ng/L)	LOD (ng/L)	MDL (ng/L, 95%)	Extraction Efficiency (%)	Apparent Recovery (%)
4tOP[a]	4	1	7	11	112	3	15	11	2	150
α-HCH[a]	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
β-HCH[a]	8	26	55	7	84	10	5	18	41	97
γ-HCH[a]	6	30	25	10	108	4	2	427	29	98
δ-HCH[a]	7	65	70	4	123	8	1	552	27	136
4nOP[a]	7	1	5	19	107	2	1	16	6	97
HHCB[a]	5	11	25	20	101	2	8	57	88	116
AHTN[a]	6	15	25	19	104	2	3	18	77	97
Clor[b]	6	25	24	33	110	2	2	n.a.	27	98
Clorf[b]	8	7	8	31	118	11	61	147	58	115
2,4'-DDE[b]	6	1	2	16	114	n.a.	n.a.	n.a.	n.a	n.a.
4,4'-DDE[b]	6	1	3	10	121	1	2	3	73	92
2,4'-DDD[b]	9	1	3	22	110	2	1	11	74	90
4,4'-DDD[b]	n.a.	n.a.	n.a.	n.a.	n.a.	1	3	7	70	88
2,4' DDT[b]	8	1	3	20	109	n.a.	n.a.	n.a.	n.a.	n.a.
4,4'-DDT[b]	n.a.	n.a.	n.a.	n.a.	n.a.	1	3	2	68	87
BBP[c]	7	6	13	22	106	7	4	8	73	100
DEHP[c]	20	15	27	13	123	22	147	792	174	194
DOP[c]	14	15	9	3	131	23	6	11	18	88

n.a.- not available; [a] Corrected with [$^2H_{15}$] MX; [b] Corrected with [$^2H_8$] 4,4'DDT ; [c] [$^2H_3$] DEHP

Limits of detection (LODs) were calculated as the average signal of the Milli-Q water samples (n=5) plus three times their standard deviation. As a general rule, the LODs obtained for all the target analytes were lower than 15 ng·L^{-1} in SR extraction except for HCH isomers (between 25-65 ng·L^{-1}). Similar detections limits were achieved in the case of SBSE, ranging from 1 to 15 ng·L^{-1} except for Chlorf and DEHP. The values obtained were similar to those found in the literature for other techniques such as SPE (Cherta et al., 2012; Pitarch et al., 2007) and other works based on SBSE (Ochiai et al., 2006; Ramírez et al., 2011; Tölgyessy et al., 2011). The method detection limits (MDLs) were calculated after spiking effluent WWTP water samples at the corresponding LOD for each analyte according to US EPA guidelines[1] and extracted using SRs and SBs. As summarised in Table 6.1, the MDL values obtained after SR extraction were between 3 and 35 ng·L^{-1} for all target compounds except for HCHs (32 - 90 ng·L^{-1}). Regarding the MDLs for samples extracted using SBs, the values obtained were at the same level than those obtained by SR extraction, i.e., between 2 – 57 ng·L^{-1} (except HCH isomers and DEHP).

6.2.5 Evaluation of the matrix effect

The accuracy of the method can be substantially influenced by the sample matrix. High levels of dissolved or suspended organic matter present in water samples may compete with the PDMS material and thus, the extraction yield might vary from sample to sample. Among the different strategies found in the literature to minimise these effects the use of deuterated analogues is the most preferred one (Iparraguirre et al., 2014). Consequently, it was evaluated the matrix effect for SB and SR extraction by triplicate comparing the responses of analytes in spiked blank water samples (at 100 ng·L^{-1}) with those obtained for target compounds spiked at the same level (at 100 ng·L^{-1}) in presence of different concentrations of humic acids (0, 50, 100, 250 and 500 mg·L^{-1}). To

[1] US EPA ,'Title 40 of the US Code of Federal Regulations, Part 136, Appendix B,' https://www.gpo.gov/fdsys/granule/CFR-2011-title40-vol23/CFR-2011-title40-vol23-part136-appB/content-detail.html (last retrieved November 2016)

assure the interaction of the target compounds with the synthetic matrix, samples were spiked and stirred for 15 min before the extraction was carried out.

Figure 6.9 shows the results (as chromatographic peak areas normalized to 100%) for one target analyte of each family with and without correction with the corresponding deuterated analogue: [$^2H_{15}$] musk xylene for octylphenols, HCHs and musks; [$^2H_8$] 4,4'DDT for Chlorp, Chlorf and DDT related isomers; and [$^2H_3$] DEHP for phthalates.. Only for a few cases (DEHP and DOP using SR) the influence of humic acids observed was not significant. In general terms, the behaviour of the rest of the analytes studied showed the same trend both for SR and SB: the higher the humic acid concentration, the higher decrease of chromatographic signal. This decrease was quite well corrected for all the target compounds with the use of the corresponding deuterated analogue (see Figure 6.9a-d) except for Chlorp and BBP. In those particular cases, the lack of correction can be attributed more to the suitability of the specific deuterated analogue used to correct the matrix effect than to the method itself.

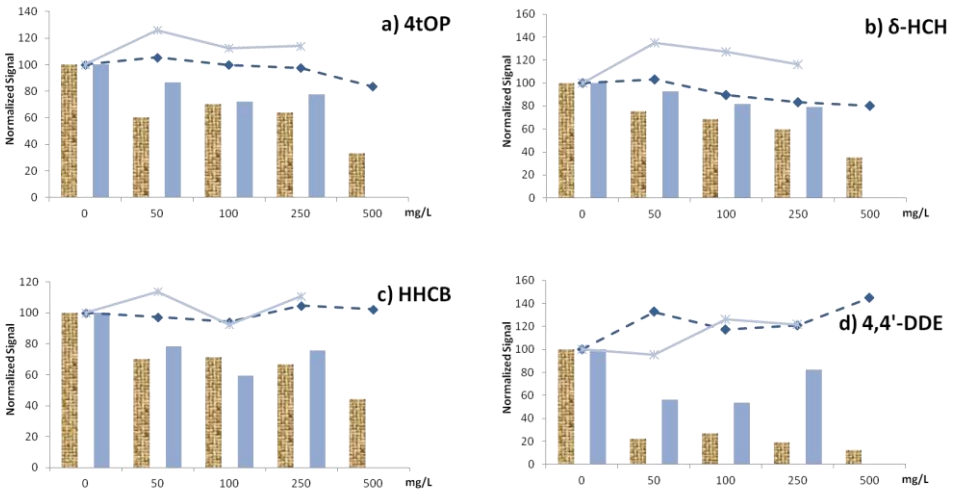

Figure 6.9. Influence of humic acids on the recoveries of SBSE and SR extraction for some of the target compounds: (a) 4tOP; (b) δ-HCH; (c) HHCB; (d) 4,4'-DDE. Non corrected data is shown as bars (framed bars for SR and solid bars for TW) and corrected recoveries using corresponding deuterated analogue are plotted as lines (dotted line for SR and solid line for TW).

6.2.6 Application to real samples

Water samples collected from the WWTP effluent were analysed by triplicate by means of the optimised SR-LVI-PTV-GC-MS and SBSE-TD-GC-MS methods. Using both methods, six of the pollutants under study were detected at ng·L^{-1} level (see Table 6.2). This can confirm the fact that most of the organic pollutants are not removed during the water treatment process, as it is well documented in the literature (Simonich, 2005). Namely, musk compounds, octylphenols and organophosphorous pesticides were the main pollutants detected at higher concentrations (ng·L^{-1}) in effluent wastewater samples. Phthalate compounds were detected in the range of 15 to 447 ng·L^{-1}. In the case of hexachlorocyclohexane isomers and organochlorine pesticides, the concentrations were much lower (between 8-55 ng·L^{-1}) while the rest of target analytes were found at concentrations lower than method detection limit, which is in agreement with those values found in the literature (Bizkarguenaga et al., 2012). The occurrence of the studied analytes in estuarine water samples was negligible since all the target analytes were below method detection limits.

Table 6.2. Concentrations expressed as ng·L^{-1} of target contaminants detected in effluent wastewater from Galindo by means of SBSE-TD-GC-MS and SR-LVI-PTV-GC-MS (n=3, 95% of confidence level).

	SR-LVI-PTV-GC-MS	SBSE-TD-GC-MS
HHCB	1270±67	1048±67
AHTN	175±7	160±22
4-tOP	17.7±0.8	44±8
4-nOP	8±1	n.a.
Chlorp	57±7	11±6
Chlorf	8±2	n.a.

n.a. not available

6.3 Conclusions

Two sorptive extraction methods based on polydimetylsiloxane acceptors have been developed for the analysis of 17 non-polar organic contaminants in environmental water samples. The first method proposed consists on a silicone rod extraction followed by a liquid desorption using ultrasonic bath and LVI-PTV-GC-MS analysis. The optimised extraction approach using SR shows acceptable precision, accuracy, and limits of detection low enough (at low $ng \cdot L^{-1}$ level) for the determination of target analytes in wastewater and estuarine samples. In addition, a second method based on stir bar extraction followed by TD-GC-MS analysis has been developed as well, achieving satisfactory results in terms of accuracy, precision and limits of detection. Additionally, the use of low cost SR polymers and the user-friendly procedures that minimise or even eliminate (in the case of SBSE) sample pretreatment, make both methods valuable for routine analysis. In the case of SR extraction, the suitability of SR for the extraction of organic compounds has been well established for the extraction of PAHs, PCBs, PBDEs and pharmaceuticals or used as passive sampling purposes, but not for the simultaneous extraction of several organic contaminants belonging to different families. Hence, this work supports the suitability of TW and SR for the extraction of hexachlorocyclohexane compounds, organochlorine and organophosphorous pesticides, polycyclic compounds, octylphenols and phthalates in environmental water samples, presenting SR as an economical but also feasible alternative to SBSE.

Moreover, the results obtained point towards a good performance of SR and SB as passive samplers for the organic compounds under study. In fact, the use of these samplers, also in the enclosed versions (Mesco SR and Mesco SB) has been already reported in the study of other contaminants. The promising results obtained in this study indicate that the application of these samplers could be extended to other types of compounds, reinforcing their role as an additional valuable tool for environmental water monitoring purposes.

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7. ASSESSMENT OF COMMERCIALLY AVAILABLE POLYMERIC MATERIALS FOR SORPTIVE EXTRACTION OF PRIORITY AND EMERGING NON-POLAR ORGANIC POLLUTANTS IN ENVIRONMENTAL WATER SAMPLES

As described in the previous chapters, in the analysis of priority and emerging organic pollutants in water samples, sorptive extraction methods can play a remarkable role in the development and application of efficient procedures of analysis. This work describes the development of sorptive extraction procedures using four commercially available and low cost polymeric materials (Raffia, Polyethersulphone, Polypropilene and Polyamide). The analysis of the stripped phase was carried out in a large volume injection (LVI) system through a programmable temperature vaporization injector (PTV) coupled to gas chromatography-mass spectrometry (GC-MS), and it included the quantification of the same target analytes considered in the previous chapter belonging to different families of emerging organic contaminants (alkylphenols, organophosphorous compounds, synthetic polycyclic musks, organochlorine pesticides and phthalates) except DEHP. Consequently, the main aim of this work is to provide a proof of concept for a simple low cost procedure to analyse environmental water samples at low detection limits (low $ng \cdot L^{-1}$ range)

Prior to the development of the extraction method, the different polymers were characterized by means of Raman spectroscopy in order to check the homogeneity of the polymeric materials employed in this study. Afterwards we proceed with the optimization of the extraction process including variables such as the desorption solvent, the ionic strength, the addition of organic modifier, or the stirring rate and the extraction time. Finally, the procedure was applied for the analysis of the 16 target compounds in estuarine water and in the effluent of a WWTP to check the feasibility of the method for the analysis of real samples.

7.1 Experimental

7.1.1 Sampling

Effluent water samples of an urban WWTP and surface water samples were collected and analysed in order to test the suitability of the method in real environmental samples. Surface water samples were collected in the estuary of Bilbao (Bay of Biscay, North of Spain) in April 2013. Final treated effluent (downstream) urban wastewater samples were obtained from the WWTP of Galindo (Barakaldo, Biscay) in June 2013. Samples were collected in pre-washed amber bottles and transported to the laboratory in cooled boxes. After reception, they were passed through 0.45 µm cellulose filters and stored in the fridge at 4˚C before treatment for a maximum of 48 hours before being extracted.

7.1.2 Sorptive materials

7.1.2.1 Preparation of sorptive materials

Cleaning and conditioning of all the used polymers is highly recommended prior to their use as sorptive materials. Thus, all the sorbents except PES were first sonicated three times, for 5 min, using fresh solvent mixture of CH_2Cl_2:MeOH (1:1, v/v). Afterwards, the polymers were dried with a lint-free tissue and thermally conditioned at their maximum working temperature (120°C for all of them) for 180 min under a nitrogen stream (ca. 30 mL·min^{-1}). The first step was suited for removing any possible interference as well as to check the chemical stability of the polymers in contact with different organic solvents (i.e., Hex, EtOAc, MeOH and cHex). No chemical alteration was observed in the studied materials. However, in the case of PES material, chemical decomposition occurred when CH_2Cl_2 was used or when it was sonicated with EtOAc or MeOH. Thus, PES material was cleaned and conditioned with EtOAc and maintained in fresh EtOAc overnight. The conditioned fibers were chemically desorbed using EtOAc and analysed by means of LVI-PTV-GC-MS in order to assure adequate blank samples.

7.1.2.2 Characterization of sorptive materials by Raman Spectroscopy

The homogeneity of the fibers was assured by means of Raman spectroscopy. They were analysed using a Renishaw InVia Raman spectrometer coupled with a Leica DMLM microscope, having a spatial resolution of 2 µm for the 50x objectives. In this work, a laser of 514 nm and a laser of 785 nm (ion-argon laser, Modu-Laser) with a holographic net of 1800 lines·mm^{-1} were used.

The spectroscopic analysis was conducted by focusing directly the polymer fibers at micron level along the length of the conditioned polymer. In all the measurements, the power of the laser (nominal power lower than 20 mW at the samples) was reduced in order to avoid the photodegradation of the polymers. Each spectrum (recorded from 100 to 3000 cm^{-1}) was collected for 10 s and 10 scans were accumulated at 10% of the maximum power of the laser used. The homogeneity of the polymers was assessed from the 10 Raman spectra obtained from each polymer along their principal axis. The software used to collect Raman spectra was WIRE (Renishaw, UK) and Omnic (Nicolet, Madison, Wis., USA) was used to process Raman spectra.

7.1.3 Sorptive extraction methodology

The extraction of the target analytes from water samples was carried out using the above mentioned fibers in 150 mL headspace extraction vessels. The optimization assays were performed with aliquots of 150 mL of ultrapure water (Milli-Q) spiked with target compounds. Each fiber was attached to a small ball of lead to assure the complete immersion in the extraction vessel. Then, the vials were closed with PTFE septa and aluminum crimp caps as it is shown in Figure 7.1.

Figure 7.1. Detail of the extraction set-up using polymers.

The optimization of sorptive extraction assays were performed using a magnetic 15 position stirring plate (Gerstel, USA) at room temperature (22 °C) and the conditions of agitation speed, ionic strength, addition of organic modifier and extraction time were systematically studied in triplicate for each polymer. Under optimal conditions, the addition of 10% of NaCl was required and the sorption of the analytes was performed overnight at 1200 rpm. Once the sorption step was over, the fibers were removed, rinsed with ultrapure water and dried with a lint-free tissue. For liquid desorption purposes the fibers were placed in amber Eppendorf vials filled with 300 µL of the corresponding desorption solvent. The liquid desorption was then accomplished by agitation for 30 min at 100 rpm. After liquid desorption, the polymers were removed, the extract was quantitatively recovered and analysed by means of LVI-PTV-GC-MS (see section 3 for the details of LVI-PTV-GC-MS analysis).

7.2 Results

7.2.1 Preliminary considerations

7.2.1.1 Selection of sorbent materials

The choice of these materials was based on their structure and compatibility with target compounds, their commercial availability and previous uses reported in the

literature. According to the literature, PP membranes provided promising results for the extraction of non-polar organic compounds such as organophosphorous flame retardants and plasticizers from water samples (García-López et al., 2008). Nefso and co-workers tested the sorptive capacity of PP and high density polyethylene (HDPE) membranes to extract organic contaminants in groundwater (Nefso and Burns, 2007). Although the sorption efficiency of HDPE was found to be lower than PP, depending on the target compound, HDPE was further used as passive sampler membranes for the analysis of polycyclic aromatic hydrocarbons, polychlorinated biphenyls, hexachlorobenzenes, and some pesticides in sediments (Allan et al., 2012). Similarly, synthetic Raffia, which is supposed to be a mixture of PP and PE, can also show promising features as sorptive material due to its high thermal and chemical stability. However, it has never been considered for sorptive extraction of organic compounds.

Furthermore, the higher polarity of PES in comparison to the previous polymers offers an interesting choice to extract a different range of compounds. PES was proposed by Prieto and co-workers as sorptive material for microextraction of emerging pollutants from water samples (Prieto et al., 2012). In that work, PES rendered higher extraction yields for polar compounds in comparison with silicone rods and PDMS stir bars. Thereafter, PES was applied to the determination of polar organic contaminants such as, perfluorinated compounds and benzotriazoles, in environmental water samples at very low $ng \cdot L^{-1}$ levels (Casado et al., 2013; Villaverde-de-Sáa et al., 2012).

7.2.1.2 Commercial polymer characterization

The fibers were characterized by Raman spectroscopy before being used. Good quality Raman spectra were obtained for raw material before and after their conditioning and, no significant differences were noticed. As an example, Raman spectra of the conditioned fibers are plotted in Figure 7.2.

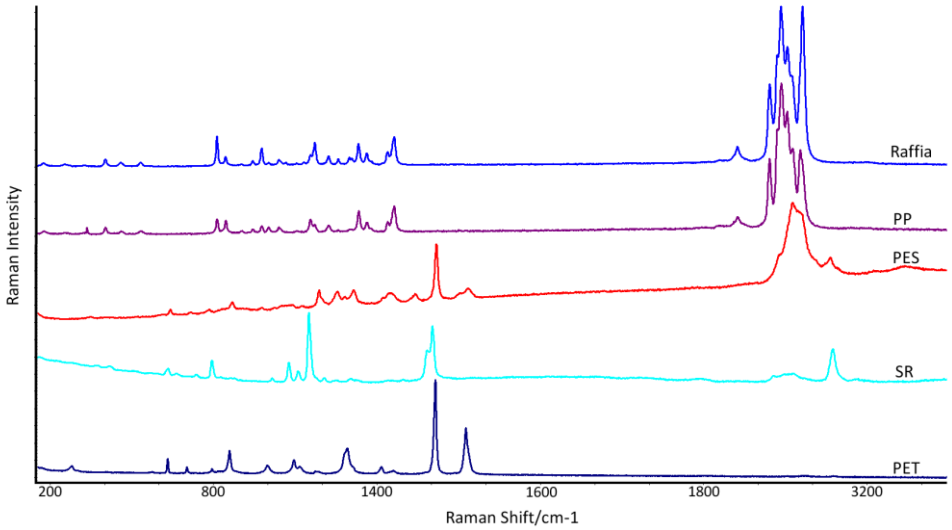

Figure 7.2. Raman spectra of the studied materials: Raffia, PP, PES, SR and PET (Y axis is shifted to show all the spectral features).

The identification of characteristic Raman bands of the tested materials was accomplished according to a Raman database of polymers (Wire software, Renishaw) and the literature (Cai et al., 2010; Gen et al., 2011; Nielsen et al., 2002; Sharma and Bijwe, 2012; Veres et al., 2012; Visentin et al., 2006; Xue, 1997). As indicated in the database, spectral fitting of 76 % and 60 % was attained for SR and PES respectively, which were identified as poly(dimethylsiloxane) in liquid state and poly(p-phenylene ether sulfone). The characteristic Raman bands for both compounds were consistent with the spectral data published in the literature (Cai et al., 2010; Sharma and Bijwe, 2012). PP and Raffia showed very similar Raman spectra, mainly attributed to the characteristic Raman bands of PP (spectral match of 80 % and 73 % for PP and Raffia according to the spectral library). The PP polymer was identified as isotactic PP characterized by the Raman bands located at 830, 972, 1151 and 1435 cm^{-1} (Gen et al., 2011; Nielsen et al., 2002).

Commercially available Raffia is known to be composed by PP and PE. However, the Raman spectra obtained for Raffia was comparable to that obtained for PP and any of the characteristic Raman bands of PE were detected (i.e., 1068, 1131, 1295 and 1416 cm^{-1} (Visentin et al., 2006). Therefore, it could be concluded that the used Raffia was another type of PP but with a different degree of crystallinity (Nielsen et al., 2002). In the particular case of PET, a laser of 785 nm was used and a spectral match of about 85% was achieved and the main Raman bands were detected (Veres et al., 2012). Anyhow, all the characterized materials were found to be homogeneous and thus, they were further used for sorptive purposes.

7.2.1.3 Preliminary performance

Batch sorption assays were performed in order to test the loading capacity of the four materials and compared with the extraction yields obtained with SR. All the experiments were conducted by triplicate and using the same procedure: each material was placed in a glass vial containing 150 mL of Milli-Q water spiked at 1 $ng \cdot mL^{-1}$ and without adding neither MeOH nor NaCl, and the extraction was performed at 700 rpm for 16 h. Once the extraction was over, the materials were chemically desorbed for 15 min using 300 µL of EtOAc and subsequently analysed by means of LVI-PTV-GC-MS. The results of these assays showed that Raffia and PP (≈ 40 mg, 1.5 cm length fibers) provided comparable extraction yields to those obtained with SR (≈ 38 mg, 1 cm length piece) for phthalates and pesticides (see Figure 7.3). Overall, PES (≈ 10 mg, 7.5 cm length piece) rendered higher extraction yields for all the studied compounds, whereas PET provided negligible chromatographic peak areas for all analytes except for pesticides. Consequently, PET was discarded and PES, PP and Raffia were considered for further experiments.

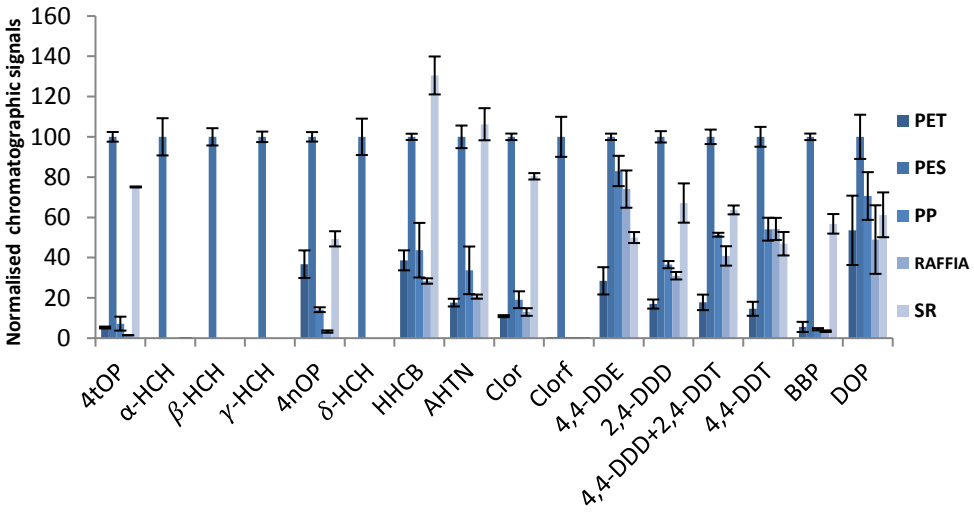

Figure 7.3. Chromatographic responses (n=3, 95% confidence level) of the four studied sorptive materials (PET, PES, PP and Raffia) for the target compounds. Normalised values to those obtained with PES.

7.2.2 Optimization of sample preparation

During micro-extraction of organic compounds with polymeric materials, several parameters affecting both extraction and chemical desorption steps were considered to get the optimum extraction conditions for each sorbent material.

7.2.2.1 Desorption solvent

The polarity and boiling point of the solvents were considered in order to choose the best leaching solvent. Ideally, organic solvents should show a high affinity for target compounds and inertness to the sorptive material. This requirement excluded chlorinated solvents, which lead to decomposition of PES polymer. Since EtOAc has been proposed as a good leaching solvent for a wide range of organic pollutants in the literature (Prieto et al., 2012), it was fixed for PES in this work. In addition to the affinity, LVI-PTV requires volatile solvents that are eliminated easily in the injection system. Thus, EtOAc, MeOH, Hex and cHex were considered for desorption of analytes from Raffia and PP polymers.

To study these solvents, the sorbents were exposed to 150 mL of spiked Milli-Q water samples (1 ng·mL^{-1}) and stirred at 1200 rpm overnight (ca. 14 h). Thereafter, the sorbents were recovered, dried with a clean tissue and chemically desorbed with 300 µL of the previously mentioned solvents by agitation for 30 min. No chemical decomposition or interferences were observed in the studied materials by using any of the tested solvents. Figure 7.4 compares the normalised responses against the highest signal for MeOH, EtOAc, cHex and Hex for Raffia and PP polymers, respectively. As observed, desorption efficiencies decreased with the polarity of the solvent for all the target analytes. Non-polar solvents (i.e., Hex) rendered higher chromatographic responses but it showed poor reproducibility mainly attributed to the swelling of the material. Moreover, cHex and EtOAc provided the best results in terms of recovery and reproducibility, but owing to the conditions required for LVI-PTV, the use of EtOAc was confirmed for further experiments.

The effects of increasing temperature or shaking vigorously the fiber are known to shorten the leaching time during the desorption step (Melo et al., 2009). Therefore, each material was desorbed three times successively with 300 µL of fresh EtOAc by agitation or sonication (using an ultrasonic bath) at room temperature for 30 min. No statistical differences ($p > 0.1$) were found between agitated and sonicated elutions obtained for any of the tested materials (data not shown). However, since direct exposure of high ultrasound energy to PES polymers leads to their degradation, agitation was preferred as a leaching aid. Desorption of Raffia, PP and PES sorbents with three consecutive extractions (n=3, 100 ng·L^{-1}) of 30 min using EtOAc as desorption solvent and agitation revealed that in the first fraction more than 86%, 81% and 92% respectively was extracted. Hence, one single desorption with 300 µL of EtOAc by agitation for 30 min was enough to assure a quantitative desorption of the target analytes.

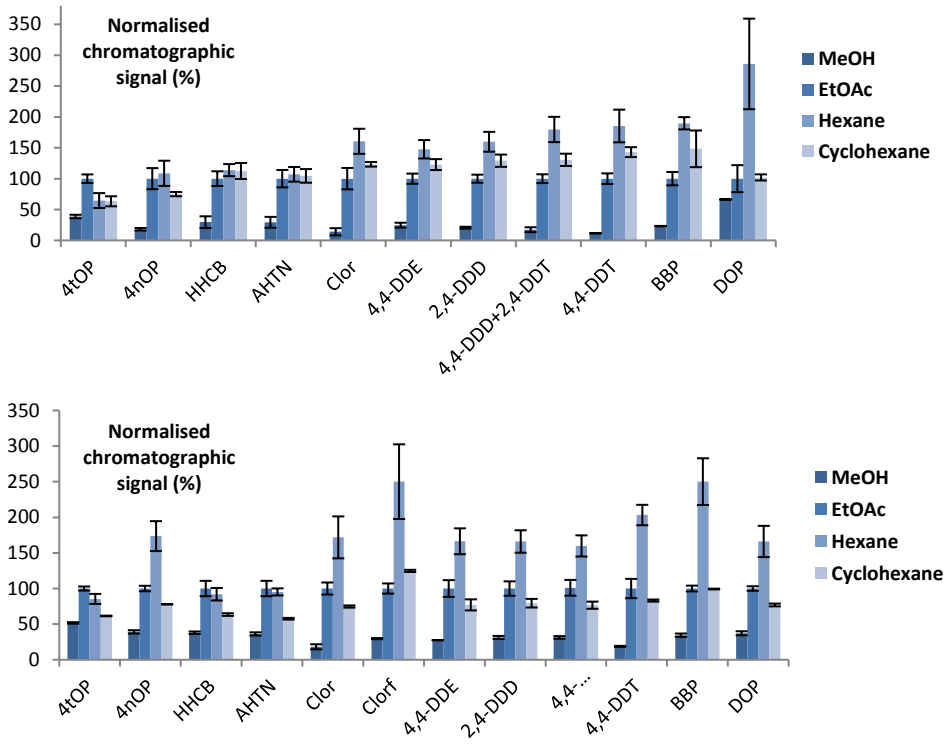

Figure 7.4. Desorption efficiencies for Raffia (showed at the top) and PP (showed at the bottom) using 300 µL of different organic solvents: MeOH, EtOAc, Hex and cHex (n=3, 95% confidence level). Results expressed as chromatographic signals normalised to the highest value.

7.2.2.2 Effect of ionic strength and addition of organic modifier

The effect of the addition of an inert salt (NaCl) and an organic modifier (MeOH) in the extraction efficiency was simultaneously studied by means of an experimental design approach for the three polymers (PES, PP and Raffia). A Central Composite Design (CCD) was performed using the Statgraphics Centurion XV program and covering a factor space of 0 – 20% for NaCl (w/v) and MeOH (v/v) respectively (with three central points, i.e., 11 experiments). These assays were carried out using fortified Milli-Q water samples (1 ng·mL^{-1}) modified with the addition of NaCl and MeOH according to each experiment

and extracted overnight (ca. 14 h). The precisions of the measurements were between 5-16 % (RSD%) for all the studied compounds using the three materials based on the three replicates of the central point.

The responses obtained for the CCD were analysed by means of analysis of variance and considering only the significant variables at 95% of confidence level (p-values < 0.05). MLR was used to build the response surfaces. Overall, as shown in Figure 7.5, the addition of MeOH was not statistically significant (p-value > 0.05) for any of the studied compounds except for Chlorp and Chlorf using PES and for octylphenols using PP, which showed lower recoveries in presence of MeOH. This fact was also reported in the literature for other sorptive extraction approaches (Camino-Sánchez et al., 2012; MacNamara et al., 2009; Prieto et al., 2012). Therefore, no MeOH was added in further experiments.

Regarding the addition of NaCl, the results showed a net decrease of the extraction efficiency for the most lipophilic compounds as already described in the literature (Prieto et al., 2012; Schellin and Popp, 2007). Some authors attribute this fact to the "oil-effect" or enrichment of non-polar compounds at the water surface leading to a lower interaction with the surface of the sorbent material (Schellin and Popp, 2007). However, the less hydrophobic analytes (i.e., organochlorine pesticides) showed the opposite effect. This behavior can be explained in terms of the salting out effect (Prieto et al., 2010). As a result, NaCl was fitted at 10% (w/v) as a consensus value for the simultaneous extraction of all the target compounds.

7.2.2.3 Stirring rate

The extraction of the analytes can be conditioned by their mass transfer rate through the boundary layer in contact with the surface of the polymeric material.

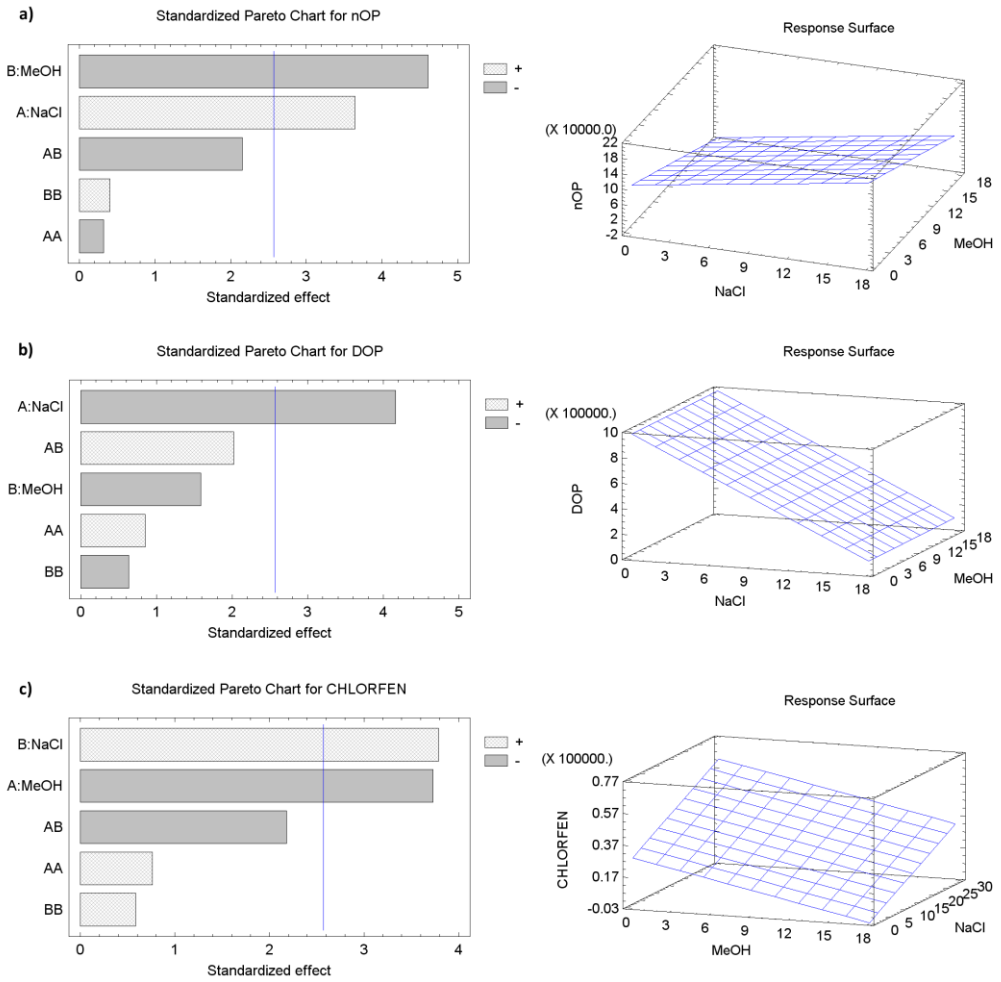

Figure 7.5. Standardised Pareto charts for the main effects and their interactions and the response surface obtained after CCD for three target compounds: a) 4-nOP using PP, b) DOP using raffia, and c) Chlorf using PES.

Thus, the effect of agitation on the extraction efficiency was studied at five levels of stirring rates: 400 rpm, 600 rpm, 800 rpm, 1000 rpm and 1200 rpm. For this assessment, extractions were performed in triplicate with 150 mL of fortified Milli-Q water samples (1 ng·mL^{-1}) under previously fixed extraction conditions (10% of NaCl, no addition of MeOH, overnight). In all the cases, high stirring speed affected positively the extraction process (see Figure 7.6) for Raffia and PES but it was non-significant for PP (for stirring rates above 1000 rpm). Consequently, all the experiments were carried out at 1200 rpm and, in all the cases, no physical damage was observed in the fibers during the extraction.

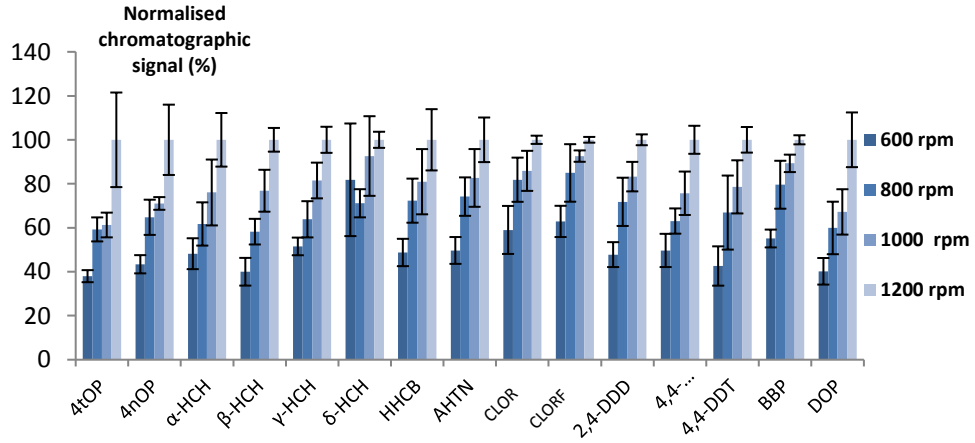

Figure 7.6. Normalised extraction efficiencies obtained for assays performed at different stirring rates using Raffia (n=3, 95% confidence level). Results expressed as chromatographic signals normalised to the highest value.

7.2.2.4 Extraction time

Extraction time profiles were evaluated in order to determine the minimum time required to reach equilibrium conditions. The kinetics of the different compounds can take from minutes to hours depending on physico-chemical properties of the target compounds and the partition coefficient between the sample and the sorbent material, among others (Ros et al., 2015). Thus, the time course of the sorptive extraction for

different sorptive materials was evaluated with 150 mL of spiked Milli-Q water (1 ng·mL^{-1}) containing 10% of NaCl with a stirring speed of 1200 rpm. Assays were performed in duplicate at 12 different extraction times between 15 min and 24 h.

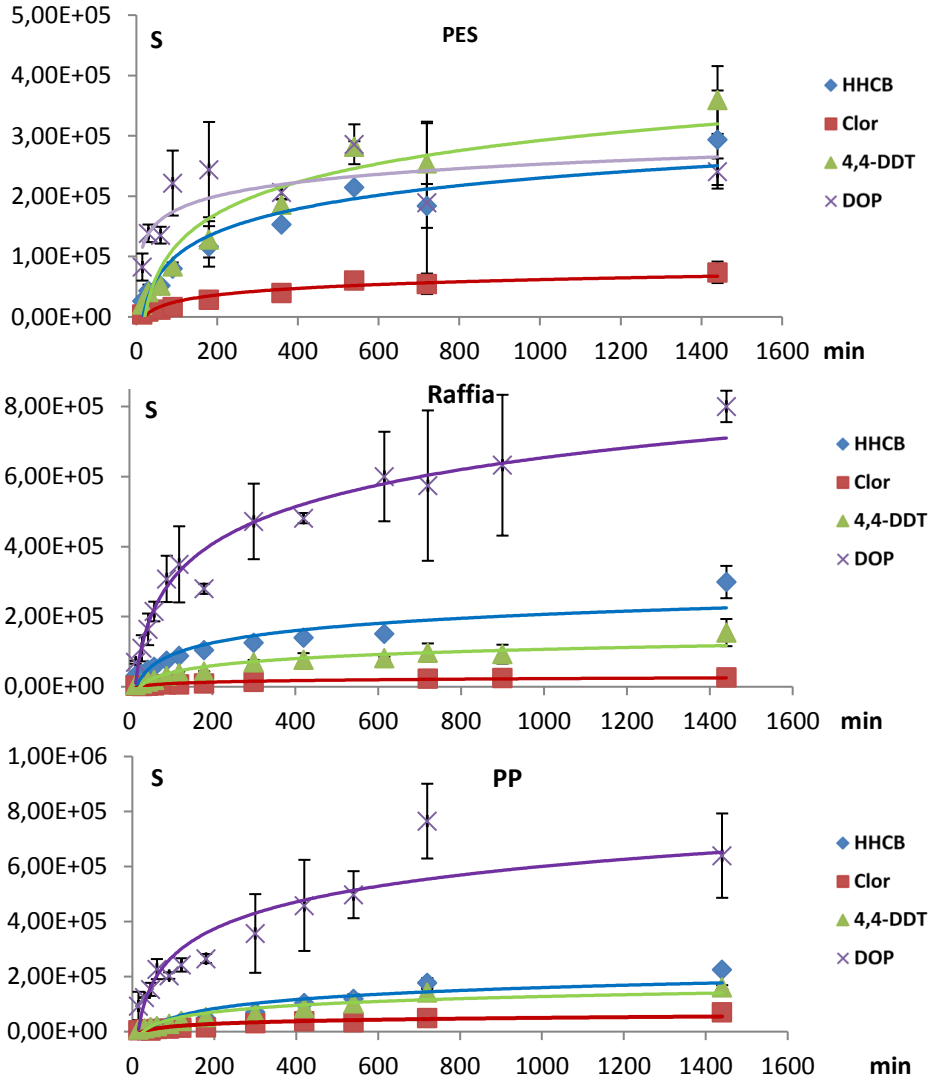

Figure 7.7. Extraction time profiles using PES, Raffia and PP for some target compounds under optimised conditions. Assays performed in duplicate (n=2, 95% confidence level).

Figure 7.7 shows the extraction time profiles obtained for four of the target compounds with three polymers. Similar trends were observed for the rest of the compounds. In agreement with results obtained in previous applications developed for other compounds (Casado et al., 2013; Prieto et al., 2012; Villaverde-de-Sáa et al., 2012), the extraction of target compounds in the PES polymer displayed a slow release, requiring more than 10 h to reach to equilibrium. PP and Raffia polymers showed similar kinetic profiles for all the compounds except for 4,4'-DDE and 2,4'-DDD pesticides for which extraction equilibrium was not reached even after a sampling time of 24h. Hence, an extraction time of 14 h (overnight extraction) was considered a right compromise between method sensitivity and practical reasons. Although this extraction time is relatively long, but still in the range of several applications using SBSE or SR extraction (León et al., 2006; Pérez-Carrera et al., 2007; Prieto et al., 2010), several samples can be extracted simultaneously overnight and thus, the method throughput is still assured.

7.2.3 Comparison of the sorptive materials

The extraction efficiencies of the three materials were compared under optimum extraction conditions and at lower concentration levels. 150 mL of Milli-Q water aliquots spiked at 100 $ng \cdot L^{-1}$ were extracted by triplicate using PES (7.5 cm length), Raffia (1.5 cm length) and PP (1.5 cm length). All the extractions were performed with the addition of NaCl (10%) at 1200 rpm for overnight. Thereafter, the sorbents were chemically desorbed with 300 µL of EtOAc by agitation for 30 min. The extracted mass of each compound was determined by external calibration and compared with the spiked concentration. Table 7.1 summarizes the extraction efficiencies obtained for each material as well as the extraction efficiency obtained in a previously optimized method using SR (see chapter 6) and SBs (Ochiai et al., 2006; Tan et al., 2008; Tölgyessy et al., 2011).

Within the studied materials, Raffia and PP materials showed similar extraction efficiencies, also comparable to those obtained with SR and SBs, which can be interpreted in terms of chemical affinity. In fact, these two polymers provided extraction efficiencies from 14 % to 59 % for the most lipophilic compounds (i.e., organochlorine pesticides). Although PP rendered better results in terms of precision compared to those obtained using Raffia (RSDs < 9% and < 14% using PP and Raffia respectively; see figures of merit section), the chromatograms were dirtier using PP after a large number of consecutive analyses. This fact endangers the life of the chromatographic column and compromises the accuracy of the results. Thus, PP was discarded further application to water sample analyses.

In addition to this, PES polymer showed a different extraction pattern in comparison to Raffia and PP. PES was more suitable for the extraction of slightly polar and non-polar contaminants. In fact, PES tube (≈14 mg) was the only polymer able to extract HCHs providing even better extraction efficiencies than SR (≈38 mg) and comparable to the features of the SBs (see Table 7.1). Consequently, PES and Raffia were considered for further method validation and application to real samples.

7.2.4 Evaluation of matrix effects

The extraction efficiency of sorptive extraction techniques can be affected by the matrix of the environmental water samples. For instance, the presence of dissolved or suspended organic matter in environmental water matrices may alter the extraction kinetics or even may compete with the sorptive material. As mentioned in previous chapters, sample dilution, sample clean-up after extraction, matrix matched calibration or the use of surrogates is the most referenced strategies to overcome the problem of matrix effect (Pérez-Carrera et al., 2007; Sánchez-Avila et al., 2010; Schellin and Popp, 2007).

Table 7.1. Extraction efficiencies obtained using PES, Raffia, PP, SR and SBs for the extraction 150 mL of Milli-Q water spiked with 100 ng·L^{-1} (n=3, 95% confidence level) for each compound.

		Extraction Efficiency (%)				
Compound	**log K_{ow}**	**Raffia**	**PP**	**PES**	**SR**	**SBs**
4 tOP	5.28	7	10	33	11[a]	30[b]
4 nOP	5.99	24	23	39	19[a]	55[b]
α-HCH	4.26	n.d	n.d	45	n.a	63[c]
β-HCH	3.68	n.d	n.d	20	7[a]	30[c]
γ-HCH	4.26	n.d	n.d	22	10[a]	53[c]
δ-HCH	3.68	n.d	n.d	25	4[a]	42[c]
AHTN	5.70	22	12	28	19[a]	n.a
HHCB	5.90	14	12	26	20[a]	n.a
Chlorp	4.96	29	26	48	33[a]	51[c]
Chlorf	3.81	n.d	29	32	31[a]	43[c]
4,4'-DDE	6.00	39	29	18	10[a]	44[b]
2,4'-DDD	5.87	47	33	26	22[a]	n.a
4,4'-DDD+2,4'-DDT	5.87/6.79	59	26	29	20[a]	38[c]
4,4'-DDT	6.79	53	57	43	n.d	47[b]
BBP	4.80	15	13	46	22[a]	59[d]
DOP	8.54	17	12	4	3[a]	n.a

n.a not available *n.d* not detected [a] see chapter 6 [b] Tölguiessy et al. 2011 [c] Ochiai et al. 2006 [d] Tan et al. 2008

In this work, the correction of matrix effect using deuterated analogues was firstly evaluated. To this purpose, 150 mL of estuarine water samples were spiked with 250 ng·L^{-1} of each target compound and the same amount of deuterated compounds was added to the sample prior to the extraction (n=3 replicates).As shown in Figure 7.8a, no matrix effect was observed using different materials for all the compounds (apparent recoveries between 81 and 115 % for PES and between 74 and 110% for Raffia) in estuarine water samples, even without being corrected with deuterated analogues. However, the extraction of target compounds was highly affected in the case of effluent wastewater samples (see Figure 7.8b).

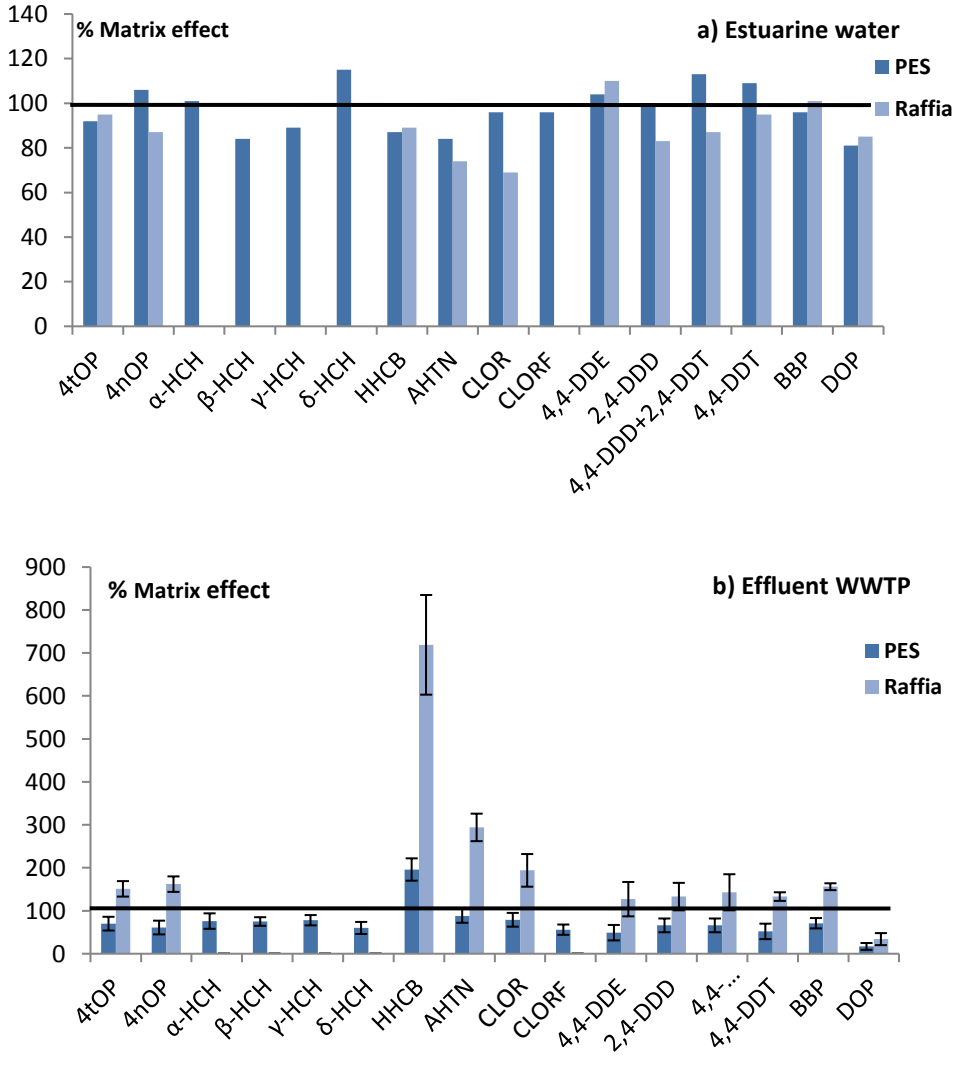

Figure 7.8. Evaluation of matrix effects (%) for different matrices calculated as the relation between spiked samples and spiked Milli-Q water (250 ng·L^{-1}, n=3) using PES and raffia respectively: a) estuarine water sample and b) effluent wastewater sample

The apparent recovery for effluent wastewater samples was calculated after correction with the corresponding deuterated analogue. Overall, this strategy could not compensate the matrix effect in analysed wastewater samples using any of the sorptive materials (see Table 7.2). Only HCHs corrected with [$^2H_{15}$] MX for PES and pesticides corrected with [$^2H_8$] DDT for Raffia provided accurate results between 80 and 105 %.

Consequently, when the use of deuterated analogues failed, the use of matrix-matched calibration was evaluated. Because of the variability existing from sample to sample, matrix matched calibrations using simulated water matrices (with equivalent Total Organic Carbon (TOC) to the analysed water samples) were performed. To this aim, two different artificial matrices were tested: matrix simulated with humic acids and matrix simulated with soluble dietary fiber. In the literature, the matrix effect has been simulated using humic acids (Lambropoulou and Albanis, 2007), but the use of soluble fiber has never been considered for this purpose. It consists on a set of complex molecules (cellulose, hemicelluloses, pectic substances, starch, gums, mucilages and non carbohydrate compounds) that are typically present in effluent sewage water (Shon et al., 2007).

Thus, both matrices were prepared according to the TOC of the analysed wastewater samples (i.e., 70 mg·L^{-1}). External calibration curves were built with a set of 5 standards containing concentration ranged from blanks (i.e. Milli-Q water sample) to 100 mg·L^{-1} and 110 mg·L^{-1} of humic acid and soluble fiber respectively in order to relate the concentration of each solution with the TOC values that were analysed using a TOC analyzer (Shimazdu). Subsequently, procedural calibration curves were built by spiking different amount of standards (from 15 to 450 ng·L^{-1}) in 150 mL of Milli-Q water (with 10% of NaCl) at the corresponding concentration of humic acid (50 mg·L^{-1}) and soluble fibre (90 mg·L^{-1}). These solutions as well as 150 mL of effluent wastewater samples spiked at

250 ng·L^{-1} (n=3 replicates) and non-spiked samples (n=3) underwent the same extraction procedure under the optimum conditions for both PES and Raffia.

Table 7.2 summarizes the apparent recoveries of the spiked effluent wastewater samples (after subtraction of the responses of the non-spiked sample) using both calibration approaches. As a rule, procedural calibration built with humic acids was not suitable for any of the target compounds when using these sorptive materials. This lack of accuracy can be interpreted in terms of the extraction yields obtained for the target compounds in the different samples. According to this reasoning the extraction yield was diminished in the presence of humic acids while the spiked wastewater samples suffered a signal enhancement in comparison to Milli-Q water samples spiked at the same concentration level. The phenomenon of signal enhancement is reported in the literature for some compounds determined in wastewater samples (Poole, 2007). On the contrary, good matrix simulation was obtained using the calibration curve built with soluble fiber which rendered extraction yields between 80 % and 116 % (except for 4nOP, 4,4'-DDD, 4,4'-DDT and DOP) using PES (see Table 7.2). Moreover, for artificial matrices built with a variable content of soluble fiber (i.e., samples with TOC values between 50 and 110 mg·L^{-1}) comparable results were also obtained (data not shown). This fact allows the simultaneous analysis of wastewater samples with different/variable TOC values using the same calibration curve. However, when this calibration was used for the quantification of spiked wastewater samples extracted with Raffia, the matrix effect was not compensated and poor results were obtained (apparent recoveries higher than 130 % for all the target analytes). Thus, since this approach cannot be considered as a useful quantification tool for the analysis of effluent wastewater samples using Raffia, standard addition approach should be performed for correct quantification.

7.2.5 Figures of merit

The figures of merit of the developed methods using sorptive PES and Raffia fibers for the simultaneous extraction of 16 organic pollutants are summarized in Tables 7.3 and 7.4, respectively. Linearity of procedural calibration curves performed with PES and Raffia was evaluated between 15 and 450 $ng \cdot L^{-1}$ (n=7 levels). Good linearity was found out over the wide range of tested concentrations as showed by the coefficients of determination (r^2): between 0.978 and 0.999 and between 0.977 and 0.999 using PES and Raffia, respectively. The repeatability of the methods was assessed using spiked Milli-Q water samples at 100 $ng \cdot L^{-1}$ for three replicates analysed in the same day. As it can be seen in Tables 7.3 and 7.4, RSD% values varied from 5 % to 14 % for assays performed using Raffia and between 3 % and 19 % using PES, which were comparable to other works dealing with sorptive microextractions of trace organic pollutants (Bonansea et al., 2013; Tölgyessy et al., 2011).

In order to estimate the accuracy of the results, 150 mL of Milli-Q water samples (by triplicate) were spiked at 100 $ng \cdot L^{-1}$ concentration level and underwent the whole extraction procedure under the optimal conditions. The apparent recovery was calculated by comparing the concentration obtained from the procedural calibration curve (i.e., built with Milli-Q spiked water samples and submitted to the whole extraction procedure) with the spiked amount of the target compounds. The obtained values are compiled in Table 7.3 and Table 7.4 for Raffia and PES, respectively. On the whole, satisfactory apparent recoveries were obtained for all the target compounds in both cases, even not using deuterated analogues: between 74 % and 125 % in the case of Raffia, whereas for PES, the range was between 66 % and 129 %.

Table 7.2. Apparent recovery obtained with different approaches studied for matrix effect correction for effluent wastewater samples using PES and Raffia sorptive materials. Samples spiked at 250 ng·L^{-1} and extractions performed in triplicate (n=3, 95% of confidence level).

	PES			Raffia		
Analyte	**Deuterated Surrogates**	**Matrix matched calibration**		**Deuterated Surrogates**	**Matrix matched calibration**	
		Humic Acids	**Dietary Fiber**		**Humic Acids**	**Dietary Fiber**
4tOP	160±22[a]	123±6	103±21	12±2[a]	131±24	208±52
4nOP	103±19[a]	221±14	131±81	28±3[a]	267±88	240±112
α-HCH	105±7[a]	105±6	94±8	n.d	n.d	n.d
β-HCH	85±3[a]	114±20	94±14	n.d	n.d	n.d
γ-HCH	91±6[a]	98±4	88±10	n.d	n.d	n.d
δ-HCH	80±15[a]	99±25	94±16	n.d	n.d	n.d
HHCB	55±17[a]	135±38	99±9	113±34[a]	397±89	307±69
AHTN	68±6[a]	182±23	96±16	194±29[a]	190±30	212±23
CLOR	70±11[a]	182±11	108±12	115±10[a]	126±19	219±21
CLORF	18±3[a]	104±4	100±8	n.d	n.d	n.ds
4,4-DDE	33±6[b]	950±204	121±51	105±12[b]	575±134	178±30
2,4-DDD	74±11[b]	569±78	115±14	107±9[b]	489±49	165±25
4,4-DDD+2,4-DDT	57±8[b]	724±70	128±40	97±8[b]	527±92	175±30
4,4-DDT	43±6[b]	876±198	145±11	94±12[b]	497±52	161±12
BBP	44±8[c]	156±8	90±8	20±3[c]	111±15	135±10
DOP	57±11[c]	177±87	72±54	60±27[c]	297±66	79±16

n.d –non detected; (a) corrected with [$^2H_{15}$]-MX; (b) corrected with [$^2H_8$]-4, 4'-DDT; (c) corrected with [$^2H_3$]-DEHP

Table 7.3. Main method parameters for the Raffia-LVI-PTV-GC-MS procedure

Raffia-LVI-PTV-GC-MS						
Analyte	r^2	Repeatability (%RSD, n=3) (100 ng·L^{-1})	Recovery		LODs (ng·L^{-1}, n=3)	MDLs (ng·L^{-1}, n=6)
			Extraction Efficiency (%)	Apparent Recovery (%)		Estuarine
4 tOP	0.977	5	7	80	5	10
4 nOP	0.987	12	24	84	5	15
α-HCH	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
β-HCH	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
γ-HCH	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
δ-HCH	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
AHTN	0.986	11	22	106	8	14
HHCB	0.991	14	14	86	8	45
Clor	0.986	13	29	95	5	79
Clorf	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4,4-DDE	0.997	10	39	95	4	49
2,4-DDD	0.992	11	47	122	4	23
4,4-DDD+2,4-DDT	0.998	7	59	125	6	14
4,4-DDT	0.999	10	53	87	22	42
BBP	0.983	14	15	74	2	16
DOP	0.997	13	17	116	2	11

Table 7.4. Main method parameters of PES-LVI-PTV-GC-MS procedure

PES-LVI-PTV-GC-MS							
Analyte	r^2	Repeatability (%RSD, n=3) (100 ng·L^{-1})	Recovery		LODs (ng·L^{-1}, n=3)	MDLs (ng·L^{-1}, n=6)	
			Extraction Efficiency (%)	Apparent Recovery (%)		Estuarine	Effluent WWTP
4 tOP	0.997	3	33	82	5	13	17
4 nOP	0.997	6	39	90	5	13	14
α-HCH	0.993	14	45	117	7	25	36
β-HCH	0.998	19	20	80	5	25	39
γ-HCH	0.995	11	22	82	9	17	60
δ-HCH	0.993	13	25	97	9	12	115
AHTN	0.999	4	28	96	4	26	38
HHCB	0.999	7	26	84	3	71	132
Clor	1.000	10	48	78	5	7	16
Clorf	0.998	9	32	106	4	15	18
4,4-DDE	0.999	4	18	98	4	31	18
2,4-DDD	0.999	8	26	91	4	10	15
4,4-DDD+2,4-DDT	0.999	11	29	88	6	17	16
4,4-DDT	0.999	16	43	111	21	29	18
BBP	0.998	15	46	80	2	38	13
DOP	0.978	4	4	80	2	15	25

Finally, the limits of detection (LODs) were experimentally determined from four blank samples (150 mL Milli-Q) and calculated with the average signal of blank plus three times the standard deviation. In the case that no signal was detected at the corresponding retention time, LODs were referred to a ratio signal-to-noise of 3. The obtained LODs were below 10 $ng \cdot L^{-1}$ for all the target compounds except for 4,4'-DDT which rendered higher values of LOD (22 and 21 $ng \cdot L^{-1}$ for Raffia and PES, respectively). These values were comparable with those obtained by SBSE (Pérez-Carrera et al., 2007; Sánchez-Avila et al., 2010; Tölgyessy et al., 2011) and the results described in the previous chapter in section 6.2.3. Afterwards, estuarine water samples and effluent WWTP water samples were spiked at the corresponding LOD of each target compound in order to calculate the method quantification limits (MDLs) as indicated in the guidance for MDL calculation proposed by USEPA[1]. As detailed in Tables 7.3 and 7.4, the MDL were in a range of 7-71 $ng \cdot L^{-1}$ using PES and 10-79 $ng \cdot L^{-1}$ using Raffia in the case of estuary water samples.

7.2.6 Application to real samples

The developed methods, PES-LVI-PTV-GC-MS and Raffia-LVI-PTV-GC-MS, were applied to real environmental water samples in triplicate, including estuarine and effluent wastewater samples, and compared with previously well established methods using PDMS (see chapter 6). Overall, the obtained values were comparable using the different extraction methods (see Table 7.5), and similar to those reported in previous works from our group (Bizkarguenaga et al., 2012). In summary, most of the target analytes were below MDL in estuarine water samples whereas higher concentration of contaminants (between 53 to 537 $ng \cdot L^{-1}$) were detected in wastewater samples.

[1] US EPA ,'Title 40 of the US Code of Federal Regulations, Part 136, Appendix B,' https://www.gpo.gov/fdsys/granule/CFR-2011-title40-vol23/CFR-2011-title40-vol23-part136-appB/content-detail.html (last retrieved November 2016)

Table 7.5. Concentrations (n=3, 95% confidence level) measured in estuarine water (Nerbioi-Ibaizabal) and WWTP effluent (Galindo) with the different sorptive materials: PES and Raffia (optimized in the present work) and SR.

Analyte (ng·L^{-1})	***Estuarine water (Nerbioi-Ibaizabal)***		***WWTP effluent (Galindo)***		
	PES	***Raffia***	***PES***	***Raffia***	***SR***
4 tOP	14±1	< MDL	73±6	< MDL	< MDL
4 nOP	< MDL	< MDL	53±5	< MDL	27±5
δ-HCH	26±5	< MDL	124±11	< MDL	173±26
AHTN	34±12	36±4	127±12	124±14	79±13
HHCB	155±12	128±46	537±43	397±14	480±61

It is worth noting that musk compounds, octylphenols and some organochlorine pesticides were the main contaminants detected in effluent wastewater samples. Briefly, among the studied analytes, the two synthetic musks (HHCB and AHTN) were the most abundant ones (see Table 7.5), which is in agreement with those values previously found in the literature (Lee et al., 2010). 4-tOP, 4-nOP and δ-HCH were detected at concentrations ranged between 27 – 173 ng·L^{-1}).

7.3 Conclusions

This work shows the suitability of PES and Raffia to carry out the microextraction of a wide range of typical contaminants in water samples. The analytical features of the methods based on the use of those two polymers are comparable to those using commercially available polymers (stir-bars) and, additionally, they are economically highly affordable.

Especially, PES showed enhanced extraction efficiencies for compounds that are poorly extracted with PDMS, as it is the case of hexachlorocyclohexane isomers. The subsequent analysis of the extracts by LD-LVI-PTV-GC-MS showed acceptable precision,

accuracy and MDLs low enough (at $ng \cdot L^{-1}$ level) for the determination of selected compounds in environmental water matrices. The efficiency of extraction step was mainly conditioned by the ionic strength, the extraction time and the matrix effect, whereas the liquid desorption of the polymers was performed with EtOAc in order to get the highest extraction yields. Variations in the yield of the micro-extraction among estuarine water samples could be effectively compensated using procedural calibrations with spiked Milli-Q water samples since no matrix effect was observed. However, a strong matrix effect was observed for PES, and especially for Raffia, when applied to wastewater samples. Although, the use of calibration curves built with simulated wastewater samples can overcome the matrix effects using PES, standard addition method would be necessary for a proper quantification using Raffia.

Finally, based on the results given in this work it is possible to design fibers that can be useful in the development of analytical microextractions. In addition to this, the behavior of these materials in passive samplers is also an added value that can be useful in future works.

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8. PASSIVE SAMPLING METHODS FOR THE DETERMINATION OF ORGANIC COMPOUNDS IN WATER

As described previously, a significant number of chemicals produced by the chemical industry is continuously released into the environment during their life-cycle, and when the hazards to human well-being or the risks to the environmental health are above the allowable thresholds, there is a need to monitor their presence in different environmental ecosystems (Seethapathy et al., 2008). As a result of these concerns, monitoring programmes are being implemented to obtain a neat picture of the risks involved in the exposure to certain pollutants. Sampling is deemed the most crucial step of many environmental monitoring programs, though it has been deeply overlooked (Alvarez and Jones-Lepp, 2010). At this initial stage any bias can be hardly corrected during the analytical procedure. In fact, the representativeness of the samples collected is sometimes called into question depending on both the sampling procedure and the monitoring characteristics.

The need of a robust and fit-for-purpose sampling procedure to assure the monitoring requirements is still an analytical challenge. In this framework, unlike active or grab sampling, passive sampling (PS) arose as a feasible alternative for many monitoring assessments. Among the most important features we can mention the estimation of time-weighted average concentrations, which are specially significant in highly dynamic compartments, and the lower analytical effort (Lohmann et al., 2012). Although 10 years ago most end users had to be persuaded about the applicability and reliability of PS, currently, there is a growing interest in the development and use of PS devices for environmental monitoring studies (Mills et al., 2014).

8.1 Passive Sampling: a general overview

PS is usually described as a sampling technique based on the diffusion of an analyte from the sampled medium to a receiving phase with no energy supply other than the difference of the chemical potential (Górecki and Namiesnik, 2002; Greenwood et al., 2009). The accumulation of target analytes in the sampling device is the result of the difference between the chemical potentials of the analyte in both media (i.e., sampled medium and receiving phase). After the accumulation of the target analytes in the receiving phase of a PS device, they are subsequently analysed in order to quantify the compounds found in the sampling medium. From these amounts, the time in which the samplers have been deployed and the kinetic-thermodynamic features of the sorption, it is possible to estimate the time-weighted average concentration (C_{TWA}) in the sampled medium.

On the contrary, active sampling procedures involve the collection of low volume spot water samples (bottle or grab) over a certain period of time (and/or space). This sampling approach is specially challenging when the concentration follows a dynamic pattern in space and in timescale (e.g. groundwaters, tidal effects or the marine coastal currents) or when the contaminants are only present at trace level but still at toxicologically relevant concentrations. In those cases, the C_{TWA} values provided by the passive samplers can offer a more meaningful environmental end-points than the spot values since the spurious effects of high or low values are limited. In addition to this, it has been emphasized the possibility of obtaining lower detection limits as a consequence of a long accumulation process and the affordability of PS, since the lab work is significantly lower. Finally, PS offers cost-effective sampling protocols since miniaturize devices with no dependence of power supply are used and hence, long and careless deployments are feasible, usually far from the lab or typical sampling stations (Miège et al., 2015; Seethapathy et al., 2008; Stuer-Lauridsen, 2005).

The back side of PS requires also a deep analysis. First of all we can mention the real meaning of the fraction that is really being measured (i.e. C_{TWA}) and its relevance, particularly the ranges of fractions going from the free-available concentration (C_{free}) to the total concentration. Moreover, we can also consider the modeling of the fate of microcontaminants and the links of these values with the accumulation in aquatic organisms, especially when bioavailability and bioaccesibility are discussed (Claessens et al., 2015a; Jin et al., 2015). Last but not least, we should also recall the low recognition of PS by the regulators (Booij et al., 2016). In the case of the EU Water Framework Directive it is considered as a complementary tool and the need of further research is explicitly addressed in the last revision of this directive (2013/39/EC). However, there is a lack of compliance to estimate the Environmental Quality Standard (EQS) for the priority contaminants since the standard values refer to total concentrations, except for metals, though the risk of toxicity for aquatic organisms is based on the bioavailable fraction (Jones et al., 2015).

Bearing in mind that sampling sites are often located far from the laboratory, it is highly desirable to develop cost-effective monitoring campaigns. Fortunately, PS devices are designed to meet this goal. In comparison to automatic samplers, PS implies the use of miniature devices with no dependence of power supply and with easy use, and hence, the sampling protocols can be easily standardised. Besides, once the sampling period is completed, the sampling devices can be transported effortless to the lab and conserved and stored in the lab until their analyses. Their analysis can be directly performed by chromatographic techniques (if the target compounds are organic contaminants).

Nonetheless, there are remarkable efforts to standardize the PS and to gain a position far beyond from a complementary tool in environmental monitoring as it has been pointed by the NORMAN position (Vrana et al., 2009; Miège et al., 2015) paper and a recent intercomparison study (Vrana et al., 2016a). In addition to this, PS is also a

promising tool in modelling the fate of contaminants in marine environments (Claessens et al., 2015a), the application of the PS together with other non-target approaches such as effect-directed analysis (EDA) and within the framework of the EU-WFD (Brack et al., 2016; Brack et al., 2017), or in the extension from the sampling to the dosing approach in toxicological studies (Claessens et al., 2015b).

8.2 Passive Sampling Devices

As reviewed before, passive samplers are devices that work in a self-governing manner sampling and accumulating the target compounds in a receiving phase. Three requirements must be fulfilled to calculate reasonable estimates of ambient concentrations of analytes from the concentrations measured in a passive sampler:

- Concentrations in the sampler must be proportional to environmental concentrations whilst the associated rate constants for chemical exchange and partition coefficients must be independent of ambient analyte concentrations.
- Calibration data (sampling rates or partition coefficients) applicable to site conditions must be available for the analytes.
- The sampling process should not significantly reduce analyte concentrations in the medium sampled.

The uptake rate of analytes depends on the sampler design, physicochemical properties of the analytes and environmental variables. Passive samplers are designed to maximise the amount of analyte sampled in order to detect the generally low levels of analytes present in water. At the same time, they must ensure a quantitative correlation between the mass of chemical accumulated and its concentration in the sampled medium (Lohmann et al., 2012). The rate at which sampler-water equilibrium is attained depends on the contaminants, deployment conditions and the PS device used. For highly hydrophobic compounds and for thick nonpolar samplers, equilibrium attainment can

take months to years. In this case, the sampling device yields a time weighted average water concentration over the exposure period. By contrast, equilibrium can be attained within hours to days for less hydrophobic compounds and for thin passive samplers. The results obtained by equilibrium extraction are comparable to those obtained by grab sampling, and therefore this type of device is unsuitable for the determination of C_{TWA} concentrations of pollutants in the environment.

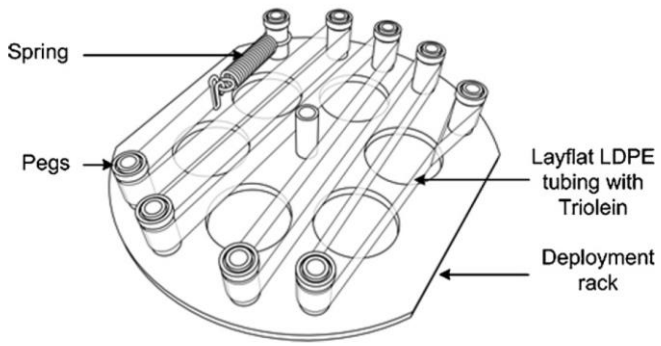

Figure 8.1. Design of a SPMD rack (Seethapathy et al., 2008).

8.2.1 SPMD

Semipermeable membrane device (SPMD) is a widely used passive sampler used for field water sampling that consists of a lay-flat LDPE tube filled with high purity triolein and sealed at both ends (see Figure 8.1). Triolein has a high accumulation capacity for compounds with a high octanol/water partition coefficient (log K_{ow}> 3). The selectivity of the sampler is based on the size of target molecules and their ability to dissolve in the receiving phase. Despite its accuracy, the main disadvantage is the complex procedure that is required to recover the target analytes from the triolein: a dialysis procedure using solvents such as hexane. An alternative to that is solvent extraction of both the LDPE membrane and the triolein. A wide range of compounds, including PCBs, PAHs, organochlorine pesticides, dioxins and furans, among many other hydrophobic

compounds, have been analysed with SPMDs. SPMDs have also been used for sampling from air and soils (Ouyang et al., 2007; Seethapathy et al., 2008).

8.2.2 POCIS

The polar organic chemical integrative sampler (POCIS) comprises a solid receiving phase material (sorbent) contained by two microporous PES membranes held with two stainless steel rings (see Figure 8.2). PES membranes have a low surface energy and thus, the effect of biofouling on their surface during field use can be reduced significantly. The type of sorbent used can be changed to target certain compounds or chemical classes specifically. Two configurations are commonly used:

- A 'generic' configuration for sampling most pesticides, natural and synthetic hormones, many wastewater related chemicals, and other water-soluble organic chemicals. It contains a mixture of three solid-phase sorbents (Isolute ENV + polystyrene divinylbenzene and Ambersorb 1500 carbon dispersed on S-X3 Biobeads).
- A 'pharmaceutical' configuration designed for monitoring drug residues. It contains a single (Oasis HLB) solid-phase sorbent (Alvarez et al., 2004).

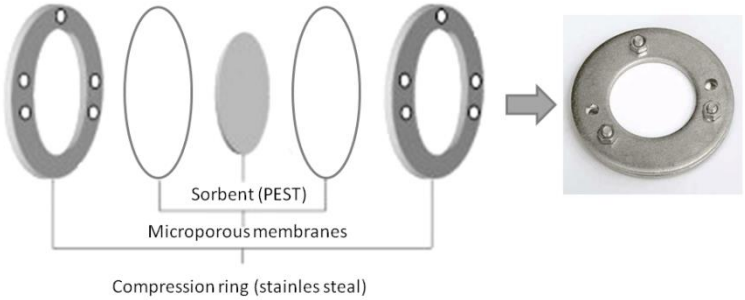

Figure 8.2. Assembly of a POCIS

After sampling, the sorbent is treated as a solid phase extraction (Mazzella et al., 2010). The uptake rates for more polar analytes are low (typically less than 1 $L \cdot d^{-1}$)

compared with the sampling of non-polar compounds by, for example, SPMDs. This can limit their usefulness in some applications, but, unlike non-polar compounds, polar compounds are usually present at higher concentrations, so that sampling rates below 1 $L \cdot d^{-1}$ are not an obstacle.

8.2.3 Chemcatcher

This device uses a diffusion-limiting membrane and a bound solid-phase receiving phase supported and sealed in place by an inert PTFE housing as shown in Figure 8.3. It was designed for sampling organic compounds from water. Accumulation rates and selectivity are regulated by the selection of both the diffusion-limiting membrane and the solid-phase receiving material (Kingston et al., 2000).

One configuration is used for the sampling of non-polar organic compounds with log K_{ow} > 4. It is based on a 47 mm C18 Empore disk as receiving phase and an LDPE diffusion-limiting membrane. The C18 Empore disk has a high affinity and capacity for nonpolar organic pollutants. Another design can be applied for the sampling of more polar organic contaminants with log K_{ow} values between 2 and 4. It combines a 47 mm C18 Empore disk as the receiving phase with a polyethersulphone diffusion-limiting membrane (Kingston et al., 2000).

Other devices are being constructed for analysing a range of emerging pollutants, including alkylphenols, antiinflammatory drugs and other pharmaceuticals, polybrominated flame retardants, steroids, sulphonamides and metals such as mercury, tin and their organometallic species (Liscio et al., 2014; Morrison and Belden, 2016; Vallejo et al., 2013).

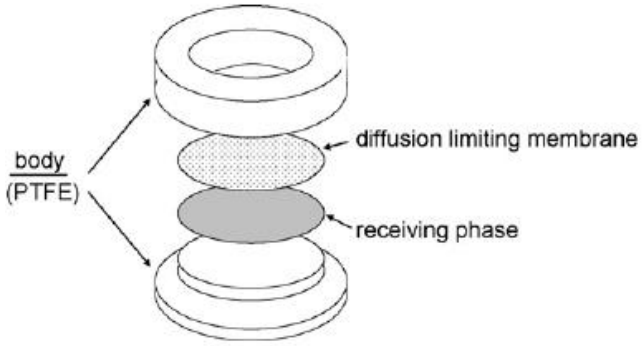

Figure 8.3. Parts of a Chemcather

8.2.4 MESCO

Membrane-enclosed sorptive coating device (MESCO) is an adaptation of the SPME technique, with the aim of enabling integrative PS of hydrophobic organic pollutants (Vrana et al., 2001). The device consists of different types of silicone collecting phases: stir bars (Assoumani et al., 2016; Vrana et al., 2001; Vrana et al., 2016b), ST (Wennrich et al., 2003), or SR (Allan et al., 2009; Paschke et al., 2006; Wennrich et al., 2003) enclosed in a protective membrane. In the earliest version, it was made of regenerated cellulose (Allan et al., 2009; Vrana et al., 2006; Vrana et al., 2001), but in latter studies cellulose was replaced by low density polyethylene (LDPE), as it turned out to be more stable against biodegradation and solvents (Wennrich et al., 2003). The receiving phases may be surrounded by air or water within the bag (Brown et al., 2001). In Figure 8.4 MESCO devices consisting of stir bars enclosed in LDPE bags are shown.

The miniature MESCO sampling system combines sampling with solventless pre-concentration. The sampler enables direct analysis of the accumulated contaminants by thermodesorption coupled on-line to GC, thereby avoiding time-consuming sample preparation and clean-up. Despite the small surface area and volume of the sampler, its sensitivity is comparable to other PS systems, since the entire amount of analyte contained in the receiving phase is introduced into GC and subsequently detected.

Apart from MESCO samplers, silicone material can also be applied without membrane protection for TWA sampling. The use of silicone sheets, mats, etc. for field sampling is usually related to a large solvent consumption for back-extraction of the trapped analytes (Booij et al., 2002). In contrast, the use of SB or small SR/ST pieces which can be processed in the solvent-free/reduced manner simplifies the sample treatment and analysis step.

In this framework, it was reported that the uptake rate of bare SR pieces are approximately ten times higher than those of the membrane-enclosed ones (Paschke et al., 2006). Recently, bare SBs have been applied for the determination of a range of pesticides in surface water (Assoumani et al., 2015; Assoumani et al., 2013).

Owing to the lower surface area compared with their analogues in sheet form, Van Pinxteren and Paschke suggested the application of this type of devices for the monitorization of contaminants in aquatic environments where elevated concentrations may be expected, such as sewage/storm water effluent (van Pinxteren et al., 2010).

In order to obtain a maximum sensitivity with this technique, the design of the PS device should have a high A/L ratio (so called badge type samplers) where A is the area and L the length of the device. Thus, since the amount of analyte sampled is directly proportional to the surface area of the sampler, tube type samplers are less sensitive than badge type samplers (Chimuka et al., 2008). However, badge-type samplers with a large surface area are more affected by fluctuations in water velocity (Vrana et al., 2005).

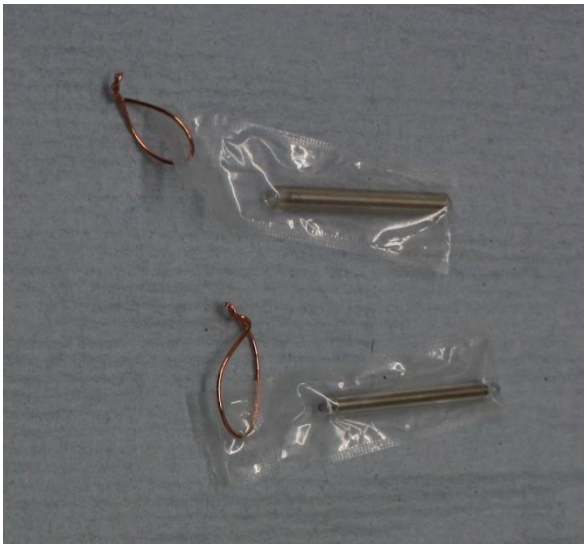

Figure 8.4. MESCO passive samplers

8.2.5 Polyethylene diffusion bags

During the accumulation of volatile organic compounds (VOCs) from groundwater, loss of volatiles hampers the achievement achieving proper estimates. In this sense, polyethylene diffusion bag (PDB) samplers help to overcome this limitation (Vroblesky and Hyde, 1997). The PDB sampler consists of a LDPE membrane sealed with the shape of a long cylindrical bag, filled with deionised water acting as the receiving phase. This bag acts as a semi-permeable membrane, allowing the diffusion of most chlorinated VOCs. Usually, PDBs take about 2 weeks to equilibrate in an aquifer (Divine and McCray, 2004).

8.3 Theoretical background

PS involves the accumulation of target analytes in an appropriate medium inside the passive sampler (a solvent, a chemical reagent or a porous adsorbent), known commonly as receiving phase. When PS is used, there is a free flow of analyte molecules from the sampled medium to a collecting medium, as a result of a difference in chemical

potentials of the analyte between the two media (Górecki and Namiesnik, 2002). The principle of operation of passive samplers is based upon mass transport, described by Fick's first law of diffusion (Equation 8.1).

$$J = \frac{dn}{dt} = -DA\frac{\partial c}{\partial x} \quad (8.1)$$

where *dn* is the amount of analyte crossing an area *A* (m^2) in time *dt*, *D* (m^2/s) is the diffusion coefficient, and *∂c/∂x* is the concentration gradient of the analyte.

The net flow of analyte molecules from the sampled medium to the receiving phase continues until equilibrium is reached in the system, or until the sampling period is stopped. Currently, two main modes of PS can be distinguished: kinetic sampling and equilibrium sampling. In the following lines, two possible approaches are detailed for describing the physicochemical processes involved in the extraction of the analytes from the medium being sampled: the kinetic approach and the mass transfer approach.

8.3.1. Kinetic approach

Pollutant adsorption or absorption from water into a passive sampler can be generally described by the pattern shown in Figure 8.5, where two regimes are differentiated: (i) a kinetic regime where a linear stage is followed by a non linear stage and (ii) an equilibrium regime after the kinetic regime (Vrana et al., 2005).

The exchange kinetics between a passive sampler and the water phase represented in Figure 8.5 can be described by the following first order mathematical model (Booij et al., 2007):

$$C_s(t) = C_w\frac{k_1}{k_2}(1 - e^{-k_2 t}) \quad (8.2)$$

where $C_S(t)$ is the concentration of the analyte in the sampler at exposure time t, C_w is the analyte concentration in the aqueous medium, and k_1 and k_2 are the uptake and release rate constants, respectively.

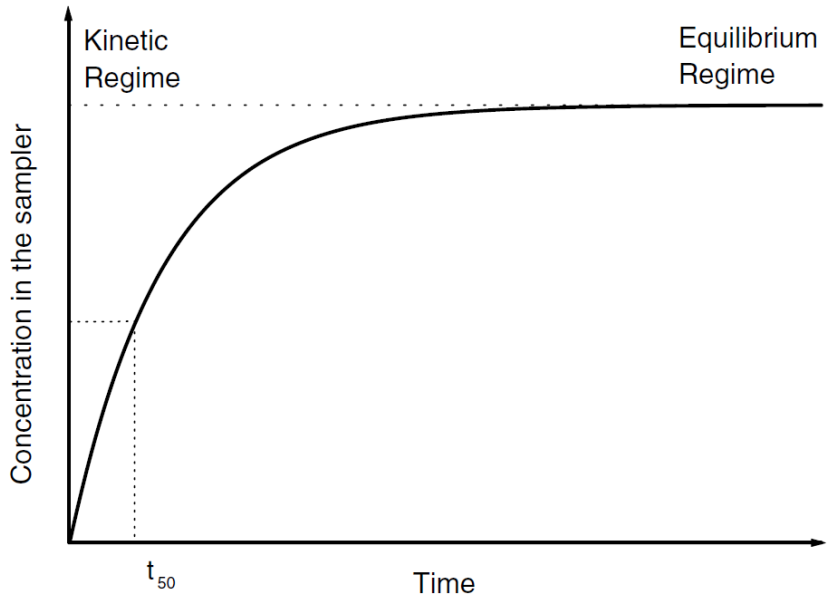

Figure 8.5. Variation of the uptake rate versus exposure time in a passive sampler (Vrana et al., 2005).

In the kinetic sampling regime, it is assumed that the rate of mass transfer to the receiving phase is linearly proportional to the difference between the chemical activity of the contaminant in the water phase and its activity in the receiving phase.

In the initial phase of the exposure, when the passive sampler works in the linear uptake regime, the desorption rate of analytes from the receiving phase to water is negligible. Thus, the amount of analyte accumulated is linearly proportional to its TWA concentration in water so that the Equation 8.2 can be simplified as follows:

$$C_s(t) = C_w k_1 \, t \quad (8.3)$$

where $C_s(t)$ is the concentration of the analyte in the sampler at exposure time *t*, C_w is the TWA concentration of the target compound analyte and k_1 is the first order contaminant uptake constant. This equation can be expressed in terms of mass accumulated $N_s(t)$ in the PS device as follows:

$$N_s(t) = C_w R_s \, t \quad (8.4)$$

where R_s is the proportionally constant (sampling rate), which is the product of the contaminant uptake constant (k_1) and the volume of water that gives the same chemical activity as the volume of receiving phase. R_s may be interpreted as the volume of water cleared of analyte per unit of exposure time by the sampler (Vrana et al., 2005).

Thus, according to the equation 8.4, if the R_s value of a target compound is previously known, the C_w can be calculated taking into account the exposure time *t* and *Ns(t)*, i.e. the exposure time and the amount of the analyte trapped in the receiving phase.

R_s values are not dependent on the C_w values for PS devices operating in the kinetic sampling mode. However, R_s values are often affected by environmental factors (water flow or turbulence, temperature and biofouling to name a few), the passive sampler design and the physicochemical properties of the analytes (Vrana et al., 2005), as it will be discussed afterwards.

In equilibrium conditions, the exposure time assures the achievement of the thermodynamic equilibrium between the sampled medium and the receiving phase. Reaching these conditions, and if the analyte concentration in the sampling medium does

not fluctuate once equibrium has been reached, it allows measuring the concentration of the analyte at the time of retrieval from the environment. (Górecki and Namiesnik, 2002). In the later situation, Equation 8.2 can be simplified to the following one (Equation 8.5):

$$C_s(t) = C_w \frac{k_1}{k_2} = C_w K_{sw} \quad (8.5)$$

According to the Equation 8.5, if the sampler-water partition coefficient K_{sw} is known, the dissolved concentration of the analyte can be easily calculated. Thus, the requirements for applying PS approach in equilibrium conditions are: (i) reaching stable concentration of analytes after a known response time; (ii) using sampler with capacity enough to avoid depletion during analyte ad/absorption; (iii) using passive sampler with shorter response time than any other fluctuation in the medium being sampled.

8.3.2. Mass transfer approach

The mass-transfer process of PS devices can be understood as a multi-stage transport process (Booij et al., 2007). As illustrated in Figure 8.6, the PS device can be described as a central sorption phase surrounded by a semi-permeable membrane. Although passive sampler is usually exposed in the environment inside a protective cage, it is very difficult to avoid the formation of biofouling film over the semi-permeable membrane of sampler during the exposure.

Briefly, in the simplest case, the mass transfer process occurs as follows. First, analytes present in the bulk water media interact with the PS device (e.g. a single polymeric phase). Close to the PS device, in the so-called water boundary layer (WBL) the transport of the analytes from the bulk phase in reduced since the convective process is minimized and the diffusional one becomes more important. Once the analytes reach to

the surface of the receiving phase, the sorptive processes can be either absorption in the new bulk phase or adsorption at the surface (Booij et al., 2007).

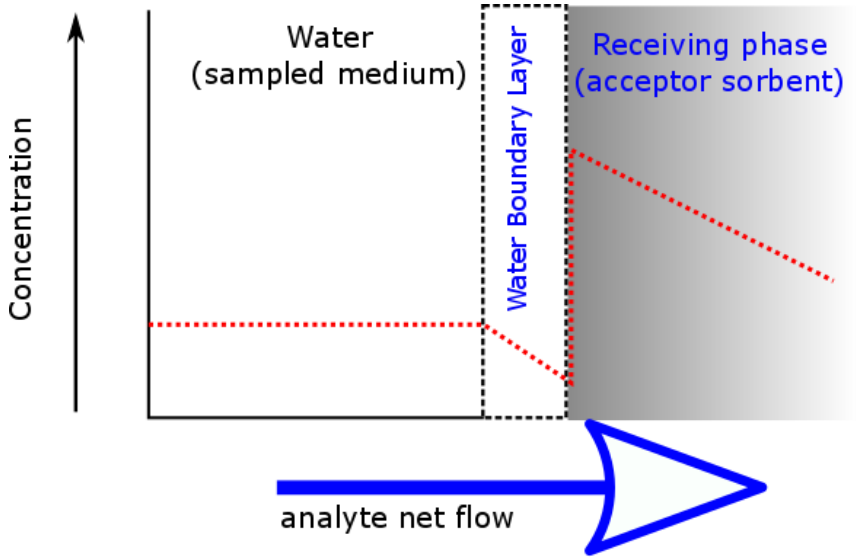

Figure 8.6. General scheme of concentration profile in a simple (one single phase) PS devices. The red dashed line represents the concentration profile from the bulk solution to the sorbent.

However, this general mass-transfer scheme may differ as new layers are interleaved. For instance, in many cases protective cages and biofouling layers are present, the receiving phase can be naked (e.g., LDPE and PDMS strip samplers or solid-phase microextraction devices) or covered by a protective membrane (e.g., MESCO and Chemcatcher samplers) (Booij et al., 2007).

Mass transfer coefficients k_i are frequently used to link the flux j_i through a phase i to the concentration difference ΔC_i between the end points of that phase, according to this:

$$j_i = k_i \Delta C_i \quad (8.6)$$

As suggested by Equation 8.6, mass fluxes j are linearly proportional to the driving force (ΔC_i). The mass transfer coefficient (k_i) can be interpreted as a conductivity term, with the dimension of a velocity (e.g., cm/day) (Booij et al., 2007).

The differential equation that governs the uptake process can be written as follows:

$$\frac{dC_s}{dt} = \frac{Ak_0}{V_s}\left(C_w - \frac{C_s}{K_{sw}}\right) \quad (8.7)$$

where C_s and C_w are the volume based contaminant concentrations in the receiving phase and in water respectively. V_s is the volume of the receiving phase, A is the sampler's surface area, and K_{sw} is the receiving phase-water partition coefficient.

The overall mass-transfer coefficient k_0 takes into account the resistance (or conductivities) of all the layers that are crossed from the feeding phase to the receiving one. For the case depicted in Figure 8.6, this can be written as follows

$$\frac{1}{k_0} = \frac{1}{k_{wbl}} + \frac{1}{k_s K_{sw}} \quad (8.8)$$

where k_{wbl} *and* k_s are the mass-transfer coefficients for the WBL and the receiving phase, and K_{sw} is the receiving phase-water partition coefficient. Hence, the resistance to mass transfer for all these layers is an additive value and does not depend one on another (K_{ow}< 4.5) (Booij et al., 2002) or on the aqueous boundary layer (when analyte log K_{ow}> 4.5). In case of samplers with a thick biofilm layer and analytes having log $K_{ow} \approx 6.0$, the biofilm can have the greatest impact on the sampling rate of the analyte (Huckins et al., 2002). Environmental conditions, such as water flow or biotic contamination of the membrane, influence the thickness of the aqueous boundary layer and the uptake rate of

the analyte under investigation. It should be noted that the maximum sampling rate can be obtained for samplers in which the limiting barrier is the aqueous boundary layer (Paschke et al., 2006).

Since the mass-transfer coefficient equals the ratio of a diffusion coefficient and an effective phase thickness δ, Equation 8.8 can also be written as:

$$\frac{1}{k_0} = \frac{\delta_{wbl}}{D_{wbl}} + \frac{\delta_s}{D_s K_{sw}} \quad (8.9)$$

A general solution to Equation 8.7 for constant C_w is:

$$C_s = K_{sw} C_w [1 - e^{-k_e t} + C_0 e^{-k_e t}] \quad (8.10)$$

where C_0 is the concentration at t = 0 and the elimination rate constant k_e is given by:

$$k_e = \frac{A k_0}{K_{sw} V_s} = \frac{R_s}{K_{sw} V_s} \quad (8.11)$$

Equation 8.10 shows that the uptake from the environment and the elimination of the initial amounts (found in the passive sampler fabrication controls) are additive. In the same way, this equation also shows that the uptake and elimination process of a particular compound are characterized by the same K_{sw} value. This observation is the basis of estimating in situ sampling rates from the dissipation rates of performance reference compounds (PRCs), as described in section 4.8.

When the initial concentration equals to 0, Equation 8.10 takes this form:

$$C_s = K_{sw} C_w \left[1 - e^{\left(-\frac{R_s t}{K_{sw} V_s}\right)}\right] \quad (8.12)$$

which in the short time limit reduces to the linear uptake:

$$C_s = \frac{C_w R_s t}{V_s} \quad (8.13)$$

Aqueous concentrations can be calculated from the amounts N_s absorbed by the sampler, the in situ sampling rate of the compounds and their sampler-water partition coefficients:

$$C_w = \frac{N_s}{K_{sw} V_s \left[1 - e^{\left(\frac{-R_s t}{K_{sw} V_s}\right)}\right]} \quad (8.14)$$

For equilibrium samplers, $\left(\frac{-R_s t}{K_{sw} V_s}\right)$ equals 1 to good approximation, and aqueous concentrations are calculated from $C_w = N_s/K_{sw}V_s$.

For kinetic samplers, operating in the linear uptake mode, the term in square brackets is approximately equal to $R_s t/(K_{sw}V_s)$, and aqueous concentrations can be calculated from:

$$C_w = \frac{N_s}{R_s t} \quad (8.15)$$

The denominations in the final equations can be interpreted as the apparent water volume that is cleared of analyte during the exposure. In the case of equilibrium sampling this volume is limited by the sorption capacity of the sampler $K_{sw}V_s$. For kinetic sampling, the apparently extracted water volume is limited by the sampling rate and the exposure time $R_s t$.

8.4 Performance reference compounds for the correction of environmental factors

Even the R_s values can be accurately determined for each target compound at lab conditions, when PS devices are exposed in the environment the uptake conditions can be slightly or even highly modified. For this reason, when PS is used under varying environmental conditions, its performance may be influenced by a range of environmental factors such as water temperature, biofouling or fluid flow across the sampler, to name a few. Consequently, these factors must be thoroughly evaluated to ensure the reliability of PS.

PRCs have been applied to account for environmental factor effects on the uptake rate of passive samplers. To meet this goal, it is necessary to calibrate the uptake kinetics in both laboratory and field situations by spiking devices prior to sampling with appropriate PRCs (Booij et al., 2002; Huckins et al., 2002). In this way, the uptake of analytes in the receiving phase and the desorption of PRCs from the sampler will be affected in the same way, and hence, the uptake kinetics in real environmental conditions can be corrected by using the offloading kinetics of PRCs (Vrana et al., 2005).

Generally, a proper correction of uptake kinetics is only performed when a sampler exhibits isotropic exchange of targeted chemicals. This means that both the uptake and release of chemicals obey first-order kinetics and elimination and uptake constants are the same as shown in Figure 8.7.

In general an appropriate PRC must ideally meet some characteristics: (i) to show similar physic-chemical parameters as the target compounds; (ii) to be an isotopic analogue of the target analyte; and (iii) not to be present in the environment. In fact, the ideal PRC would be an isotopic analogue of the target analyte. Besides, the sorbent in the passive sampler should have low enough sorption strength for depuration of the PRC into the sample matrix when it is exposed. For this reason, the use of PRCs in passive samplers

containing sorbents based on adsorption, such as activated carbon, is ruled out, but they can be useful when using devices such as SPME and SPMD, where analyte partitioning is involved (Seethapathy et al., 2008).

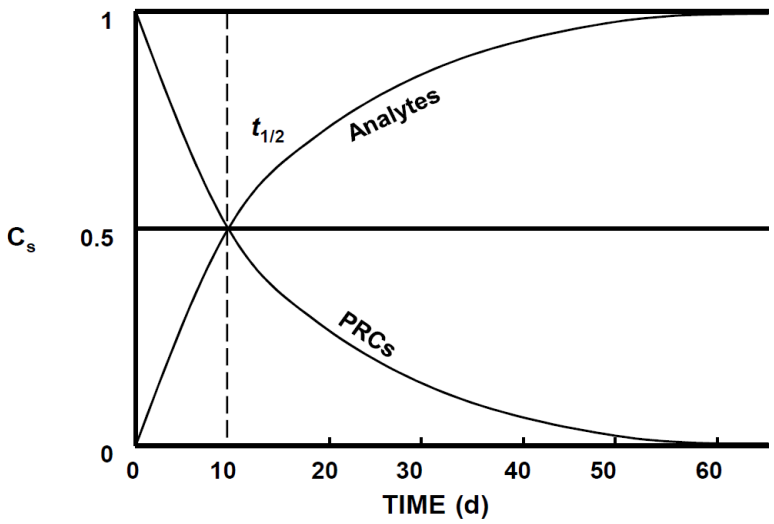

Figure 8.7. Illustration of isotropic exchange kinetics (Huckins et al., 2005).

More in detail, the elimination of PRCs from the receiving phase is inferred from Equation 8.2 as follows (note that M_0 initial mass and $C_w = 0$ since PRCs are loaded to the sampler prior to exposure):

$$M_s(t) = M_0(e^{-k_2 t}) \quad (8.16)$$

where M_s is the mass of PRC in the receiving phase after the exposure and M_0 is the initial mass of PRC present in the sampler before being exposed.

Then, corrected R_s values are calculated using the Equation 8.17, where $k_{2,lab}$ is the elimination constant determined under controlled conditions in the laboratory, and $k_{2,field}$ is the elimination constant obtained in the field measurements:

$$R_s(corrected) = R_s\left(\frac{k_{2,field}}{k_{2,lab}}\right) \quad (8.17)$$

Unfortunately, PRC approach cannot be used when PS devices are applied for polar organic compound analysis. In this case, the device is based on analyte diffusion through microporous membranes and on the strong sorption of the polar contaminants to a selective adsorbent material. In fact, the accumulation of polar organic compounds by adsorbents is more complex than the absorption of hydrophobic chemicals in non-porous polymers such as LDPE or PDMS. Adsorption distribution coefficients are obtained from sorption isotherms and they depend on the concentration of the analyte. Besides, competitive adsorption of non-target analytes cannot be ruled out (Lohmann et al., 2012). In this sense, the application of PS to measure pharmaceuticals, PCPs and other polar chemicals such as polar pesticides or alkylphenols in various aquatic matrices is a challenge nowadays.

A further drawback is that there is a lack of theoretical models able to predict the uptake of a chemical compound into a POCIS or Chemcatcher®, based on the physicochemical properties of the compound. There is a scarcity of reliable uptake rates available in the literature for polar compounds, so extensive calibration experiments in the laboratory are required to measure compound specific uptake rates, and to evaluate the effects of temperature, turbulence and salinity, before the samplers can be used in the field to measure TWA concentrations (Morin et al., 2012). Consequently, the utility of these samplers is limited beyond screening or semi-quantitative assessment of pollutants.

However, the scientific community is constantly attempting to overcome these inconveniences. Some groups have reported that deuterated (d5) deisopropylatrazine can be preloaded in the receiving phase to act as PRC for POCIS (Mazzella et al., 2010). Others

suggested a modification of the sorbents to allow the use of less sorptive substances, like C18 and silicone, as well as mini SPMDs mounted in POCIS rings to act as PRCs for correction of the uptake rates (Seethapathy et al., 2008).

Another alternative, instead of performing laboratory calibration experiments, in which it is impossible to simulate the variable conditions that characterise the real scenarios being sampled, is the performance of in situ calibrations. If the field concentration of a substance is known to be relatively constant, then in situ calibration is a possibility Although it implies a tedious procedure in the field, it provides useful data, especially when correction of field measurements is not feasible using PRCs. It is a useful technique for compounds that are difficult and expensive to obtain for laboratory analysis, such as human metabolites of pharmaceuticals. Typically, samplers can be deployed in the influent or effluent of a WWTP to obtain such calibration data. In situ calibration may also be attractive in other complex matrices such as estuarine, halo-saline and marine environments where salinity may influence uptake kinetics (Mills et al., 2014).

8.5 Main environmental factors that affect the uptake

It is worth noting that all passive samplers are influenced by environmental parameters, to a greater or lesser extent depending on their configuration (in particular the type of membrane, porous or semi-permeable for either polar or hydrophobic chemicals), targeted analyte characteristics (polar or hydrophobic) and variability of the studied parameters in each exposure case (Lissalde et al., 2016). Among potential factors that affect uptake kinetics into passive samplers, hydrodynamics and fouling are the most important ones (Jálová et al., 2013).

Biofouling occurs when unprotected surfaces submerged in water become colonised by different bacteria, flora and fauna, leading to the formation of a biofilm. The thickness of this biofilm varies from not only exposure to exposure but also spot to spot

on the same diffusion-limiting membrane, and its composition depends on the aquatic system (Vrana et al., 2005). Due to the increase of the thickness of the membrane in the passive sampler, biofouling can affect the overall resistance to mass transfer, blocking any water-filled pores in the diffusion-limiting membranes.

When the membrane of a sampler is made of a degradable material, colonising organisms may damage its surface. For this reason, the selection of suitable construction materials for the sampler device may allow to reduce to some extent the effect of biofouling in PS. For example, polyethersulphone used in one design of the Chemcatcher and in the POCIS is less likely to fouling than polyethylene used in SPMDs (Alvarez et al., 2005). Another solution to fight against biofouling relies on the use of solvent-filled membrane devices, which are based on a slow release of an inhibiting solvent, such as hexane, from the sampler during the exposure. In addition, some protective screens made of copper or bronze mesh have also been proposed to inhibit biofouling; however, their use is restricted when monitoring heavy metals.

The other main factor that affects the uptake kinetics during the exposure is the water flow or hydrodynamics. In this sense, several authors have analysed the impact of hydrodynamics on the sampling rates. Vermeirssen et al. reported for POCIS that the sampling rate of polar compounds increases with increasing flow rate (Vermeirssen et al., 2008). Regarding the non-polar compounds, Huckins et al., found that changes in the flow velocity-turbulence of the exposure medium affect the effective thickness of the external boundary layer of a PS device. Since mass-transfer resistance is directly proportional to boundary layer thickness, the sampling rates of analytes will vary with the hydrodynamics of the deployment site. Under boundary layer control, sampler design features other than the external surface area for chemical exchange will have little or no effect on linear uptake rates (Huckins et al., 2002). In the case of MESCO, it was found that MESCO samplers are minimally or even not affected by the variation of the water flow. However,

they did not exclude the possibility of a greater effect of hydrodynamics under exposure conditions very different from their study, e.g. in highly turbulent water (Vrana et al., 2006).

Other factors that have influence in the uptakes are the temperature and the salinity of the sampling media. Though the "salting-out" effect has been considered negligible in terms of the variation of the partition coefficients in solid-phase microextraction (SPME) studies (DiFilippo and Eganhouse, 2010; Lohmann, 2012), the effect of the variation of temperature and salinity it has been recently pointed with silicone rubber samplers (Jonker et al., 2015) based on partition coefficients. According to this last work, the partitioning of hydrophopic organic contaminants increases with a decrease in temperature and an increase in salinity. In both cases, the aqueous solubility of non polar compounds is reduced, as a result of their hydrophobicity, and thus their affinity for a hydrophobic phase, such as a polymer, increases (Jonker et al., 2015).

In this sense, researchers have suggested and performed adjustments of K_{sw} values to site- or experiment-specific conditions, based on calculations using the Van 't Hoff equation to correct for the effect of temperature (Lohmann, 2012) and the empirical Setschenow relationship to correct for the effect of salinity (Perron et al., 2013). Others have attempted to experimentally quantify the actual effects for a specific polymer (Booij et al., 2003). Although one would expect the effects to be similar for different polymers, as they presumably are mostly driven by an alteration of the aqueous solubility and not by a change in polymer characteristics (Lohmann, 2012), contrasting information has been obtained by different researchers for different polymers. In summary, there is no scientific consensus on the existence, extent, and generality of the effects of temperature and salinity on the partitioning of HOCs between water and different polymers.

On the other hand, uptake calibration experiments in salt water were recently performed in order to assure the suitability of Chemcatcher® to monitor hydrophobic pollutants in fresh and salt water (Petersen et al., 2015). In addition, Arp and coworkers concluded that for the POM polymer based samplers, regardless of the mechanism, the influence of temperature is similar in magnitude to other passive samplers, being within a factor of 2 per 10°C (Arp et al., 2015).

8.6 Application of PS devices

PS devices must be calibrated for each analyte in the laboratory in order to provide data about the concentration of the target analytes of the field during the sampling campaign. In this sense, the calibration methodology varies depending on the sampling regime, this is, kinetic or equilibrium conditions.

Working at equilibrium conditions imply reaching thermodynamic equilibrium of the analyte between the sampled medium and receiving phase, which supposes long sampling periods from days to months depending on the sampling device used. The concentration of analytes found with this strategy is often statistically comparable with those obtained by grab sampling techniques, and hence, it cannot be used for the determination of TWA concentration of pollutants in the environment (Vrana et al., 2005).

However, in many PS methodologies the equilibrium conditions are not immediately achieved so that working in the linear uptake regimens (i.e. in kinetic conditions) is often preferred. In this situation, it can be assumed that the rate of mass transfer or sampling rate R_s remains constant throughout the sampling period, and the relationship between C_w (the concentration of target analytes in water) and N (the amount of analytes in the receiving phase) are proportional to R_s. Thus, to determine the TWA concentration of the target analytes with kinetic samplers, the calculation of R_s values is a must. In this sense, the use of equilibrium passive samplers is extended because a sampling campaign can be

accomplished without performing the calibration experiments required for calculating R_s values. Data about partitioning between the receiving phase and water is available for several pollutants.

R_s constants are carefully determined at lab conditions, where contaminant exposure conditions (i.e., water temperature, water flow rate and nominal concentration of the target analyte in exposure system) are accurately controlled (Kot-Wasik et al., 2007). In the simplest approximation, sampler devices are exposed to a continuous flow of contaminant at a constant flow rate of contaminants to determine R_s values(Gunold et al., 2008; Vermeirssen et al., 2012; Vrana et al., 2006), although other approaches based on the use of semi-empirical correlations between mass-transfer coefficients, physicochemical properties (mainly diffusivities in various media) and hydrodynamic parameters can also be used. However, the complexity of the water flow around PS devices during exposure (usually non-streamlined objects) makes it difficult to estimate uptake parameters from first principles (Distribution constants, hydrophobicity…) (Vrana et al., 2005).

On the other hand, the use of passive samplers can provide information about the physicochemical distribution of organic pollutants between particulate, dissolved and colloidal phases in the sampled matrix. In order to sample a desired fraction of an analyte among the different phases, the selectivity of sampling devices can be adjusted by selecting membrane materials with the required properties (e.g., pore size and charge on the surface). Most passive samplers accumulate only the truly dissolved fraction of chemicals, since only dissolved molecules are sorbed by the receiving phase. In addition, when samplers contain a membrane that separates water from the receiving phase, the truly dissolved molecules get released from colloids and particles during their diffusion across the membrane (Vrana et al., 2005; Vrana et al., 2014).

After a monitoring campaign pre-concentrated extracts are obtained from the elution of receiving phases of passive samplers (those used to measure organic pollutants). Apart from being used for the quantification of the target compounds, these extracts can be combined with a variety of bioassay procedures in order to assess the level and the biological effects of water contaminants (Emelogu et al., 2013b; Sabaliünas et al., 1998). For instance, in some in vitro bioassays used to evaluate the health of an ecosystem, it can be difficult to obtain suitable water samples for testing. Actually, most hydrophobic organic contaminants are present in aquatic environment only at trace levels (i.e., < 1 µg/L). Therefore, to be able to perform the bioassay, several litres of water should be extracted to yield sufficient amounts of the analyte. Fortunately, the use of extracts from passive samplers can overcome this problem (Emelogu et al., 2013a; Emelogu et al., 2013b).

Besides, the concentration of contaminants separated by passive samplers can provide valuable information about the baseline toxicity of chemicals, based on total body residue estimate (Leslie et al., 2004).

8.7 Selection of suitable materials for PS devices

As reviewed before, the characteristics of the acceptor phase and its surrounding membranes have a direct influence in the performance of the passive sampler. The selectivity and accumulation capacity are defined by the diffusion properties of the membrane and the affinity of the receiving phase to the analytes. In this sense, the study of the performance of different materials both for membranes and collecting phases will improve the operation of existing and forthcoming passive samplers. For instance, a more polyvalent sampler which allows the collection of a larger number of analytes with different polarity in the same device will result in lower cost and sampling efforts.

The most common polymers employed in the design of passive samplers are silicone and polyethylene. Apart from these materials, recently polyoxymethylene (POM)

has been satisfactorily used for the PS of polar and non-polar compounds. POM presents high physical and chemical stability and a smooth and hard surface, which make it less sensitive to suffer from the trapping of particles or biofouling, in comparison to other samplers with rough or sticky surfaces (Cui et al., 2013). Nevertheless, little research on the use of this polymer is still published and thus, further studies will be required.

On the other hand, PES polymer, tipically used as diffusion limiting membrane in some devices, has been studied as an alternative to POM. It has shown a good performance acting as extracting polymer (Blanco-Zubiaguirre et al., 2014). Therefore, the suitability of PES as passive sampler should also be tested. In Figure 8.8 some of the passive samplers developed in this work using polymers in different formats are shown.

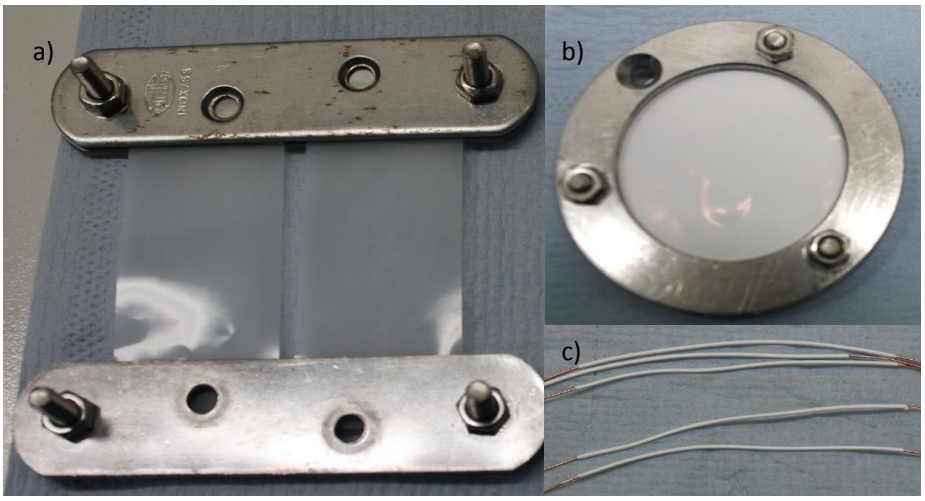

Figure 8.8. Single-phase polymer based passive samplers analysed in this work: a) POM strips, b) PES membranes and c) PES tubes

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9. UPTAKE CALIBRATION OF POLYMER-BASED PASSIVE SAMPLERS FOR THE MONITORISATION OF PRIORITY AND EMERGING ORGANIC NON-POLAR POLLUTANTS IN WWTP EFFLUENTS

In this chapter the uptake calibration of more than 12 non-polar organic contaminants by three polymeric materials is shown: bare polydimetilsiloxane (PDMS, stir-bars), polyethersulfone tubes and membranes (PES) and polyoxymethylene membranes (POM), both in their free form and Membrane Enclosed Sorptive Coating (MESCO). The calibration process was carried out exposing the samplers to a continuous flow of contaminated water at 100 $ng \cdot mL^{-1}$ for up to 28 days and, consequently, the sampling rates (R_s, $ml \cdot day^{-1}$) of several organic micro-contaminants were provided for the first time. In situ R_s values were also determined disposing the samplers in the effluent of a wastewater treatment plant. Finally, these passive samplers were applied to monitor the effluents of two wastewater treatment plants. This application leads to the confirmation of the presence of galaxolide, tonalide and 4-tert-octylphenol at high $ng \cdot mL^{-1}$ levels, as well as the identification of phthalates and alkylphenols at levels below the detection limits for active sampling methods.

9.1 Passive samplers

9.1.1 Polymeric materials

Three types of polymeric materials have been studied as passive samplers: PDMS, PES and POM. The PDMS stir-bars (SB hereafter, Gerstel, Mülheim an der Ruhr, Germany) used as passive samplers were 20 mm length and 0.5 mm film thickness size. The exposure of SBs to water samples was carried out in two forms: (i) free, held by a stainless steel wire, and (ii) enclosed in a LDPE membrane bag (4 cm x 1 cm) embedded in air as it is described elsewhere (Vrana et al., 2001; Wennrich et al., 2003). The former design is known as Mesco-Stir-bar (M-SB) design.

PES polymer was used as passive sampler in three different formats: (i) free PES tube (PES_t), (ii) PES tube enclosed in LDPE membrane (M-PES_t) and (iii) free PES membrane (PES_m). PES_t (0.7 mm o.d., Membrana GmbH, Wuppertal, Germany) were

carefully cut to obtain pieces of 7.5 cm length. All of them were accurately weighted (~ 40 mg) and those with mass differences higher than 10% were discarded. Then, a 0.5 mm diameter stainless steel wire was inserted inside the tubes to ensure a safe and reproducible support in different lab and field exposure assays. PES_t were enclosed in LDPE membrane bags (9 cm length x 1 cm width) embedded in air to build M-PES_t passive sampler. PES_m, commonly used as filtering disks (Supor® -100, 0.1 µm, 47 mm), were obtained from Pall Life Sciences (New York, USA) and they were supported in home-made stainless steel O-rings holders to build the sampler. The diameter of the surface area exposed was 42 mm^2.

POM based passive sampler was made using a thin sheet of POM (76.2 µm thick) purchased from CS Hyde Company (Illinois, USA). 60 mm long POM sheets were exposed fixed one next to the other using four stainless steel support plates tighten using four screws and nuts. The surface of POM exposed using this design was 40 mm long x 25 mm wide in each POM strip.

9.1.2 Passive sampler conditioning and preparation

All the polymers were cleaned before being used. SBs were cleaned with Acetonitrile (AcN): Methanol (MeOH) (80:20, v/v) mixture in an ultrasonic bath for 3 h and then, thermally conditioned under nitrogen stream (ca. 30 $mL \cdot min^{-1}$) at 290 °C for 3 h in a thermal condition unit (TC2 tube conditioner, Gerstel). PES_t and PES_m were kept in pure EtOAc for 72 h, renewing the solvent several times during that period. In the case of PES_m, a second cleaning step was accomplished using MeOH for 24 h. Finally, POM sheets were precleaned with pure EtOAc in an ultrasonic bath for 2 h and then maintained in clean EtOAc for 72 h. After the immersion of polymers in solvents or thermal conditioning, all the materials were dried with a lint-free tissue and stored in closed flasks to avoid contamination until being used as passive samplers.

Once SBs were conditioned they were exposed to a mixture of five deuterated PAHs (used as general purpose PRCs) before being used as passive samplers. SBs were immersed in chromatographic inserts containing 300 µL of a MeOH:H_2O (80:20, v/v) solution fortified with 100 ng of deuterated PAH. The vials were kept shaking for 100 h according to the procedure suggested in the literature (Allan et al., 2009; Booij et al., 2002). In the case of PES_t, 10 mL test tubes sealed with a single use cap and containing 7 mL of the solution indicated above were employed to upload the deuterated PAHs.

9.1.3 Exposure tank

Preconditioned passive samplers were placed in a stainless steel carrousel described in detail in previous works (Gunold et al., 2008; Vallejo et al., 2013). Afterwards, the carrousel was immersed in a stainless steel tank containing ~50 L of fortified water. The exposure of the passive samplers was performed in a dynamic flow system. The tank has two feeding inputs, one with fresh (tap) water with a flow of 5 $L \cdot h^{-1}$ and the second one with a cocktail of chemicals at the nominal concentration (100 $ng \cdot mL^{-1}$) in MeOH and with a flow of 10 $mL \cdot h^{-1}$ (Liquino, Metrom, Switzerland). In order to assure the steady state of the nominal concentration in the tank both inputs were mixed with the bulk water solution of the tank for 72 h before the exposure of the passive samplers. The carrousel was stirred at a constant rate of 50 rpm and the experiments were carried out at room temperature (18±2 °C).

9.2 Calibration of passive samplers

9.2.1 Laboratory calibration of passive samplers

The calibration studies were conducted over 14 days for SB, M-SB, PES_t, M-PES_t and over 28 days for PES_t, PES_m and POM. The samplers were deployed in a staggered consecutive design at 2, 4, 6, 8, 10, 12 and 14 days for SB, M-SB, PES_t and M-PES_t, and at 2, 4, 6, 8, 10, 12, 14, 20 and 28 days for PES_t, PES_m and POM. All the samplers were exposed using the exposure system described above, arranged in the carrousel in a

tangential way according to the rotation, equidistant from one to another and in series. Every time a sampler was retrieved, two passive samplers from each different design were collected. In addition, water aliquots of 500 mL from the exposure tank were collected over the exposure period in order to check any deviation from the nominal concentration. Finally, all the calibration experiments and sampling campaigns included field blanks, which accompanied the samplers during transportation, deployment and retrieval from the sampling sites. In all the cases, the amounts of analytes detected in the field blanks were negligible compared to the ones quantified in the samplers, making unnecessary blank corrections.

9.2.2 Field measurements and in-situ calibration of passive samplers

In order to test lab calibrated passive samplers and to supplement the laboratory calibrations, passive samplers were deployed in the WWTPs of Galindo and Gernika, both located in Biscay. The WWTP of Galindo collects industrial and urban wastewater from the metropolitan area of Bilbao and it includes a physical, biological and advanced treatment lines. On the contrary, the WWTP of Gernika is much smaller, collecting urban, industrial and rural wastewaters and includes only physical treatment lines.

For sampling purposes, all the studied passive samplers (i.e., SB, M-SB, PES_t, M-PES_t, PES_m and POM) were placed in a stainless-steel canister and deployed in the effluent of the WWTP under real environmental conditions for 7, 14 and 28 days (2 replicates per passive sampler were collected) in December 2013. Passive samplers were transported at ~4°C and stored at -20°C until analysis. Field blanks were taken in duplicate to the study site and exposed to air during deployment and retrieval of passive samplers. Together with the passive samplers, water grab samples were taken when passive samplers were deployed and removed (i.e. at days 0, 7, 14 and 28). Grab water samples were collected in pre-cleaned amber glass bottles and transported to the laboratory in

cooled boxes (~4°C). Samples were filtered through 0.45 µm cellulose filters (Whatman, Kent, UK) and kept in the fridge at 4°C before analysis, which was performed within 24 h.

For the *in-situ* calibration experiment, the passive samplers were immersed in the same exposure system described above, i.e. the tank, the pump and the carrousel were arranged next to the effluent output of the WWTP. This way, the effluent fed the tank at a constant flow and the carrousel was stirred at the same speed. SB, M-SB (both containing preloaded PRCs) and PES_t were exposed over 14 days to effluent fresh water under controlled hydrodynamic conditions. Two passive samplers of each type were retrieved every time on separate days: 1, 4, 6, 8, 11 and 14 days. 24 hour composed water samples collected by an automatic sampler were available for some of the sampling days, provided by the staff of the WWTP. The concentrations obtained from these samples were considered in the calculation of the *in-situ* sampling rates.

PDMS based samplers were directly analysed by thermal desorption – gas chromatography – mass spectrometry (TD-GC-MS) and the rest of polymers were done by large volume injection – programmable temperature vaporizer – gas chromatography – mass spectrometry (LVI-PTV-GC-MS). Water samples were analysed by means of a fully optimised method based on stir-bar sorptive extraction (SBSE) followed by TD-GC-MS. A detailed description of those procedures is available in Section 3.4.

9.3 Results and discussion

9.3.1 Dissipation of PRCs

Attending to the features of PRCs described in the literature (Huckins et al., 2002), the uptake of the target compounds and the elimination of their isotopically labeled analogues take place under isotropic exchange kinetic conditions. It is advisable that the mass release from the sampler during the exposure to be between 20 and 80% of the loaded mass to avoid either a complete depletion or negligible losses (Booij et al., 2007; Söderström and Bergqvist, 2004). Additionally, the regression fit to equation 8.16

(p. 186 in logarithmic way) should satisfy the statistical requirements for the estimation of the dissipation rate constant. Therefore, experimental regression fits with determination coefficients (r^2) below 0.90 were discarded.

The results obtained in this work with the 5 deuterated PAHs showed two different scenarios. On the one hand, some deuterated PAHs fulfilled the conditions to be used as PRCs for PDMS based samplers, either for free SBs and M-SBs. In fact, as shown in Figure 9.1, d-Phen provided dissipation rates within the expected range and with good fitting parameters during the complete exposure time (i.e., 14 days). In the case of d-Ace a high dissipation rate was obtained since the mass released was over 80 % after 4 days. Consequently, d-Ace offered an acceptable performance for sampling periods no longer than 3 days. Though both the losses and fitting standards for d-Cry and d-Per were within the acceptance criteria they showed high uncertainties and were disregarded. On the other hand, the deuterated PAHs did not meet the established criteria to be used as PRCs for PES_t samplers. In fact, all the studied PRCs showed very low dissipations (a net release < 1% in 14 days) and poor fittings ($r^2 < 0.4$).

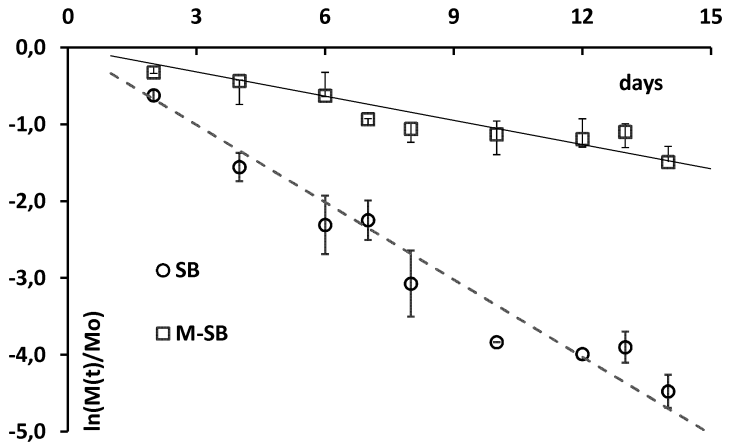

Figure 9.1. Dissipation of deuterated phenantrene from SB (k_2= -0.34±0.01) and M-SB (k_2= -0.105±0.005).

The two different patterns observed in this work respond to the different physical uptake/release mechanisms of the target compounds in the studied polymeric materials. In fact, PDMS accumulates the non-polar compounds through an absorptive process and it is assumed that adsorptions is the main retention mechanism when using POM and PES. An essential condition for using PRCs is that the overall uptake and release rates of chemicals are governed by first-order kinetics and the loss rates of the PRCs are under the same variability than the uptake of the analytes. This means that the resistance to mass transfer is the same for the chemical flux in and out of the sampler and thus, the receiving phase should have low sorption strength for releasing the PRC into the sample matrix during exposure (Alvarez et al., 2007; Assoumani et al., 2014). Consequently, this excludes the use of PRCs for sorbents based on adsorption mechanism due to the strong sorptive properties resulting in an anisotropic exchange, but allows their use in devices where analyte partitioning is involved (Seethapathy et al., 2008).

In order to overcome the lack of a direct correction mechanism for the application of these materials in environmental waters and to supplement the estimations obtained from those samplers in which the PRC correction was feasible, in-situ calibration of passive samplers was also performed in the effluents of WWTPs in order to obtain sampling rates directly from field conditions.

9.3.2 R_s calibration

All the R_s values obtained in this work are collected in Table 9.1 including the uncertainties (relative standard deviation, RSD). From those results, it can be highlighted that SBs showed a linear uptake for nine of all the target compounds (musks fragrances, organochlorine pesticides (4,4'-DDE, 2,4'-DDD, 4,4'-DDD, 4,4-DDT), phthalates (BBP, DOP) and octylphenol (4nOP)) with low uncertainties (RSD < 12%). As an example of this pattern, Figure 9.2 shows the linear uptake for 4,4’-DDT. For the rest of the tested compounds (i.e., 4tOP, HCHs, Chlorp and Chlorf) the linear fit was inadequate to provide

reliable R_s values (shown as lack of fit (l.f.) in Table 9.1). As mentioned in the literature (Paschke et al., 2006, Vrana et al., 2009), good linear fitting for PDMS polymer was obtained for those compounds that shown log k_{ow} > 5.0 (see Tables 3.1 and 3.2 from section 3), whereas the affinity of this sorptive material for more polar compounds (log k_{ow} < 5.0) was not so adequate.

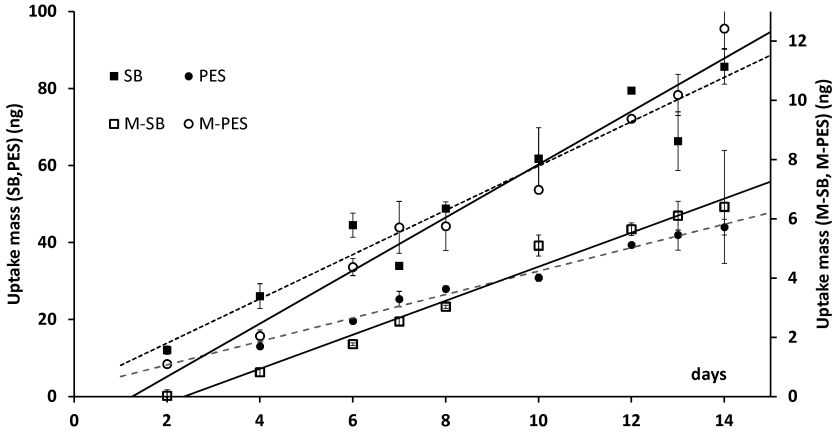

Figure 9.2. Uptake profiles obtained for 4,4'-DDT using SB and PES_t both in the free and enclosed modes. Left axis is for SB and PES_t and right axis is for M-SB and MPES.

Though this kind of experimental studies has been reported in the literature with other HOCs and more specifically with PDMS samplers (Assoumani et al., 2014; Vrana et al., 2001; Wennrich et al., 2003), most of the R_s values are reported for the first time. It is remarkable the fact that R_s values of HCH isomers were determined by Wennrich et al. (Wennrich et al., 2003) in MESCO-silicon tube and silicon-rod samplers with values ranging 0.7 – 5.5 mL·day^{-1}, and these results are below the lowest experimental R_s values reported in this work for any compound and using the same polymer.

Table 9.1. Sampling rates (in mL·day^{-1}) obtained for each type of passive sampler and including the log $K_{POM/W}$.

	SB		M-SB		PES_t		M-PES_t		PES_m		POM		
	Rs	RSD%	Rs	RSD%	Rs	RSD%	Rs	RSD%	Rs	RSD%	Rs	RSD%	log$K_{POM/w}$
4tOp	l.f.	l.f.	l.f.	l.f.	22	9	l.f.	l.f.	l.f.	l.f.	15	73	4.3
α-HCH	l.f.	l.f.	l.f.	l.f.	n.e.s.	n.e.s.	n.e.s.	n.e.s.	l.f.	l.f.	l.f.	l.f.	
β-HCH	l.f.	l.f.	n.e.s.	n.e.s.	n.e.s.	n.e.s.	l.f.	l.f.	l.f.	l.f.	l.f.	l.f.	
γ-HCH	l.f.	l.f.	n.e.s.	n.e.s.	l.f.	l.f.	l.f.	l.f.	l.f.	l.f.	l.f.	l.f.	
δ-HCH	l.f.	l.f.	l.f.	l.f.	l.f.	l.f.	n.e.s.	n.e.s.	l.f.	l.f.	l.f.	l.f.	
4nOP	29	12	l.f.	l.f.	l.f.	l.f.	l.f.	l.f.	25654	22	l.f.	l.f.	
HHCB	274	6	44	6	l.f.	l.f.	58	7	l.f.	l.f.	l.f.	l.f.	
AHTN	314	5	43	5	n.e.s.	n.e.s.	63	6	l.f.	l.f.	l.f.	l.f.	
Clor	n.a.	n.a.	17	15	99	10	38	2	1820	20	230	39	5.4
Clorf	l.f.	l.f.	l.f.	l.f.	9	15	n.e.s.	n.e.s.	l.f.	l.f.	l.f.	l.f.	
4,4'-DDE	254	4	65	4	104	3	51	5	3071	11	960	55	6.2
2,4'-DDD	384	4	35	10	119	5	54	3	3125	11	1000	34	6.1
4,4'-DDD	282	4	46	4	100	3	39	4	3795	10	980	40	6.2
4,4-DDT	306	4	25	5	131	4	34	3	3694	9	1670	35	6.4
BBP	253	8	l.f.	l.f.	85	8	l.f.	l.f.	2446	20	220	40	5.4
DOP	38	5	l.f.	l.f.	l.f.	l.f.	l.f.	l.f.	976	12	280	80	5.7

n.a. not available, *l.f.* lack of fit, *n.e.s.* not enough sensitivity

M-SBs showed a similar pattern to that seen with bare SBs for all the target compounds, but the R_s values were significantly lower than those estimated for SBs. In addition to the lack of fit observed in some cases with free SBs, we want to stress on the plots with not enough sensitivity (n.e.s. in Table 9.1). In those cases (e.g. β/γ-HCH), the full range of mass uptake (roughly 3-4 ng in 14 days) was comparable to the experimental uncertainty of any single analysis, so the sampling rates were highly questionable and were not provided though the fits were above the minimum requirements.

PES_t and M-PES_t showed also a very close pattern regarding the compounds with reliable R_s values, as shown in Figure 9.2 for 4,4'-DDT. Compared to SBs, we were able to get some R_s values for 4tOP and Chlorf. In addition to this, as it was observed with SBs, R_s values were higher for PES_t than for M-PES_t and these two were much lower than those obtained with PES_m. Regarding MESCO, the additional LDPE layer explains the lower values and in the later case, the size of the sheets (mass/volume of the receiving phase) is much higher and therefore the sampling rates are also higher. Finally, as shown in Figure 9.3, PES_m showed the highest R_s of all the PES samplers due to the higher surface (ca. 1400 mm^2 of the membranes against the 52 mm^2 of the tubes) and they are also significantly higher than POM sheets of almost the same surface (1000 mm^2).

Finally, as illustrated in Figure 9.3, POM sheets showed very slow uptake kinetics for the uptake profile of most of the target compounds and, since the uptake profile was fitted to the exponential function described in equation 8.12 (p. 175), the estimation of both the R_s values and the partition coefficients ($K_{POM,w}$) was required, as shown in Table 9.1.

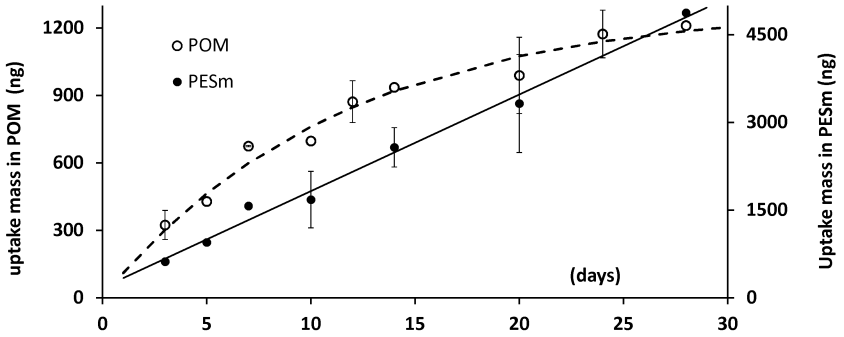

Figure 9.3. Uptake profile of chlorpyrifos obtained for POM sheets and PES membranes

9.3.3 In-situ calibration

As in constant flow laboratory calibration experiments it is difficult to include all the sources of variations that take place in real sampling scenarios, in-situ calibrations are gaining importance. Even though this procedure implies a tedious procedure in the field, it provides useful data especially when correction of field measurements is not feasible using PRCs. In fact, in-situ calibration can be a good strategy for passive sampling approaches performed with PES material. In this sense, an in-situ calibration was accomplished in the two WWTP effluents.

This experiment involved the calibration of three passive samplers: SB, M-SB and PES_t. The selection of these three sampling devices was based on the results obtained previously during the lab assays. This calibration was carried out under constant hydrodynamic conditions, but samplers were exposed directly to the effluent stream containing high load of dissolved organic matter (DOM).

R_s values were only determined for the main analytes detected in wastewater: HHCB, AHTN, Chlorp and 4tOP. As it is shown in Figure 9.4, satisfactory linearities (r^2 > 0.90) were obtained in the calibration curves for AHTN in both uptake modes (SB and M-

SB). In addition, quite low standard deviations for the R_s values were achieved (see Table 9.2).

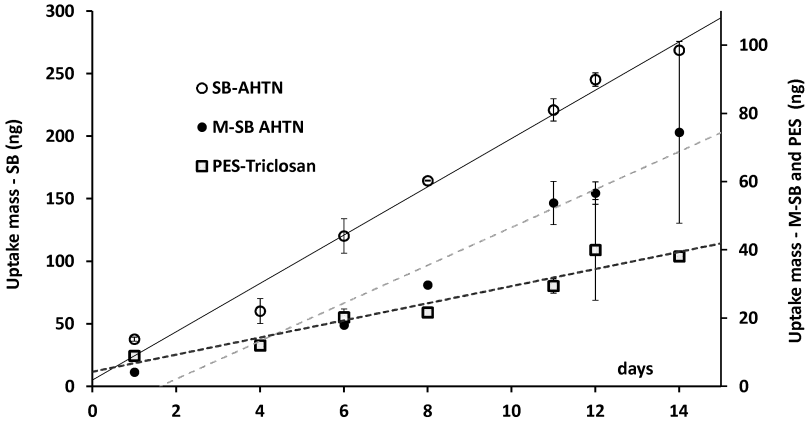

Figure 9.4 In situ uptake of AHTN with SB and M-SB, and triclosan with PES

In Figure 9.4 we have also included the uptake of triclosan in PES tubes. Though we were able to calibrate non-polar compounds with PES, we did not find the expected linear pattern in the effluents for the above mentioned compounds (HHCB, AHTN, Chlorp and 4tOP). However, more polar compounds such as triclosan were successfully accumulated following a lineal model which emphasizes the feasibility of PES as a passive sampler of slightly polar compounds.

Table 9.2 Sampling rates for analytes detected during in-situ calibration

	SB		M-SB		PES_t	
	R_s(mL·day^{-1})	RSD%	R_s(mL·day^{-1})	RSD%	R_s(mL·day^{-1})	RSD%
4tOp	n.d.	n.d.	n.d	n.d	3.8	8
HHCB	77	4	27	9	l.f.	l.f.
AHTN	105	3	29	9	2.1	19
Chlorp	56	3	23	7	7.4	13

n.d. not detected, l.f. lack of fit

9.3.4 Comparison of samplers

The R_s values obtained for the passive samplers of different materials and different shapes were compared. In order to make this comparison the experimental R_s were normalised. On the one hand, R_s values obtained for PDMS were normalised to the volume of the sampler since the accumulation of the analytes takes place through a partitioning process. On the other hand, R_s values obtained for PES and POM polymers were normalised to the area exposed since the adsorption of the analytes takes place in the surface of the polymer. Uptake results expressed in this way allow comparing adequately the sorptive capacities within samplers.

In Figure 9.5 normalised R_s for analytes with adequate fittings ($r^2 > 0.85$) are shown for each sampler. The highest values were achieved for SBs with a satisfactory precision in all the cases (RSD < 12%). It is important to show that in-situ-SB values are much lower than bare SBs but close to those estimated for M-SB. This fact may suggest that the in-situ values are under the effect of a fouling layer that reduces the transport of analytes to the polymeric sampler quite in the same way as the LDPE layer does in M-SBs. On the other hand, the R_s values obtained for PES are overall close to M-SB or in-situ SB. Additionally, the normalised R_s of PES_m and PES_t are of the same size, which suggests that the shape of the polymeric layer is not a limiting factor. However, due to the higher sampling area of PES_m, this device could allow the detection of some target analytes at lower concentration levels during 14 days of exposure. Although this advantage, the stripping of PES_m is more tedious and the repeatability of the overall procedure is slightly lower than that obtained for PES_t (see Table 9.1). M-PES_t sampling rates are slightly lower than the previous ones and higher than in-situ PES. Finally, POM values (not included in the plots) are of the same size than those shown for PES_m.

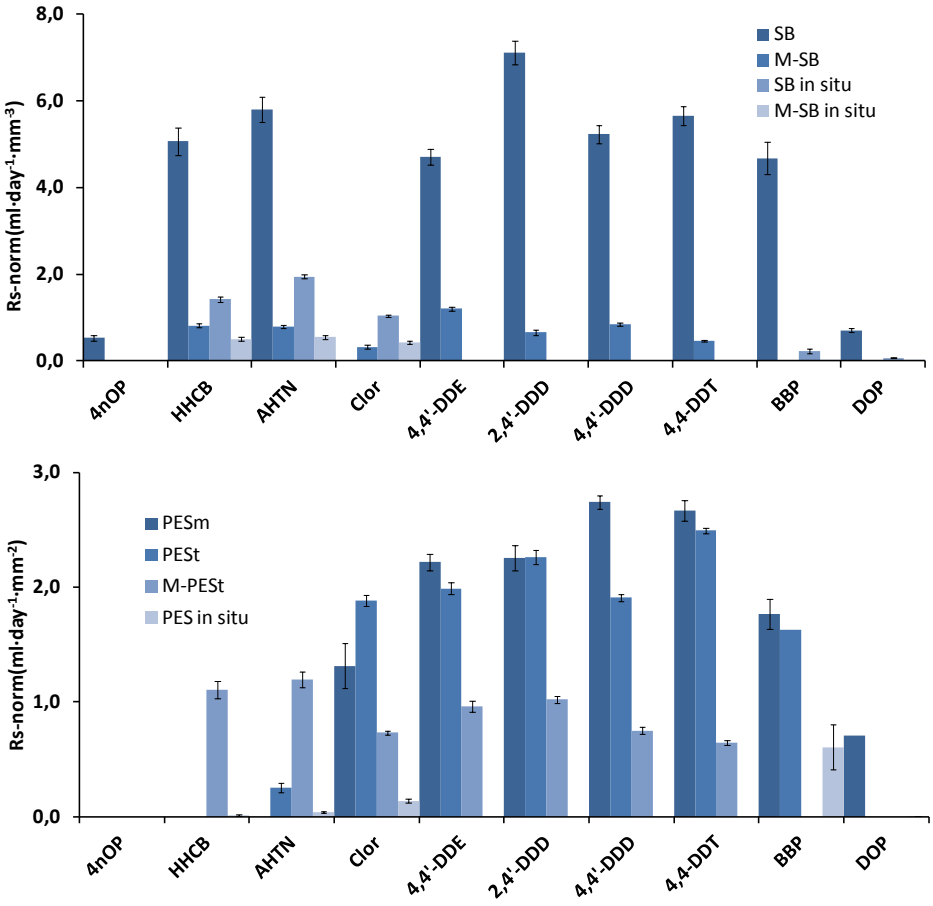

Figure 9.5. Comparison of normalised R_s to area of exposure: PDMS based samplers (up) and PES based samplers (down).

9.3.5 Application to WWTPs

During the sampling campaigns grab samples were also collected together with the retrieval of passive samplers. In this way, TWA concentrations obtained from the passive samplers can be compared with concentrations obtained through grab samples. In addition to this, 24-h composed water samples were collected during the in-situ calibration of passive samplers in the WWTP of Galindo. Although composed and grab samples are from different campaigns and passive samplers reflect the free concentrations, this comparison allows to highlight the representativeness of grab samples in a dynamic system as it is the effluent of a WWTP.

In Figure 9.6 the concentrations obtained in grab samples (n=3) and the concentrations obtained from composed effluent wastewater samples (n=4) collected in Galindo are shown. HHCB, AHTN, and DOP were detected along all the sampling days whereas 4tOP, Chlorp and BBP were only in few days. All the target compounds were quantified and satisfactory intra-day repeatability (RSD < 24%) was also obtained. Although the same concentration levels were observed during the whole sampling campaign, grab concentrations and composed ones are very different reflecting the complexity of taking representative samples.

Finally, the TWA concentrations in the WWTP effluent of Galindo were estimated based on the two types of calibrations (lab and in-situ) and including the PRC correction. Though POM, PES_t and PES_m only provided laboratory sampling rates, SB and M-SB values were calculated using both in-situ and laboratory calibration data, which were subjected to a further correction with the PRC (i.e. d-Phen). In all the cases, the values collected in Table 9.3 were calculated from the passive samplers retrieved after 7 days of exposure.

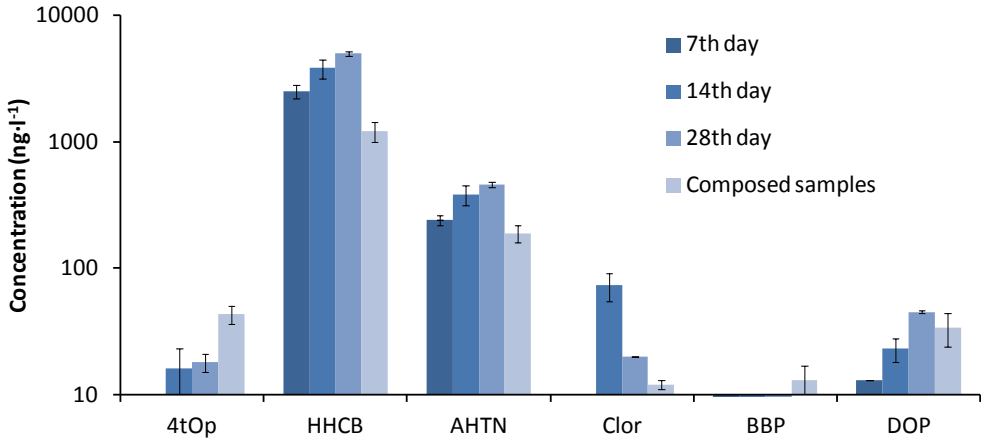

Figure 9.6. Concentrations (ng·L^{-1}) at 95% of confidence level obtained in grab (n=3) and composed samples (n=4) from the effluent of Galindo WWTP.

The use of passive samplers allowed the determination of the presence of 4tOP, 4nOP, and Chlorp, which were found below method detection limits (MDL) in grab samples (see Table 9.4). Other compounds were detected using both sampling strategies. For instance, musk fragrances HHCB and AHTN were the main pollutants present in water samples at µg·L^{-1} and ng·L^{-1} levels respectively, followed by BBP and DOP phthalates in a lower extent, all of them at ng·L^{-1} levels. Comparable concentration results were obtained for musk fragrances using both laboratory R_s corrected with PRCs (d-Phen) and R_s from the in-situ calibration. TWA concentrations estimated through non-corrected laboratory R_s underestimated the grab values and, on the contrary, in-situ calculated and, above all PRC corrected R_s values lead to overestimated concentrations. This fact may suggest that the PRC correction over in-situ calibration R_s values may introduce an overfitting factor that should not be considered any longer. Finally, in the case of phthalates, all the samplers presented underestimated values around 5-10 times lower. Finally, organochlorine pesticides and Chlorf were under detection limits both for grab and passive sampling.

Table 9.4 Method detection limits estimated by PES and SB extractions for the water analyses.

	MDL (ng/L)	
	PES	SB
4tOP	13	11
α-HCH	25	n.a.
β-HCH	25	18
γ-HCH	17	427
δ-HCH	12	552
4nOP	13	16
HHCB	71	57
AHTN	26	18
Chlorp	7	n.a.
Chlorf	15	147
4,4'-DDE	31	3
2,4'-DDD	10	11
4,4'-DDD	17	7
4,4'-DDT	29	2
BBP	38	8
DOP	15	11

In the case of the effluent wastewater from Gernika only the grab samples and the lab R_s and lab R_s corrected values were available, as shown in Table 9.5. As it was carried out in the previous effluent, the TWA concentrations were calculated from the passive samples retrieved after 7 days of exposure.

Table 9.3. Comparison of composed sample concentration ranges and TWA concentration ranges in the effluent of the WWTP of Galindo.

	Composed samples C ($ng \cdot L^{-1}$)	SB	M-SB	PES_t	PES_m	POM	
		Range of C_{TWA} ($ng \cdot L^{-1}$)					
4tOP	29 - 57			31 - 33			Rs lab.
				180 - 192			Rs *in-situ*
4nOP	<MDL	1.8 - 2.1			0.1 - 0.2		Rs lab.
		5.6 - 6.4					Rs corrected[1]
HHCB	780 - 1660	463 - 841	3421 - 3530				Rs lab.
		1584 - 2879	4343 - 4481				Rs corrected[1]
		1643 - 2986	5533 - 5709	1455 - 1814			Rs *in-situ*
		4579 - 8322	18236 - 18818				Rs corr. (*in-situ*)[2]
AHTN	130 - 250	40 - 91	370 - 370				Rs lab.
		138 - 311	470 - 470				Rs corrected[1]
		121 - 271	545 - 545	52 - 57			Rs *in-situ*
		336 - 755	1797 - 1797				Rs corr. (*in-situ*)[2]
Clor	<MDL		14 - 22	3.9 - 4.1	1.1 - 1.2	5.2 - 5.6	Rs lab.
			18 - 28				Rs corrected[1]
		5 - 12	11 - 16				Rs *in-situ*
		13 - 34	35 - 54				Rs corr. (*in-situ*)[2]
BBP	5 - 21				0.6 - 0.7	9.0 - 9.2	Rs lab.
							Rs corrected[1]
DOP	14 - 54	0.9 - 1.1			1.5 - 1.7	0.8 - 0.8	Rs lab.
		2 - 5					Rs corrected[1]

1. *In-situ* sampling correction : k_2(Stir-bar) = -0.273 ±0.006; k_2(Mesco-Stir-bar) = -0.11 ± 0.01
2. *In-situ* calibration dissipation constants: k_2(Stir-bar) = -0.098 ±0.004; k_2(Mesco-Stir-bar) = -0.083 ± 0.008

Table 9.5. Comparison of composed sample concentration ranges and TWA concentration ranges in the effluent of the WWTP of Gernika.

	Grab samples	SB	M-SB	PES_t	PES_m	POM	
	C ($ng \cdot L^{-1}$)	Range of C_{TWA} ($ng \cdot L^{-1}$)					
4tOp	<MDL			450 - 700			Rs lab.
4nOP	<MDL	9 - 10					Rs lab.
		67 - 70					Rs corrected
HHCB	2970 - 4160	118 - 173	577 - 1110				Rs lab.
		1036 - 1067	1126 - 1360				Rs corrected
AHTN	295 - 435	11 - 16	69 - 108				Rs lab.
		92 - 102	127 - 134				Rs corrected
Clor	MDL - 24		15 - 21	18 - 21	6.8 - 7.2	16 - 18	Rs lab.
			25 - 29				Rs corrected
BBP	<MDL	0.5 - 0.9			7 - 9	23 - 27	Rs lab.
		4.8 - 5.5					Rs corrected
DOP	<MDL	1.3 - 1.5			1.6 - 1.7	1 - 2	Rs lab.
		9.8 - 10.4					Rs corrected

The most concentrated contaminants found in Gernika were HHCB, 4tOP and AHTN, at higher concentrations than those found at the WWTP of Galindo, which can be understood based on the higher efficiency of wastewater treatments in Galindo. Regarding the concentrations obtained from the passive samplers, it is clearly observed the key importance of R_s correction since the corrected and non-corrected estimations are very different. In this particular case, though the R_s corrected estimations are higher than the non-corrected ones, those estimations are significantly lower than the grab sample concentrations. Finally, though the data collected from this station is very limited to support strong conclusions, it can be highlighted that the concentrations of HHCB and AHTN obtained through R_s corrected values from SB and M-SB are very close, as well as the concentrations of Chlorp obtained from the M-SB, PES_t and POM.

9.4 Conclusions

In this section several sampling rates have been calculated for the first time for several priority and emerging organic pollutants for polymer based passive samplers. In fact, the feasibility of PES material for passive sampling purposes has not been assessed previously. In addition, correction procedures required to ensure more realistic TWA concentrations have been applied. In this sense, the use of PRCs for absorption based polymeric samplers allows a satisfactory correction of the results obtained in this study. In-situ calibration has been also presented as another method for improving the accuracy of the results. However, despite being an appropriate method, it is a tedious procedure which requires a previous effort prior to regular sampling campaigns. Finally, TWA concentrations of non-polar pollutants in the effluents of two WWTP have been provided and the results were in good agreement with those obtained by grab sampling.

9.5 References

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10. APPLICABILITY OF POLYDIMETHYLSILOXANE AND POLYETHERSULFONE AS PASSIVE SAMPLERS OF MORE HYDROPHOBIC ORGANIC COMPOUNDS IN INTERTIDAL ESTUARINE ENVIRONMENTS

As stated before, the application of passive sampling in estuaries can show the problem of the periodic variation of the salinity and the temperature. Though the "salting-out" effect has been considered negligible in terms of the variation of the partition coefficients in solid-phase microextraction (SPME) studies (DiFilippo and Eganhouse, 2010; Lohmann, 2012), the effect of the variation of temperature and, specifically, the slight effect of salinity has been recently pointed with silicone rubber samplers (Jonker et al., 2015) based on partition coefficients. Moreover, uptake calibration experiments in salt water were recently performed in order to assure the suitability of Chemcatcher® to monitor hydrophobic pollutants in fresh and salt water (Petersen et al., 2015). However, we believe that the variation of salinity in estuaries requires a deeper attention and it is worth studying this effect, especially when high and periodic gradients of salinities may affect the uptake and release kinetics of the contaminants. The scope of this chapter, therefore, is based on the kinetic pattern i.e. following a thorough estimation of the R_s values and the elimination rate constants (k_e) of the PRCs at different salinities instead of studying the variation of the partition coefficients.

Consequently, following the passive sampling set up previously used to study the performance of different passive samplers for hydrophobic compounds (see section 9, Posada-Ureta et al. 2016), we wanted to study the effect of the salinity to support the suitability of PDMS (stir-bars, SBs), both free or naked stir-bars and MESCO/stir-bars (M-SBs), and polyethersulphone polymers (PES_t) to monitor a broad variety of hydrophobic organic contaminants often found in estuarine and coastal media. The list of contaminants includes organochlorine pesticides (DDT related), organophosphorous pesticides, hexachlorohexanes (HCH related), phthalates, antimicrobials, alkylphenols, musk and fragrances.

The calibration procedure was accomplished by exposing the samplers to a continuous flow of fortified seawater for up to 14 days under laboratory conditions. Prior to the exposure SBs and M-SBs were loaded with a known amount of deuterated PAH mixture to study the feasibility of those compounds as performance reference compounds (PRCs) in seawater. Sampling rates (R_s, mL·day^{-1}) were calculated for each sampler under several experimental conditions. On the one hand, experiments were performed at different concentrations in order to check if there is any influence of the concentration in the calculation of R_s. On the other hand, another experiment was carried out at a half of the salinity level found in the seawater with the aim of studying the effect of the salinity level on the R_s. A sampling campaign of seawater was performed in order to assess the presence of non polar organic compounds in the coastal environment. Among all the tested passive samplers SBs were used estimate the time-weighted average concentration of some of those contaminants in the feeding seawater of the experimental aquaria at the Marine Station of Plentzia (PiE, Plentziako itsas Estazioa).

10.1 Passive samplers and exposure system

In this work we studied the feasibility of some of the passive samplers used in the previous chapter (section 10.1.1). The PDMS stir-bars and PES polymer tubes were the same ones described in the previous chapter. In the case of stir bars, they were exposed in two forms: free (SB) and enclosed (M-SB). On the other hand, PES tubes were only exposed free (PES_t). The procedures followed for passive samplers conditioning and preparation are described in the previous chapter (see section 9.1.2). PES_t were not fortified with deuterated compounds because it was worthless as we could conclude in the previous work (Posada-Ureta et al., 2016).

Passive samplers were exposed in a continuous flow calibration system described elsewhere (Posada-Ureta et al., 2016; Vallejo et al., 2013). Samplers were placed in a stainless steel carrousel immersed in a stainless steel tank containing ~50 L of fortified

water (see Figure 10.1). Two different tanks were prepared in order to evaluate the effect of salinity for passive sampling calibration purposes: seawater (30‰ of salinity) and seawater diluted to a 15‰ of salinity (i.e. a 1:1 mixture of sea and fresh water). The tank has two continuous feeding inputs, one with seawater with a flow of 5 $L \cdot h^{-1}$ and the second one with a cocktail of chemicals in MeOH and with a flow of 10 $mL \cdot h^{-1}$ (Liquino, Metrom, Switzerland). In order to assure the steady state of the nominal concentration both inputs were mixed with the bulk water solution of the tank for 72 h before the exposure of the passive samplers. The carrousel was stirred at a constant rate of 50 rpm and water temperature was fairly constant (19±1 °C) through the entire experiments.

All the calibration experiments were carried out up to 14 days, and during that period two replicates of each sampler (SB, M-SB and PES_t) were removed from the tank at 6-8 different exposure times (days). All those experiments were set in series of different nominal concentrations (25 and 50 $ng \cdot L^{-1}$) and salinities (15 and 30 ‰).

Finally, passive samplers were placed in the big aquaria (5000 L) of the PiE to estimate the background concentration of the studied compounds. These samplers were deployed for up to 5 and 10 days under a constant renewal of filtered seawater (≈ 1000 $L \cdot h^{-1}$) directly pumped from the sea.

Finally, after the exposure, passive samplers were carefully disassembled, washed using Milli-Q water in order to remove salt from the surface, and dried using a clean tissue. The storage, extraction and analysis procedures are detailed in the previous chapter (see section 9.2.2). Tank water samples were analysed by means of a fully optimised method based on stir-bar sorptive extraction (SBSE) followed by TD-GC-MS. A detailed description of those procedures is available in Section 3.4.

Figure 10.1 Stainless steel carrousel containing SB, M-SB and PES_t

10.2 Results and discussion

10.2.1 Dissipation of PRCs

As mentioned before, PRCs are used to overcome the differences in the hydrodynamic regime between the calibration and the field measurements. Following the procedure and constraints used in a previous work (Posada-Ureta et al., 2016), but with seawater as exposure medium, it was studied whether an appropriate release of preloaded deuterated compounds from SBs and M-SBs was achieved.

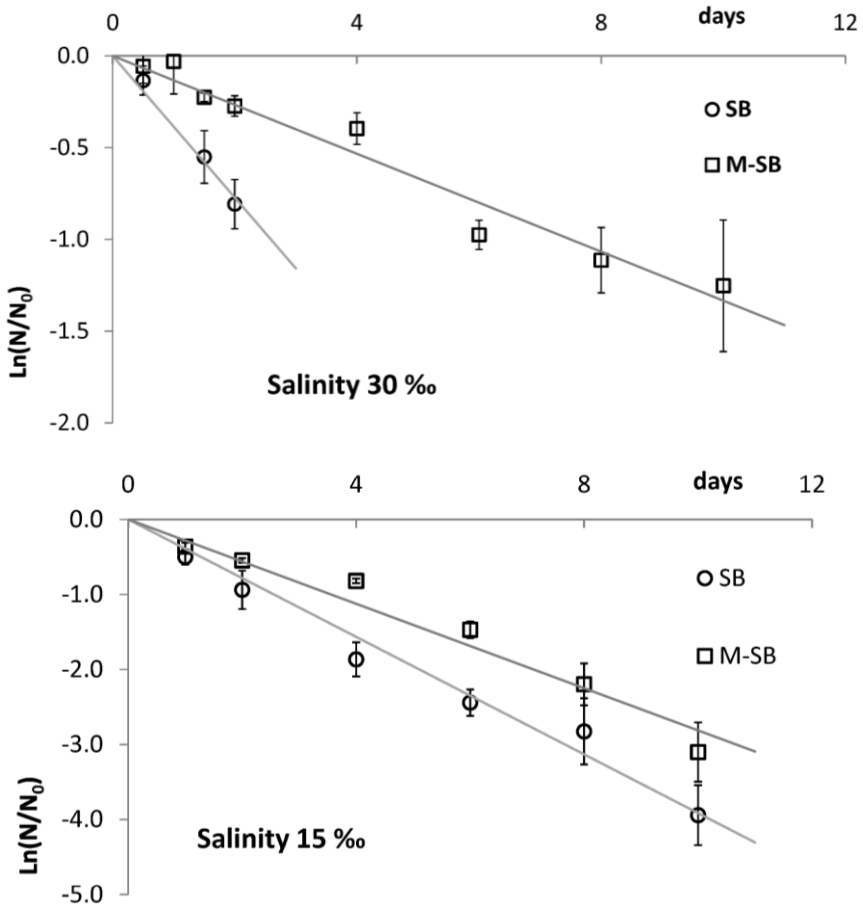


Figure 10.2. Dissipation of deuterated phenantrene from: SB (k_2= -0.31±0.03) and M-SB (k_2= -0.13±0.01) at 30‰ salinity (up); SB (k_2= -0.39±0.01) and M-SB (k_2= -0.28±0.01) at 15‰ salinity (down).

It is generally assumed that any PRC candidate can be accepted if the regression lines of dissipation show fittings with r^2 above 0.90 and the mass losses during the dissipation step are between 20-80% as shown in Figure 10.2 for d-Phe. Among all the studied deuterated compounds, d-Phe showed the best features as PRC in terms of dissipation curve fitting (r^2 > 0.97), mass released (over 20% for SB and over 30% for M-SB

respectively, after 8 days) and repeatability, in terms of relative standard deviation of the two samplers (RSD < 18% and 16% for SB and M-SB respectively). However, in the case of 30‰ salinity and with the naked SBs only three experimental measurements fulfilled those conditions and in the case of 15‰ salinity the linearity of the release plot was far beyond the 80% limit. The rest of deuterated compounds were discarded as PRC candidates because any of those constraints was not satisfactorily fulfilled. Particularly, a high dissipation rate was obtained for d-Ace since the mass released was over 80% after 2 days. On the other hand, though d-Cry and d-Per showed good dissipation curve fittings (r^2 close to 0.9), poor repeatabilities (RSD > 125%) were obtained.

From the elimination constants (k_e) obtained for SB in this work at different salinities and in a previous chapter (Posada-Ureta et al., 2016) with no salinity (i.e., -0.39 ± 0.02, -0.39 ± 0.01 and -0.34 ± 0.01 ( ) at 30‰, 15‰ and 0‰ of salinity respectively), we cannot conclude a defined trend since the overall variation is close to the extended uncertainty of the k_e values (i.e.). However, in the case of M-SB (i.e. -0.13±0.02, -0.28±0.02 and -0.105 ± 0.005 at 30‰, 15‰ and 0‰ respectively) the differences were more significant than the experimental uncertainties and a clear non-linear trend is observed. In this last case, at 15 ‰ of salinity, the elimination of PRCs was found to be much higher than the values determined for 0‰ and 30‰ of salinity. This pattern is not explained following the salting out models described in the literature (Endo et al., 2012; Lohmann, 2012) since slight linear trends are typically observed when partition coefficients are plotted against salinity or ionic strength. The fact that the diffusion from the M-SB sampler includes the silicone, the air gap, the LDPE membrane and the water boundary layer (WBL) before reaching to the external bulk seawater should explain the observed pattern. Since the variation of salinity only affects to the external diffusion, this unexpected higher dissipation might be explained as a lower resistance to the transport across the WBL surrounding the LDPE layer of the MESCO membrane due to the moderate presence of NaCl and other dissolved salts in the water.

10.2.2 Comparison of R_s obtained at different conditions

Before calculating the R_s values obtained using the different samplers at the assayed salinities, the concentration of each analyte in the exposure tank during the sampling period was controlled. The experimental concentrations in the exposure tanks of some of the target compounds during the exposure are shown in Figure 10.3. Since the RSD% values of all the target analytes were < 20%, we assumed that the steady state of water concentration in all the exposure tanks was attained.

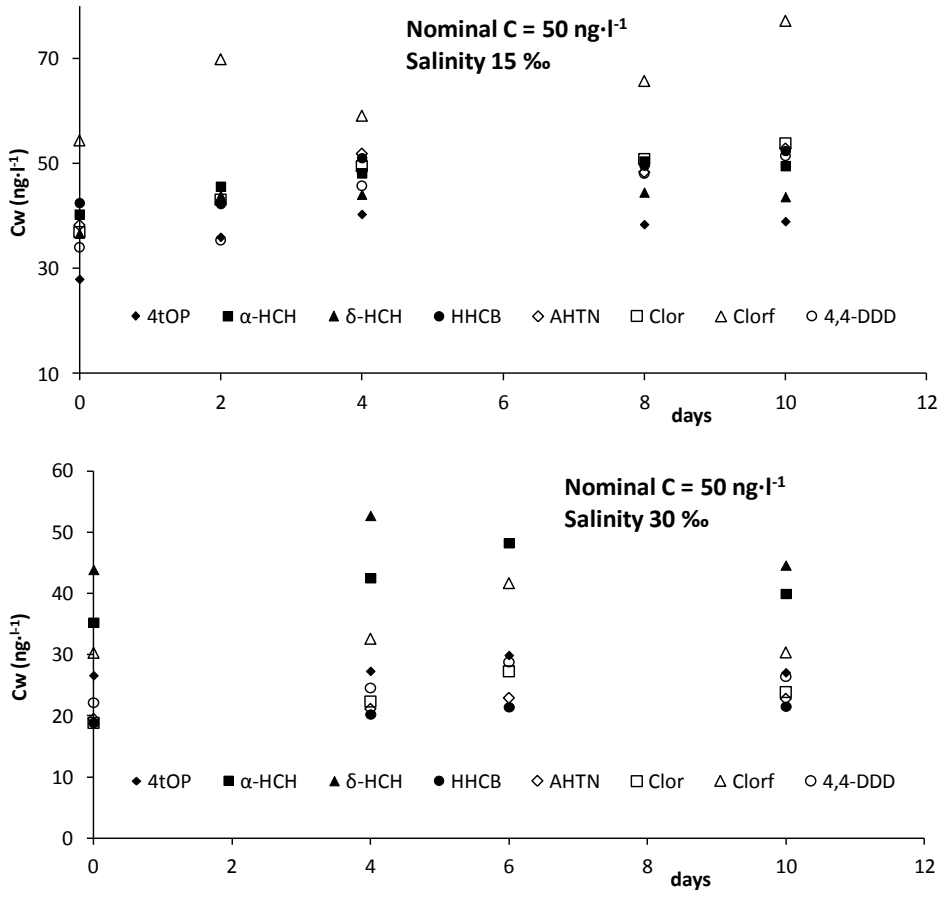


Figure 10.3. Variation of the concentrations of six target analytes in the exposure tank along two of the uptake experiments

Table 10.1. Sampling rates obtained for each type of passive sampler at different salinities (30‰ and 15 ‰ and 0 ‰ (from chapter 9).

	30‰		**15‰**		**0‰**	
SB	R_s (mL·day^{-1})	%RSD	R_s (mL·day^{-1})	%RSD	R_s (mL·day^{-1})	%RSD
4tOP	l.f.		l.f.		l.f.	
4nOP	n.e.s.		n.e.s.		n.e.s.	
α-HCH	n.e.s.		n.e.s.		n.e.s.	
β-HCH	n.e.s.		n.e.s.		n.e.s.	
γ-HCH	n.e.s.		n.e.s.		n.e.s.	
δ-HCH	n.e.s.		n.e.s.		n.e.s.	
HHCB	239	14	280	9	274	6
AHTN	241	14	283	9	314	5
Chlorp	340	14	307	9	306	30
Chlorf	66	34	65	31	68	14
4,4'-DDE	325	10	387	5	254	4
2,4'-DDD	323	11	359	6	384	4
4,4'-DDD	338	11	353	7	282	4
4,4'-DDT	320	7	333	11	306	4
BBP	271	15	220	13	253	8
DOP	35	30	30	8	38	5

	30‰		**15‰**		**0‰**	
M-SB	R_s (mL·day^{-1})	%RSD	R_s (mL·day^{-1})	%RSD	R_s (mL·day^{-1})	%RSD
4tOP	l.f.		l.f.		l.f.	
4nOP	n.e.s.		n.e.s.		n.e.s.	
α-HCH	n.e.s.		n.e.s.		n.e.s.	
β-HCH	n.e.s.		n.e.s.		n.e.s.	
γ-HCH	n.e.s.		n.e.s.		n.e.s.	
δ-HCH	n.e.s.		n.e.s.		n.e.s.	
HHCB	115	5	128	4	44	6
AHTN	114	5	129	5	43	5
Chlorp	186	5	167	4	17	15
Chlorf	l.f.		l.f.		l.f.	
4,4'-DDE	347	4	476	5	65	4
2,4'-DDD	259	3	312	2	35	10
4,4'-DDD	239	4	284	4	46	4
4,4'-DDT	260	8	355	4	25	5
BBP	17	16	11	9	1	9
DOP	41	11	15	6	1	29

	30‰		15‰		0‰	
PES	R_s (mL·day^{-1})	%RSD	R_s (mL·day^{-1})	%RSD	R_s (mL·day^{-1})	%RSD
4tOP	21	28	53	32	22	18
4nOP	n.e.s.		n.e.s.		n.e.s.	
α-HCH	n.e.s.		n.e.s.		n.e.s.	
β-HCH	n.e.s.		n.e.s.		n.e.s.	
γ-HCH	n.e.s.		n.e.s.		n.e.s.	
δ-HCH	n.e.s.		n.e.s.		n.e.s.	
HHCB	l.f.		l.f.		l.f.	
AHTN	n.e.s.		n.e.s.		n.e.s.	
Chlorp	77	15	114	10	99	21
Chlorf	14	17	20	32	9	30
4,4'-DDE	n.a.		n.a.		n.a.	
2,4'-DDD	84	9	161	9	119	10
4,4'-DDD	148	9	180	8	100	6
4,4'-DDT	78	6	244	5	131	7
BBP	48	10	118	12	85	16
DOP	33	22	24	8	l.f.	

n.a. not available, *l.f.* lack of fit, and *n.e.s.* not enough sensitivity

Though most of the calibration experiments lasted 14 days, the kinetic regime was generally assured up to 10 days. In Table 10.1, all the experimental R_s values and their RSDs are shown including the reasons to disregard some results when they did not fulfill some quality control prerequisites.

All target compounds (musk fragrances, organochlorine pesticides and phthalates) except HCH isomers, 4tOP and 4nOP provided a linear uptake using SB as passive sampler, with fitting precision below 12% and with a wide range of mass uptake (10 – 65 ng), much higher than the experimental uncertainties. In the case of M-SB, the uptake of the analytes showed also an analogous trend, and the fitting precision were below 13% for all the analytes except for alkylphenols and chlorfenvinphos, which did not show a linear uptake over the sampling period, regardless the salinity. The mass uptake for M-SB was moderately lower, between 50% and 80% lower, than the uptake for naked SBs. Finally, regarding the PES_t polymer, a less number of the target compounds followed a linear trend. Above all, 4tOP, Chlorp, Chlorf, 2,4'-DDD, 4,4'-DDD, 4,4'-DDT, BBP and DOP

were successfully calibrated in a linear range of 2 to 18 ng with a fitting precision below 26%. In Figure 10.4, it is shown the linear uptake of 4,4-DDD at two different experimental conditions and with SB, M-SB and PES_t. In Figure 10.5 the uptake plots of Chlorp are also shown.

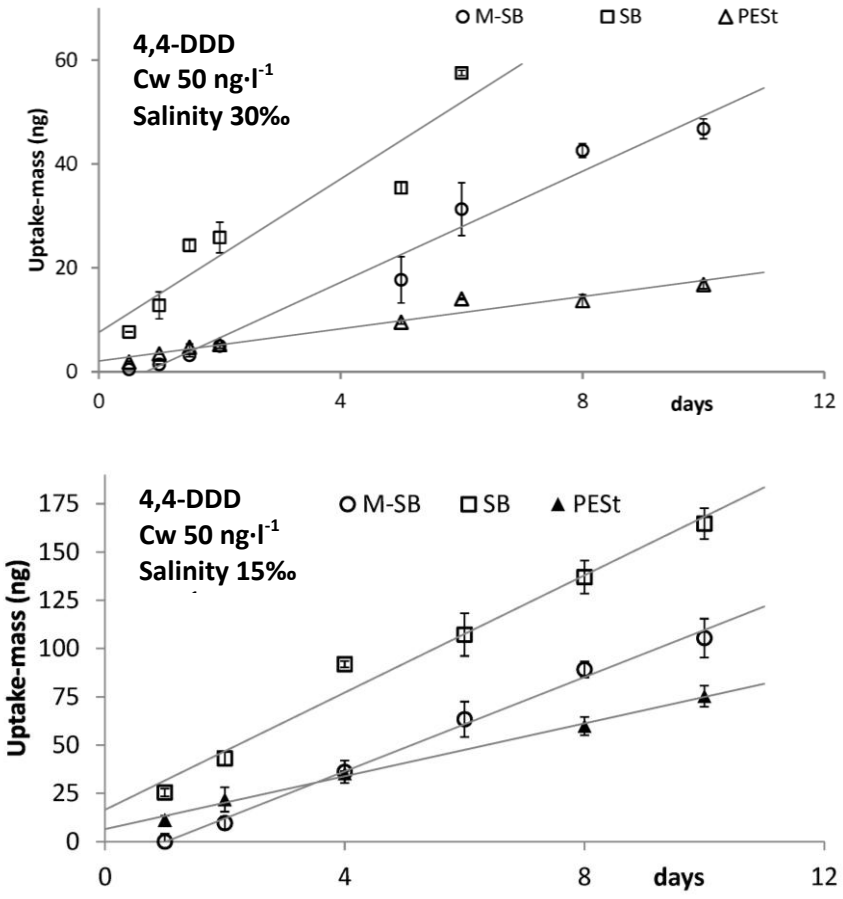


Figure 10.4. Uptake profiles obtained for 4,4-DDD using the three samplers and at different salinities. The uncertainties are the standard deviations of the two replicates.

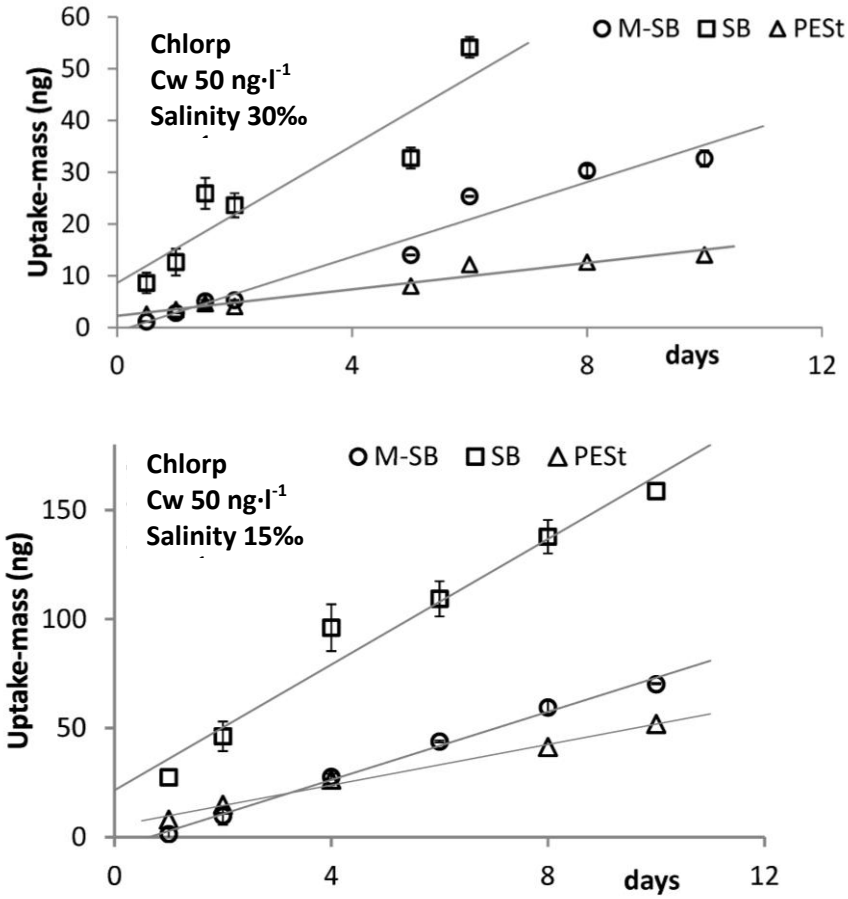


Figure 10.5 Linear uptake plot of Chlorp at three different experimental conditions and with the three passive samplers (SB, M-SB and PESt) at two salinities, 30‰ (top) and 15 ‰ (bottom).

Though most of the target compounds are accumulated following a first-order kinetic model (linear uptake), in some cases, patterns described lag effects (Belles et al., 2014), as shown for 2,4-DDT and Chlorp in Figure 10.6. The lag effect was particularly observed with M-SBs and especially when the nominal concentration was the lowest (25 $ng \cdot L^{-1}$). This effect can be explained in terms of a higher diffusion resistance of the LDPE polymer in the MESCO approach.

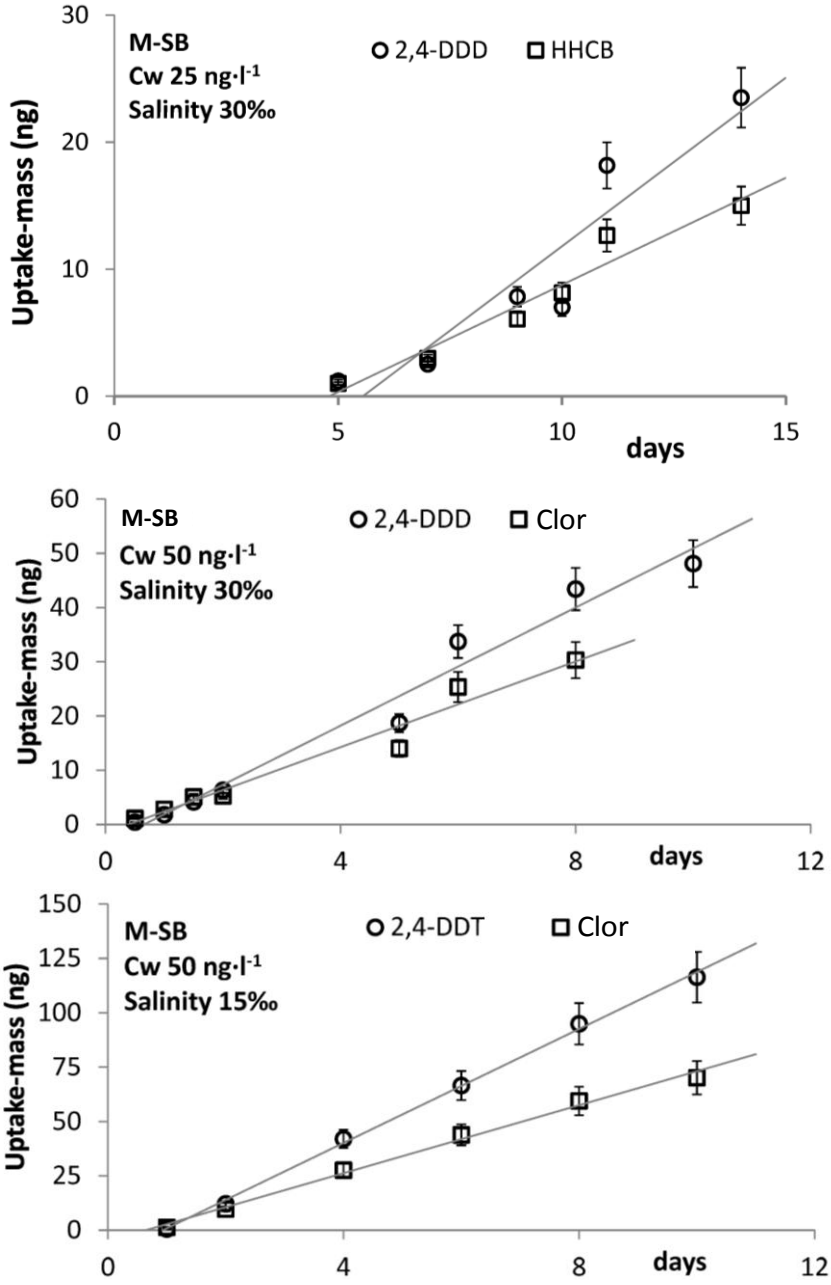
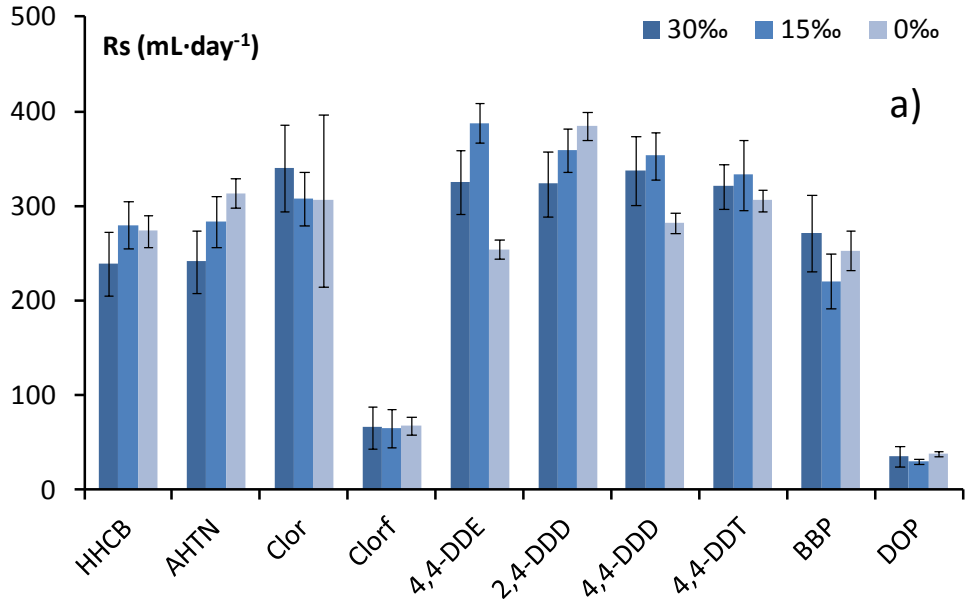
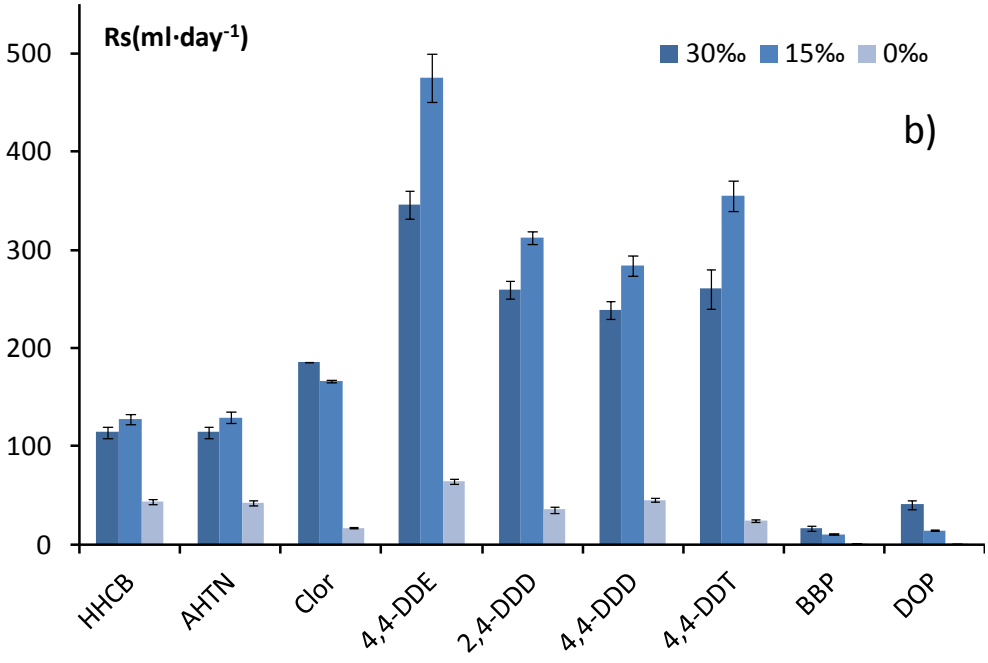


Figure 10.6 Linear uptake plots of 2,4-DDD 2,4-DDT, HHCB and Chlorp with M-SB systems in which the presence of a lag effect can be observed.

Regarding the efficiency of the samplers since both SB and PES_t have similar volumes and surface areas exposed (54 mm^2 in SB vs. 52 mm^2 in PES_t), the comparison of the sampling capacity of the three samplers can be done directly without any further normalization. As expected, the sampling rates obtained with SB were higher (R_s values between 35 – 340 $mL \cdot day^{-1}$) than those obtained with M-SB (R_s values between 10 - 347 $mL \cdot day^{-1}$). However, the results obtained with PES (R_s values between 14 - 148 $mL \cdot day^{-1}$) are significantly lower than those obtained with SB, and this difference may be attributed to the different uptake mechanism or the different affinities of the analytes with the sorbent polymers. Though the log K_{ow} of the target analytes (4.30-8.62) and their molecular surface (257-716 $Å^2$) and polar surface areas (9-52 $Å^2$) were considered to find a pattern in the variation of the R_s values, in the way it has been used by Bäuerlein et al. (Bäuerlein et al., 2012) to describe the sorptive features of common solid phase extraction materials, we were not able to build any sound empirical model.

In order to answer to our main question regarding the effect of salinity variations on the performance of passive samplers, or the feasibility of using laboratory calibrated samplers (fresh water) to be applied on estuarine or coastal media, all the passive samplers were exposed at two salinity levels. To this purpose, apart from exposing passive samplers to raw seawater (around 30‰ salinity), they were also exposed to a 1:1 mixture of fresh and seawater prepared daily (15‰ salinity). In addition, data calculated from previous laboratory calibrations with fresh water was employed to compare three levels of salinity. In Figure 10.7, the R_s values obtained at the three salinities are shown using the different sampling devices.


Rs (mL·day-1)
30‰
15‰
0‰
a)
500
400
300
200
100
0
HHCB
AHTN
Clor
Clorf
4,4-DDE
2,4-DDD
4,4-DDD
4,4-DDT
BBP
DOP


Rs(ml·day-1)
30‰
15‰
0‰
b)
500
400
300
200
100
0
HHCB
AHTN
Clor
4,4-DDE
2,4-DDD
4,4-DDD
4,4-DDT
BBP
DOP

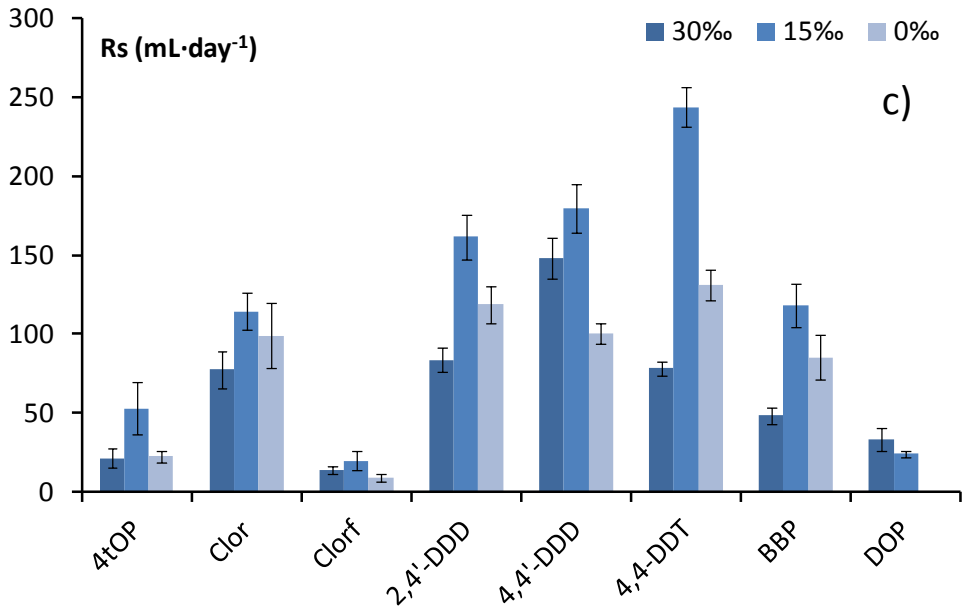


Figure 10.7. R_s values (mL·day^{-1}) obtained for target compounds at different salinity levels using a) SB, b) M-SB and c) PES_t respectively.

The comparison between SB and M-SB shows an interesting different pattern. In the case of SB, as shown in Figure 10.7a, all the values are broadly within the experimental uncertainty, so it has been shown the negligible effect of salinity. In the case of M-SB (Figure 10.7b), however, the effect is dramatic especially when the values obtained in this work at 15 ‰ and 30 ‰ are compared to those obtained previously at 0 ‰. As it was pointed before with the PRCs, this high difference can be attributed to an increased diffusion rate across the WBL when the salinity or ionic strength is increased. Actually, as it happens with the organochlorine pesticides, the R_s values of M-SB at 15‰ or 30 ‰ are comparable to those obtained with naked SB.

Consequently, the use of naked SB is likely the most robust option when estuary waters are monitored since the sampling rates show a low variability against a full range variation of the salinity (0-30‰). On the contrary, though M-SB samplers are more robust against biofouling and hydrodynamic fluctuations (Vrana et al., 2006) further studies should be addressed to assure the remarkable effect of salinity in the diffusion rate and the sampling rates and whether this effect increases the options of M-SB as efficient passive samplers in coastal environments, where the salinity variations are much less significant.

In the case of PES_t the effect of salinity is also significant especially when the salinity is 15‰ (see Figure 10.7c). This fact was observed in the work of Shi and coworkers (Shi et al., 2014) with POCIS and several antibiotics and endocrine disrupting chemicals. According to the results obtained in this work, it seems likely to attribute that pattern to the PES sheet that covers the sorptive phase, where the analytes are also retained.

In addition to the effect of salinity, another basic assumption of passive samplers is the one compartment sorption rate model. As it has been pointed in the general literature (Booij et al., 2007), most of the kinetic models, and especially for silicone based materials such as SB, are based on a first order kinetics equation, which is not dependent of concentration. In order to verify if this assumption is valid also for PES based samplers, we have determined the sampling rates of SB and PES at two levels of concentration, i.e. 25 $ng \cdot L^{-1}$ and 50 $ng \cdot L^{-1}$, as shown in Figure 10.8. As expected, there is no difference between the values of R_s obtained with SB (p-level > 0.1) except for 4,4'-DDE. In the case of PES_t, the number of compounds that fulfilled the requirements to provide robust R_s values was rather limited and the results showed significant differences for 4-tOP (p-level < 0.05) and non-significant ones for Chlorp and 2,4-DDD (p-level>0.05).

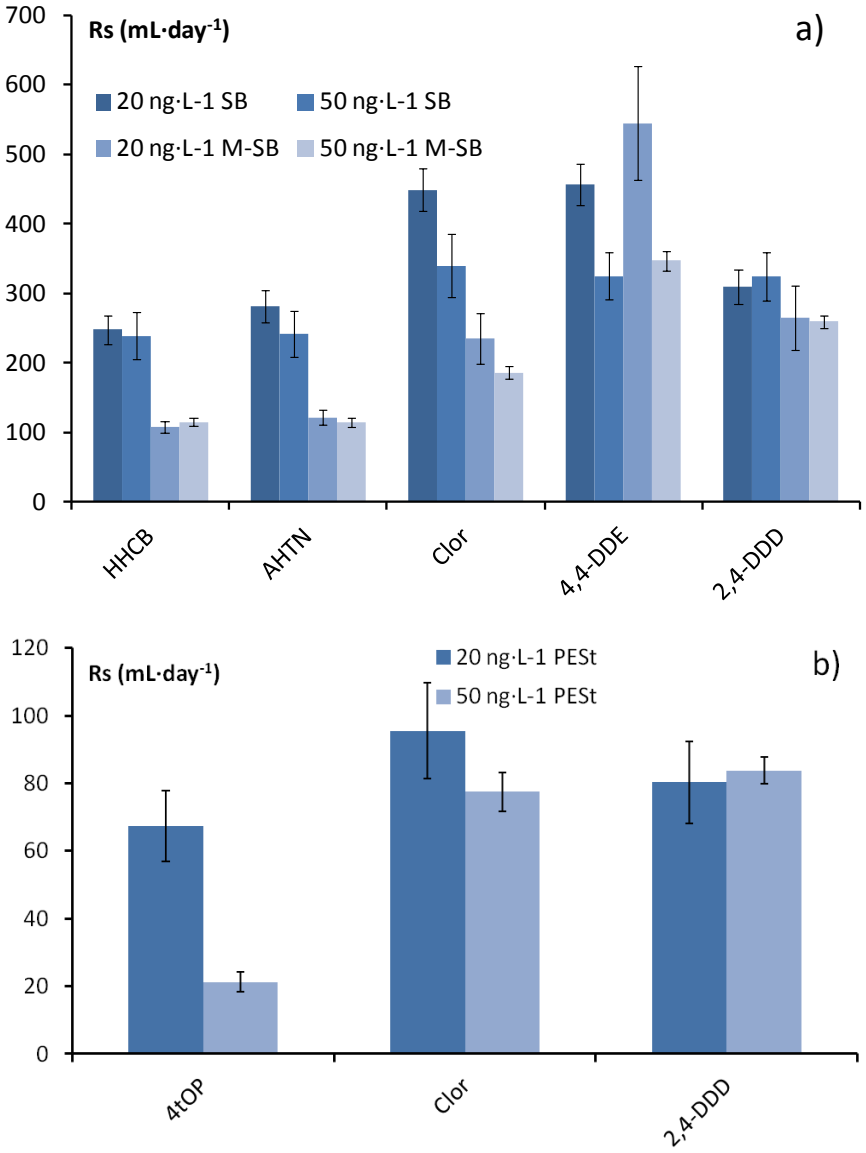


Figure 10.8. R_s values (mL·day^{-1}) obtained at different exposure concentration levels for a) SB and M-SB and for b) PES.

10.2.3 Application to seawater

The passive samplers were used to estimate the concentrations of the target contaminants in the seawater that enters to the Plentzia sea station (PiE). We used one of the 5000 L tanks to expose the passive samplers (SB, M-SB and PES_t) and retrieved three replicates of each sampler at the 5th and the 10th days. 0.5 L of seawater were also collected to measure the concentration in the tank and to compare with the C_{TWA} values.

Table 10.2. C_{TWA} concentration ranges obtained (using R_s and PRC corrected R_s) by means of SB in the feeding seawater of the Plentzia Marine Station (PIE).

	C_{TWA} (5 days) ng·L^{-1}	C_{TWA} (10 days) ng·L^{-1}	
HHCB	0.20 - 0.30	0.11 - 0.15	R_s
	0.23 - 0.36	0.13 - 0.18	R_s corrected
AHTN	0.10 - 0.17	0.06 - 0.08	R_s
	0.12 0.20	0.07 - 0.09	R_s corrected
Chlorp	0.08 - 0.14	0.1 - 0.3	R_s
	0.09 0.17	0.1 - 0.3	R_s corrected

In the case of the active sampling, all the signals were below detection limits (< 10 ng·L^{-1} for all the analytes with the exception of β and γ-HCHs, which showed much higher detection-limit values > 130 ng·L^{-1}). On the contrary, among the passive samplers, only SB provided measurable concentrations of HHCB, AHTN and Chlorp as shown in Table 10.2. The other two passive samplers (i.e. M-SB and PES_t) did not provide measurable signals. Since the water of tank was continuously renewed at high rate (1000 L·h^{-1}) there are slight differences between the samples retrieved at the 5th day and

those retrieved after 10 days, but a reasonable range of concentrations were obtained for two musks fragrances (HHCB and AHTN) and one pesticide (Chlorpyrifos). The use of PRC corrected concentrations included in Table 10.2 was also considered as explained in the previous chapter (Posada-Ureta et al., 2016) and as can be seen, the concentrations were close to the non-corrected ones. Finally, it is worth mentioning the low concentration levels of target compounds that can be detected using SB passive samplers in comparison to those detected by grab sampling.

10.3 Conclusions

The main aim of this work was to study the effect of salinity in the suitability of three passive samplers and therefore we have determined the R_s of several non-polar contaminants in seawater media at different salinities. In general terms we observed different patterns. On the one hand, free or naked SBs show constant R_s values in brackish media ‰ and, on the other hand, M-SBs and PES_t show a sharp variation of the R_s values for almost all the target compounds.

As a consequence, SB passive samplers can be deployed in estuarine and coastal media because of the robustness of the R_s values against the periodic variations in salinities. In addition to this, the hydrodynamic fluctuations can be overcome with a proper PRC correction. Though M-SBs are not suitable when large salinity variations take place or when the concentrations are still too low, the use of M-SB also showed interesting features in marine media since the sampling rates are significantly much higher than in fresh water and the SBs are better protected against biofouling and the absorption of interfering compounds. Finally, PES_t are the less interesting samplers basically due to the hydrophobicity of the target compounds (log K_{ow} > 3.6). In the specific case of SB, passive samplers allow detecting at low concentration levels in intertidal estuarine environments, mainly when the detection of contaminants cannot be achieved using spot sampling strategies.

10.4 References

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11. CONCLUDING REMARKS

Do we miss any important clue in this Thesis? To suggest another view, it is time to look back to see the main achievements and to discuss them critically and in a proper context. Though the initial references or checklist will be the main objectives of this work, we would like to share as well all the lessons we learned including those that are not explicitly written in this memory.

First of all, this work did not come out of the blue. The research group had a long experience in the GC analysis of legacy contaminants (PAHs, PCBs, lindane...), the development of analytical methods was part of our skills, and we had the Gerstel MP2 system on top of a GC-MS, which offered a lot of possibilities to run stir-bar, headspace or purge¬trap analysis in a semi-automatic way.

In this context, the two main objectives included in this PhD Thesis, namely the development of analytical procedures for the analysis of non-polar organic pollutants in water and the development of passive sampling techniques for some of the previously considered compounds, were given to follow the tracks that were previously opened and to tackle a long desired challenge.

Regarding the first objective, the first task was the development and validation of a method based in membranes for quantifying musk fragrances in water. In this particular case, we were pursuing a simple method to get started in the analysis of non-polar contaminants in the environment. Later on, we found the first hurdle in our way: the matrix effect. Hopefully, we could deal with it using deuterated compounds and we were able to measure the concentrations of HHCB and AHTN in WWTP effluents. Though the ubiquitousness of these compounds has been widely described in the literature, it was the first time they were reported in the WWTPs of Galindo and Gernika.

Since membrane assisted extractions required great skills, we continued investigating other alternatives for the microextraction of the main musks (tonalide and galaxolide) and other persistent and not so well known contaminants (pesticides, alkylphenols and phthalates). On the one hand, we chose stir-bars since they allowed routine extractions and analysis in an automatic set-up, and also because PDMS is a good polymer for the analysis of non polar contaminants. Due to these reasons, stir-bars were considered as a safe way to accomplish this analysis though they were not performed before in our lab. As stir bars are expensive materials which have to be carefully treated, we tried to find a cheaper and single-use device with similar properties. Our first option was the silicone rod. Although its performance was good enough, it has a great disadvantage: it contaminates our GC-MS instruments, and thus, thermal desorption should be always avoided. Nonetheless, we confirmed that both PDMS devices could be used for the second purpose of developing passive sampling techniques for our set of target contaminants.

The combination of cheap polymers to run microextractions and the possibility to become passive samplers was very interesting. In this sense, we continued working with other materials in order to have more choices when developing an analytical method. In this scenario, we included a broader spectrum of pollutants with different chemical properties (broadly referred as polarity) and we also tried to find another feasible sampler both for microextraction and passive sampling. We tested four alternative materials, but only PES seemed to be an adequate choice, as it offers an advantage in comparison to silicone: PES can perform the extraction of the most polar compounds of our selection of chemicals, better than silicone materials do. In addition, we could find again musks and other contaminants in the WWTP effluent and also in estuary water using these new polymers.

Once we gained knowledge about the occurrence of contaminants and the behaviour of the materials we were using, and once we got a reliable method for the analysis of our target compounds at $ng \cdot L^{-1}$ concentrations, we faced our second challenge: to study the presence of these contaminants in water by means of passive sampling.

This was not the first time our research group tackled the use of passive sampling though, but, eventually, this is the first time we run the whole way from a thorough calibration to a monitoring assessment. First of all, we developed a calibration system for passive samplers in our laboratory. We were able of applying all we learned from our colleagues at UFZ, in Leipzig. We determined for the first time the sampling rates of the contaminants from our set of contaminants. Some of them had been already measured by stir-bars or silicone in other formats, but in general, there was no previous data neither for the polymers (PES and POM), nor for the pollutants. We gained a lot of technical skills in the dynamic flow systems to run the calibrations but, thankfully, we learned the most during the edition of the published papers. However, there are still some questions with fuzzy answers. For instance, how to validate or correctly interpret the C_{TWA} values obtained with passive samplers is still an intriguing issue that requires successive trials.

Feeling self-confident about our own capacities, and with a clear lack of awareness about the risks involved, we designed a new calibration set-up to work with seawater in the Plentzia Marine Station (PiE). The risky game came when we tried to combine the passive sampling calibration with the simultaneous exposure of mussels and all the expected bonuses of a complex set-up. Since mussel capacity to stand for a long exposure to a cocktail of contaminants was uncertain, we all learned some deadly effects and the virtues of a prompt spawning. Nonetheless, the analytical achievements were assured as described in chapter 10 and the bioanalytical issues were part of the learned lessons for future applications.

The effect of salinity variations in the suitability of passive samplers (SB, PES and M-SB) is not a trivial problem, especially in estuarine waters. As described, we determined the Rs values of our target contaminants at different salinities and, once again, the hydrodynamic fluctuations could be corrected with PRCs. In addition to this, we observed different patterns. SB passive samplers are robust enough to be deployed in estuarine and coastal media, regardless the periodic variations in salinities. In contrast, M-SBs and PES_t showed a sharp variation of the Rs values for most of the pollutants. Generally speaking, SBs are the best option, though M-SBs could be applied when there are not large salinity variations, and PES could be an interesting choice for contaminants more polar than ours. We applied the passive samplers in seawater obtaining measurable concentrations for musks and Chlorp with SB after five days. However we could not detect the target analytes with M-SB or PES_t. Anyway, the MESCO version could be the best choice for longer sampling periods since the Rs values are higher than in fresh water and the SBs are better protected against biofouling and the absorption of interfering compounds.

Once a door is closed, new windows are opened. Novel microextraction procedures have been applied for new families of pollutants in more complex matrices (soils, vegetables, food packages, etc.). Following the tracks of passive sampling new challenges have been achieved in new born projects: modified POCIS systems have been applied for the analysis of emerging contaminants or the use of PDMS sheets in passive dosing experiments is under scrutiny. Additionally, from the failing experience with mussels, we gained the experience not only to expose fishes but to work with biologist in complex toxicological issues and to sail in omic seas.

In this sense, the paths taken in this thesis are being continued by our colleagues, who are obtaining relevant results in this area. We must also strengthen the collaborations and the opportunities with scientists from other disciplines to make sense of our virtuous measurements.

In this work, several procedures have been developed to accomplish the analysis of a wide range of non-polar pollutants in estuarine media. The applicability of passive sampling approaches has been also thoroughly studied. Hopefully, this work will contribute to a better understanding of the presence and distribution of contaminants in estuarine waters and to the effects that may happen.

The experimental works of this PhD Thesis have been carried out at the Department of Analytical Chemistry (Faculty of Science & Technology) and the Plentzia Marine Station (PiE) of the University of the Basque Country, and at the Centre for Environmental Research (UFZ, Leipzig, Germany).