Study of microplastics role in seawater based on collected samples across the oceans during the Barcelona World Race 2015

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DOCTORAL THESIS

Title	Study of microplastics role in seawater based on collected samples across the oceans during the Barcelona World Race 2015
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A la meva família

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We must have perseverance and, above all, confidence in ourselves

Marie Curie

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Mireia Singla Milà, 11 de Maig de 2020

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Abstract

Study of microplastics role in seawater based on collected samples across the oceans during the Barcelona World Race 2015

Microplastics have become a huge environmental concern in recent years. The overproduction and excessive use of plastic have made difficult a proper manage and that is why it has become the fastest growing segment of the waste stream. Plastic debris, through several physical, chemical and biological processes can degrade or breakdown resulting in microplastics. In addition to these it can also find other type of microplastics which are those originally and intentionally manufactured in that size. Although several research studies have been published demonstrating the presence of microplastics in localised coastal regions, any of them show a global scenario about this environmental concern. Here we present the development of a new methodology for microplastics sampling and retention of pollutants present in seawater. In this sense, we have collaborated with the Barcelona World Race (BWR) organization and the "Fundació de Navegació Oceànica de Barcelona" (FNOB). Throughout this collaboration, we have developed a device, named COA device, installed in a racing boat of the BWR 2015 which collects microplastics and pollutants on superficial seawater from the different locations of the world going through four oceans (Mediterranean Sea and Atlantic, Pacific and Indian Oceans).

Furthermore, we performed the characterization of the microparticles collected during the BWR 2015. The analysis, based on microscopic techniques, of their morphology, composition and distribution has allowed us to know better the level of pollution of the marine environment and which is the global impact of having that particles in the oceans. In addition to that, we demonstrate the microplastics concentration effect of persistent organic pollutants. We also developed a reproducible analytical methodology based on a new approach for the release and quantification of different families of pollutants from polymeric microparticles.

Finally, different analytical methods have been optimized for the analysis of several pollutants solved in seawater. The elution of pollutants retained in the SPE cartridges used in the BWR 2015 has been performed.

In conclusion, this thesis provides information about the overall status of the oceans in terms of microplastics and their consequences at present. The study of the role of microplastics in seawater in a global way have been helpful to understand the actual environmental situation.

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Resumen

Study of microplastics role in seawater based on collected samples across the oceans during the Barcelona World Race 2015

Los microplasticos se han convertido en un gran problema medioambiental. La sobreproducción y el uso excesivo del plástico ha dificultado mucho su tratamiento y esto provoca que sea el sector con un mayor crecimiento en la generación de residuos. Los desechos plásticos, a través de varios procesos se degradan o rompen en partículas más pequeñas dando lugar a los microplasticos. También se pueden encontrar otro tipo de microplasticos, esos originados y fabricados en ese tamaño de forma intencionada. Aunque ya se han publicado varios artículos científicos demostrando la presencia de microplasticos en zonas localizadas, ninguno de ellos muestra una visión global acerca de este problema medioambiental.

En esta tesis presentamos el desarrollo de una nueva metodología de muestreo de microplásticos además de la retención de otros contaminantes orgánicos suspendidos en agua de mar. Para ello, hemos colaborado con la Fundación de Navegación Oceánica de Barcelona (FNOB), entidad organizadora de la Barcelona World Race (BWR) y con el Grupo Sailing Technologies. A través de esta colaboración, hemos desarrollado un nuevo dispositivo de muestreo instalado en uno de los barcos participantes en la BWR 2015 que es capaz de colectar microplasticos y contaminantes orgánicos de agua superficial en varias localizaciones del mundo pasando por cuatro océanos (Mar Mediterráneo y Océanos Atlántico, Pacífico y Índico).

Hemos realizado la caracterización de los microplasticos muestreados durante la BWR 2015. El análisis de su morfología, composición y distribución nos ha permitido conocer el nivel de contaminación y el impacto de tener este tipo de micropartículas en los océanos. También hemos demostrado el efecto concentrador que poseen los microplasticos. Hemos desarrollado un método analítico reproducible para la extracción y cuantificación de varias familias de contaminantes orgánicos de distintos tipos de micropartículas poliméricas.

Por último, se desarrolla una metodología para la elución de los cartuchos SPE usados en la BWR 2015. Además, se ha realizado un análisis PCA y se han agrupado las muestras en función de varios parámetros como las corrientes, la localización o su posición respecto al ecuador.

En conclusión, esta tesis proporciona información sobre el estado global de los océanos en relación con los microplasticos y sus consecuencias. El estudio del efecto de los microplasticos en los océanos de forma global es de ayuda para comprender la situación medioambiental actual.

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Resum

Study of microplastics role in seawater based on collected samples across the oceans during the Barcelona World Race 2015

Els microplàstics s'han convertit en un gran problema mediambiental. La sobreproducció i l'ús excessiu del plàstic ha dificultat el seu correcte tractament y això provoca que sigui el sector amb un major creixement en la generació de residus. Els residus plàstics, a través de diversos processos es degraden i es trenquen en partícules mes petites donant lloc als microplàstics. També es poden trobar un altre tipus de microplàstics, aquells originats i fabricats d'aquesta mida de forma intencionada. Tot i que s'han publicat diversos articles científics demostrant la presencia de microplàstics a zones mol localitzades, cap d'ells mostra una visió global sobre aquest problema mediambiental.

En aquesta tesis presentem el desenvolupament d'una nova metodologia de mostreig de microplàstics a més de la retenció d'altres contaminants orgànics suspesos en l'aigua de mar. Per això, hem col·laborat amb la Fundació de Navegació Oceànica de Barcelona (FNOB), entitat organitzadora de la Barcelona World Race (BWR) y amb el grup Sailing Technologies. A través d'aquesta col·laboració, hem desenvolupat un dispositiu de mostreig instal·lat en un dels vaixells participants en la BWR 2015 que es capaç de col·lectar microplàstics i contaminants orgànics en aigua superficials en varies localitzacions del mon passant per quatre oceans (Mar Mediterrani i Oceans Atlàntic, Pacífic e Índic).

Hem realitzat la caracterització dels microplàstics mostrejats durant la BWR 2015. L'anàlisi de la seva morfologia, composició i distribució ens ha permès conèixer el nivell de contaminació i l'impacte de tenir aquest tipus de micropartícules en els oceans. També hem demostrat el efecte concentrador dels microplàstics. Hem desenvolupat un mètode analític reproduïble per l'extracció i quantificació de varies famílies de contaminants orgànics de diferents tipus de micropartícules polimèriques.

Per últim, es desenvolupa una metodologia per l'elució dels cartutxos SPE utilitzats durant la BWR 2015. A més, s'ha realitzat un anàlisis PCA i s'han agrupat les mostres en funció de diferents paràmetres com les corrents, la localització o la seva posició respecte l'equador.

En conclusió, aquesta tesis proporciona informació sobre l'estat global dels oceans en relació als microplàstics i les seves conseqüències. L'estudi del efecte dels microplàstics en els oceans de forma global ajuda a comprendre la situació mediambiental actual.

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List of Abbreviations

ABS	Acrylonitrile butadiene styrene
BWR	Barcelona World Race
BPA	Bisphenol-A
COA	Clean Ocean Analysis
DeBDPE	Decabromodiphenylether
DDT	Dichlorodiphenyltrichloroethane
ESI	Electrospray ionization
ECD	Electron capture detector
FTIR	Fourier transform infrared
FNOB	Fundació Navegació Oceànica de Barcelona
GC	Gas chromatography
HCHs	Hexachlorocyclohexane
HLB	Hydrophilic-Lipophilic balance
HDPE	High density polyethylene
HRMS	High resolution mass spectrometry
IR	Infrared
LDPE	Low density polyethylene
MP	Microplastics
OcBDPE	Octabromodiphenylether
POPs	Persistent Organic Pollutants
PP	Polypropylene
PS	Polystyrene
PVC	Polyvinyl chloride
PET	Polyethylene terephthalate
PMMA	Polymethyl methacrylate
PA	Polyamide
PCS	Polycarbonate
PUR	Polyurethane
PCBs	Polychlorinated biphenyls
PBDEs	Polybrominated diphenyl ethers
PAHs	Polycyclic aromatic hydrocarbons
PeBDPE	Pentabromodiphenylether
PFACs	Perfluorinated compounds
PCA	Principal component analysis
QTOF	Quadrupole-time of flight
SPE	Solid phase extraction
SPME	Solid phase microextraction
SBSE	Stir bar sorptive extraction
SEM	Scanning Electronic Microscopy
UHPLC	Ultra-high-performance liquid chromatography
UV	Ultraviole

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Chapter I.

Motivation and Aims

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Plastic impact

The present chapter summarizes the current environmental situation due to the abusive fabrication and use of plastics. Besides that, the bad management of plastic leads to a high amount of plastic debris in the marine environment that over the years turns to microplastic particles. Not only is the presence of microplastics a concern, but to this must be added the presence of organic pollutants coming from the pollution or from the compounds added during the plastic manufacturing. This thesis will focus on the study of the consequences of having the microplastic particles and POPs in the oceans around the world.

I.1 Motivation and Aims

First invented in the 1860s and developed for industry in the 1920s, plastic production exploded in the 1940s becoming one of the fastest-growing global industries. Plastic is a general term to describe a wide range of synthetic or semi-synthetic materials. A family of organic polymers derived from natural gas or petroleum sources[1].

Plastics are extremely versatile materials. The relatively low density of most plastics gives plastic products the advantages of light weight. Although most have excellent thermal and electrical insulation properties, some plastics can be made to conduct electricity. They are corrosion resistant to may substances which attack other materials, making them durable and suitable for use in harsh environments. Some are transparent, making optical devices possible. They can easily be modulated into complex shapes, allowing other materials to be integrated into plastic products, and making them ideal for a wide range of functions. Furthermore, if the physical properties of a given plastic do not quite meet the specified requirements, its balance of properties can be modified with the addition of, for example, reinforcing fillers, colours, foaming agents, flame retardants or plasticizers, to meet the demands of the specific application. Besides this, plastic is relatively inexpensive. Those are attractive qualities that makes plastic one of the most common used materials both in industrial applications and in human activities (see Figure I-1).

The same properties that have turned the plastics a commonly used material of everyday life happen to be the reasons why plastics are a serious hazard to the environment[2]. World production of plastic surpassed the 320 million tons mark in 2016, most of which is intended for packaging, i.e., for immediate disposal[3].

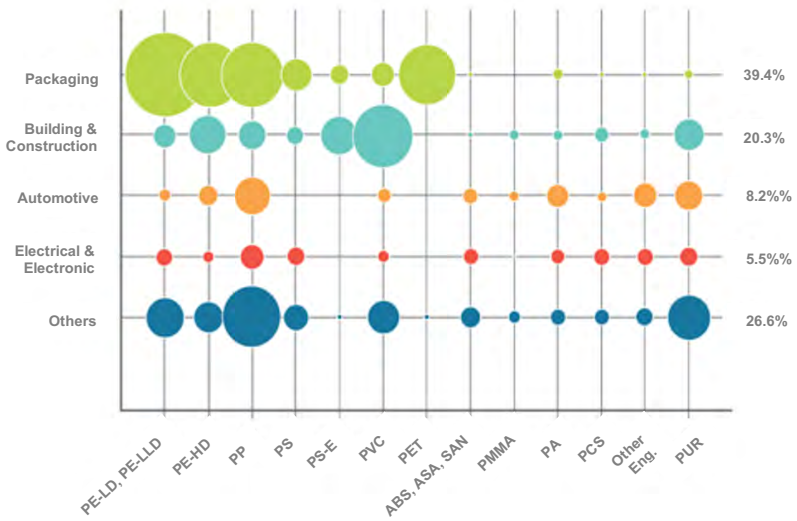

Figure I-1. European plastics demand by resin type and industrial sector in 2012. PE-LD is the low density polyethylene, PE-HD is the high density polyethylene, PP is polypropylene, PS is polystyrene, PVC is polyvinyl chloride, PET is polyethylene terephthalate, ABS is acrylonitrile butadiene styrene, ASA is acrylonitrile styrene acrylate, SAN is styrene acrylonitrile, PMMA is polymethyl methacrylate, PA is polyamide, PCS is polycarbonate and PUR is polyurethane[4]

Over the last few decades, plastic contamination has become a major cause of concern among scientist, politicians and the public. The overproduction and excessive use of plastic have made difficult a proper manage and that is why it has become the fastest growing segment of the waste stream. It is not possible to obtain reliable estimates of the amount of plastic debris that reaches the marine environment, but the quantities are nevertheless quite substantial[5–7].

In 1975 the world's fishing fleet alone dumped into the sea approximately 135,400 tons of plastic fishing gear and 23,600 tons of synthetic packaging material. It is estimated that merchant ships dump 639,000 plastic containers each day around the world, and ships are therefore, a major source of plastic debris. Recreational fishing and boats are also responsible for dumping a considerable amount of marine debris. Moreover, the abusive use and the management of the plastic by the industries and the human activity is not always the adequate so ends up reaching the oceans. Plastic materials also end up in the marine environment when accidentally lost, carelessly handled or left behind by beachgoers. They also reach the sea through the rivers and municipal drainage. There are major trades of plastic litter in densely populated or industrialized areas[8–10].

Plastic debris could be differentiated according to the size, origin, composition or shape. When the plastic debris classification is made as a function of the composition, it refers to the polymer type. Knowing the composition or origin of the plastic can help to determine where the particles will be found depending on their density. Quantifying plastic debris in the ocean includes the floating plastic particles, the ones in the sediment and mid-water plastic. Plastic debris can be classified in function of their composition which will also determine their location in the water

column. This requires establishing the relation between the plastic and sea water density (density of sea water is approximately 1025 Kg/m^3 at 25ºC, salinity of 35 g/Kg and 1 atm). Some examples of the plastics, typically used in the marine environment, that has a specific gravity lower than that of seawater are low-density polyethylene (LDPE), high-density polyethylene (HDPE), polypropylene (PP) or polystyrene (PS). Denser varieties of plastics such as nylons or polyvinyl chloride (PVC) tend to submerge in the water and even reach the coastal sediment (see Table I-1)[11].

Table I-1. Classes of plastics that are commonly encountered in the marine environment[11]

Resin type	Specific gravity	Common applications
Polyethylene	0.91-0.95	Plastic bags, storage containers
Polypropylene	0.90-0.92	Rope, bottle caps, gear, strapping
Polystyrene (expanded)	1.05	Cool boxes, floats, cups
Polystyrene	1.04-1.09	Utensils, containers
Polyvinyl chloride	1.16-1.30	Film, pipe, containers
Polyamide or Nylon	1.13-1.15	Fishing nets, rope
Polyethylene terephthalate	1.37	Bottles, strapping
Polyester resin + glass fibre	>1.35	Textiles, boats
Cellulose Acetate	1.22-1.24	Cigarette filters

Plastics are divided as first-generation plastics or second-generation plastics. First-generation plastics are those which are found in their original or close-to-original form when they are collected, such as bottle caps, resin pellets or plastic bags. Second-generation plastics are those that comes from first-generation plastics, i.e., are degradation products from the original plastic or small pieces of plastics that come from the breakdown of the first-generation plastics during the years. Besides this, macroplastics cause a health risk to aquatic animals, including fish, turtles, seals and birds, because of possible entanglement or ingestion, that may cause internal bleeding, abrasion and ulcers, as well as blockage of the digestive tract[12,13].

Through some physical, chemical and biological processes such as UV-light, wave action, ocean currents, suspension and resuspension of plastics, large plastic debris fragments can degrade into micro-sized plastic commonly referred to as Microplastics (MP). Are the result from the breakdown of large plastic items, e.g. from fishing gears, ships, recreational activities or transport of plastic products. Microplastics were first reported in the scientific literature in the early 1970s, and later publications described studies identifying plastic fragments in birds in the 1980s. It is unclear when the term microplastic was first used in relation to marine debris. It was mentioned by Ryan and Moloney (1990) describing the results of surveys of South African beaches, and in the 1990s by Thompson et al. (2004) describing the distribution of plastic fragments in seawater[14,15]. There is no a unique definition for microplastics but generally refers to plastic particles in the size range of 1 μm to 5 mm.

The scientific community have focused their researches in the study of these type of microplastics but, in addition to these we can also find other type of microplastics which are those originally and intentionally manufactured in the size range of 1 μm to 5 mm and have applications in personal care products like toothpaste, shower gels, scrubs, peelings or cosmetics[16,17].

In general, it is extremely difficult to identify and point out the ultimate sources of microplastics due to their fragmentation and degradation nature of the debris occurring in small and heterogeneous assemblages. It is not possible to observe the microplastic particles that float below the surface of the seawater by flight observations or satellite and there is no accurate data estimating the global plastic inputs into the ocean and the parts that sink to the ocean floor. The geographical coverage of microplastics is growing on a yearly basis[18,19].

The scientific community is currently focusing on microplastics more than macroplastics and study their abundance and effects. Microplastics tend to pose a greater threat to marine biota and increasing changes in the integrity of the habitats at alarming rate globally[20]. For instance, microplastic ingestion has been recorded in a wide variety of marine biota resulting in physiological disorders[15,21,22]. Because of its high mobility and specific hydrology microplastic debris has practically permeated the global marine environment, including the polar regions, mid-ocean islands, and the deep sea.

Due to its size, microplastics tends to exhibit a relatively large ratio of surface area to volume and combined with their nature, have a significant ability to accumulate (sorb) persistent organic pollutants (POPs). POPs are chemicals of global concern due to their potential for long-range transport, persistence in the environment, ability to bio-magnify and bio-accumulate in ecosystems, as well as their significant negative effects on human health and the environment. Humans are exposed to these chemicals in a variety of ways, through the food we eat or through the air we breathe. Many products used in our daily lives may contains POPs, such as flame retardants, surfactants or oils. The most commonly encountered POPs are polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), polycyclic aromatic hydrocarbons (PAHs), DDT or Hexachlorocyclohexane (HCHs) from water and the atmosphere onto the surface. The highest concentrations of POPs are thus found in organisms at the top of the food chain[23–26]. The sorption of contaminants on those particles is particularly high. Within a few weeks microplastic particles can accumulate pollutants on the particle surface at concentrations that are orders of magnitude greater than in the surrounding water[27,28]. Following sorption onto the particles, the contaminants are carried along with the plastics from their origin. Sorption will tend towards equilibrium between the plastic and seawater. The size of microplastics, polymer type and hydrophobicity of the contaminant will all exert an influence.

Moreover, a growing number of studies demonstrate that, under the right conditions, many species of marine organisms will ingest microplastic particles. As organisms consume a mixed diet consisting of a variety of particles, including perhaps microplastics, both microplastics and organic pollutants will be introduced into the trophic chain potentially affecting human health (see

Figure I-2). For some compounds and scenarios, even to low levels of POPs can lead, among others, to increased cancer risk, reproductive disorders, alteration of the immune system, neurobehavioral impairment, endocrine disruption, genotoxicity and increased birth defects.

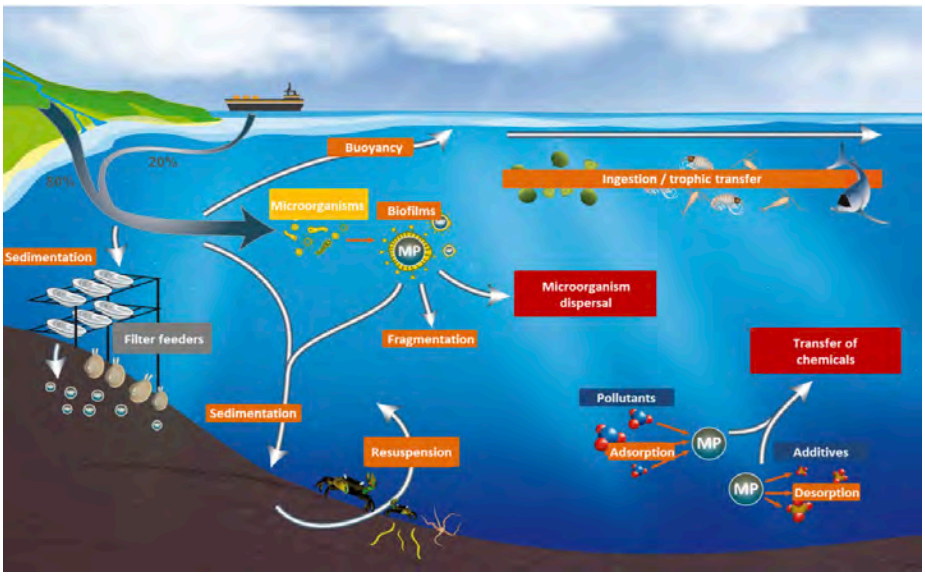

Figure I-2. Possible consequences of the presence of microplastics in the marine environment[29]

So, oceans microplastic pollution has become a growing environmental problem. The adverse effects of this particles in the marine environment and the introduction of them to the tropic chain as well as their capacity of adsorbing POPs is still an unfinished research topic.

The increase in scientific publications, as can be seen in Figure I-3, demonstrates that the understanding about microplastics has advanced considerably over the last decade, but is still in the beginnings and the knowledge of the relative importance of various sources, spatial trends in distribution and abundance, temporal trends, or effects on biota are still quite limited.

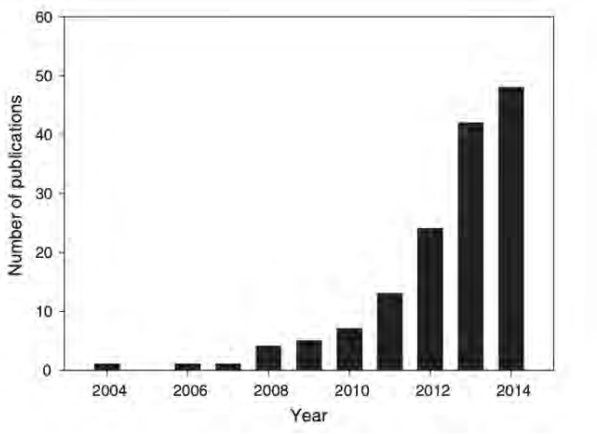

Figure I-3. Number of publications related to microplastics from 2004 to 2014[30]

For the moment, the research studies referred to seawater microplastics show a huge diversity of results and are normally focused in specific seawater locations from different oceans which are interesting due to their proximity to treatment plants, coastal zones or urbanized regions. Until now, do not exist a global picture about this problematic in the marine environment. That does, therefore, raise the question of the possibility to develop a study to give to the scientific community a global vision about which the actual state of the oceans is regarding to microplastic particles.

Thus, the main objective of this thesis is providing information about the overall status of the oceans in terms of microplastics at present. Study the role of microplastics in seawater in a global way will be helpful to understand the actual environmental situation. To achieve this goal, samples from different locations along the world oceans have to be taken. In this sense, we have collaborated with the "Fundació de Navegació Oceànica de Barcelona" (FNOB) which is one of the organizations which is part of the Barcelona World Race (BWR). Through this collaboration, we have developed a device installed in a racing boat of the BWR 2015 which collects microplastics on superficial seawater from the different locations of the world going through all the oceans.

To achieve the main goal of this work, this thesis has been structured in the following work plan:

- Development and optimization of the device installed in the racing boat of the BWR 2015 **(Chapter II).** We design and optimize a device that will be installed in a racing boat that participates in the Barcelona World Race 2015. This device has to be capable to collect more than 100 samples in different locations of the world oceans, with a high simplicity and easy to manipulate by the crew. We do a study of which are the best filters to collect microplastics and the optimal pore sizes. We also add to the device, a solid extraction cartridge that will collect the pollutants solved in the seawater in each point.

- Characterization of microplastics collected. Qualitative and quantitative analyses of microplastics **(Chapter III).** The treatment of the samples collected once they arrive at the laboratory and they subsequent analysis constitutes the central axis of Chapter III. To achieve that a qualitative and quantitative analysis of the particles retained in the different sizes filters have done. A count of the microplastic particles (microplastics) as well as the microplankton particles have been performed. The particles composition by infrared microscopy and the particles morphology by SEM microscopy have been also studied. All the results extracted will allows us extract conclusions about the distribution of microplastics around the world oceans in addition to the proportion of microplastics versus microplankton particles to learn more about the existing problem.

- Understanding the role of light microplastics as concentrators of organic pollutants and develop analytical methods to analyse them **(Chapter IV).** There is a question that arises

in the scientific community related with the microplastics concern. In Chapter IV, we have focused on the study of the adsorption of organic pollutants on microplastic particles acting this way as transporters for POPs. We present the development of an experimental method to study the adsorption of high environment impact organic pollutants and the demonstration of the importance of the analytical process for an optimal release.

- Study and optimization of the elution process of POPs from solid extraction cartridges and development of analytical methods to analyse them **(Chapter V).** Once all the studies related with the microplastics are finished, we discussed in the Chapter V of this thesis the best way to extract the POPs retained in the solid extraction cartridge placed in the BWR 2015 device. The elution process is optimized, and a list of high impact organic pollutants have been analysed using last generation chromatographic techniques.

In summary, the impact of plastic debris in the marine environment have been a huge concern for the last decades. The most recent studies have been focused on the presence of first generation microplastics or those microplastics result of the plastic debris degradation (second-generation microplastics) in certain locations. The accumulation of microplastic debris in the water leads to an additional problem to world oceans pollution, the microparticles, which are polymeric, can adsorb on their surface organic pollutants that are in suspension in the water due to their hydrophobicity. In this work, we want to answer the question that then arises, which is the global state of our oceans resulting from the presence of microplastics. Furthermore, we will study the microplastics concentration effect of POPs. All the results will help us to understand better in what state the situation is at this moment.

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Chapter II.

Microplastics sampling device

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Microplastics sampling device

This chapter describes the development of a new sampling device capable to collect microparticles and organic pollutants from superficial seawater. Until now, the existing sampling methodologies are not so practical and require several steps in terms of laboratory sample processing. We create a device that improves the existing methods and fulfil the requirements previously established. Furthermore, the device developed, in collaboration with Sailing Technologies R+D, has been installed in a racing boat that participates in the Barcelona World Race 2014-2015. Samples from different locations along the oceans have been collected.

II.1 Introduction

As previously discussed in Chapter I, the over manufacturing and the excessive use of plastic has caused a negative impact on the environment which increases over the years. The bad management of plastic has hindered the process of recycling causing the accumulation of floating plastic debris that reaches the most remote areas of the planet, including the surface waters and open oceans[1,2].

Plastic debris results in a smaller plastic pieces known as microplastics. The term microplastics generally refers to plastic particles in the size range of 1 μm to 5 mm. As have been mentioned, part of the microplastics can be originated as a result of the fragmentation and degradation of larger plastic particles due to physical, chemical and biological processes such as UV-light, wave action or ocean currents. In addition to these, other type of microplastics can be found in seawater which are those originally and intentionally manufactured in the size range of 1 μm to 5 mm and have applications in personal care products like shower gels, scrubs or cosmetics[3].

In addition of microplastics, other types of pollutants can be found in seawater such as hydrophobic persistent organic pollutants (POPs). POPs are chemicals of global concern due to their potential for long-range transport, persistence in the environment, ability to bio-magnify and bio-accumulate in ecosystems, as well as their significant negative effects on human health and the environment. POPs coming from wastewaters due to a bad management or from the human activity and can accumulate and end in the oceans. Those are pollutants such as polycyclic aromatic hydrocarbons (PAHs), pesticides or polychlorinated compounds (PCBs) or polybrominated diphenyl ethers (PBDEs). In addition to these, suspended in seawater, other type of pollutants can be found. The growing use of pharmaceuticals or personal care products, named as emerging pollutants, has become a new environmental problem since in the last years, traces of this compounds have been detected in seawater.

The global concern about this type of pollutants is their potential for long-range transport, persistence in the environment, ability to bio-accumulate in ecosystems and their significant negative effects on human health. The presence of these plastic microparticles in seawater and their ability of adsorbing POPs in combination with the fact that some studies have demonstrated that many species of marine organisms can ingest them due to they have the same size of microplankton becomes microplastics a huge environmental problem[4,5]. The adverse effects of having these pollutants in the oceans as well as the consequences of their adsorption into microplastic particles will be explained in further detail in Chapter IV and Chapter V.

The analysis of microplastics therefore, has become a new challenge for the scientific community. Although first steps in the development of methodologies for their analysis and quantification and which are the consequences of having them in the oceans have been made, there is still a lack of information in many aspects related to this type of particles[6–8].

Several studies have been published about methodologies for identifying and quantifying microplastics. Reviews address microplastics in the marine environment, fresh-water environments, sediments, biota or soils. However, the main focus of these studies was on comparing published articles or on analytical issues; only little attention has been payed to sampling and sample preparation.

The amount of studies published in the last years about microplastics clearly shows that intensive research is still necessary to develop and strengthen the methods used for sampling and sample preparation of microplastics. Therefore, comparing the results of different studies is not yet possible an harmonization of methods and the establishment of standards are needed[9]. Thus, the first step for the subsequent analysis is the optimization of the sampling process.

One of the main problems of large-scale spatial and temporal comparisons is the fact that a wide variety of approaches have been used to identify and quantify microplastics. Furthermore, microplastics comprise a very heterogeneous assemblage of pieces that vary in size, shape, coloration, specific density, chemical composition, and other characteristics. Microplastics sampling in the main marine environments (water surface, water column or sediments) requires different approaches[10]. The most commonly used sampling methods can be classified in three general groups. These methods are presented below:

- **Selective sampling:** consists of a direct extraction from the environment of items that are recognizable by the naked eye, usually on the surface of sediments. However, when microplastics are mixed with other debris or have no characteristic shapes there is a risk to overlook them.

- **Bulk sampling:** samples where the entire volume of the sample is taken without reducing it during the sampling process. Most appropriate when microplastics cannot be easily identified visually. However, this methodology requires a several steps of sample processing in the laboratory.

- **Volume-reduced sampling:** used for sediment and superficial seawater samples. In this methodology the volume of the bulk sample is reduced during the sampling process, preserving only that portion of the sample that is of interest. However, for superficial seawater samples, volume-reduced methodology is usually obtained by filtering large volumes of water with nets. This methodology, as bulk sampling, require further processing in the laboratory.

The selection of any of the approaches mentioned above will be made based on the microplastics sample location, that is to say, water sampling or sediments sampling.

Microplastics are distributed in the water column dependent on their properties, such as density, shape, size, adsorption of chemicals and on environmental conditions such as water density, wind, currents and waves. Thus, quantity and quality of microplàstics recovered are highly dependent on sampling location and depth. Sampling and processing methods are similar for both fresh and saltwater samples. However, differences can be found in the distribution of microplastics in each system, influenced by environmental characteristics, such as differences in density of fresh and seawater.

In contrast with water microplastics sampling, the distribution of microplastics on sediments is uneven, largely influenced by their properties and environmental factors, such as winds and currents. Results will be largely dependent on the sampling area and depth since some areas may contain higher concentrations of microplastics. Collection of microplastics on beaches include direct sampling, sieving and collection of sediment samples[11].

A brief summary of the advantages or disadvantages of the instruments and methodologies used for the microplastics sampling based on their location is presented below in Figure II-1.

Sample	Type	Advantages	Disadvantage
Water	Neuston and Manta nets	Easy to use; Sample large volumes of water; Largely used (good to compare between locations); Produces large numbers of microplastics for further testing.	Expensive equipment; Requires boat; Time-consuming; Potential contamination by vessel and tow ropes; Lower limit of detection is 333 μm.
	Plankton net	Easy to use; Lowest limit of detection 100 μm; Quick to use; Samples medium volumes of water.	Expensive equipment; Requires boat; Static sampling requires water flow; May become clogged or break; Sampling of lower volumes of water than Manta trawl.
	Sieving	Does not require specialized equipment nor boat; Easy to collect samples.	Laborious and time consuming; Samples medium volumes; Manual transfer of water with buckets
	Pumps	Samples large volumes of water; Effortless; Allows choice of mesh size.	Requires equipment; Requires energy to work; Potential contamination by the apparatus; May be difficult to carry between sampling locations.
	Filtration or Sieving *ex situ*	Easy to collect samples; Known volume of water; Allows choice of mesh size.	Sampling of low volumes; Transportation of water samples to the lab; Potential contamination by the apparatus; Time consuming depending on mesh size.
Sediment	Beach sediment collection	Easy to implement; Rapid sampling; Allows collection of large volumes of sample or replicates.	Variation with sampled area and depth.
	Seabed collection (Grab sampler, box corer, gravity core)	Easy to use; May allow replicates.	Expensive equipment; Requires boat; Variation with sampled area and depth; Sampling may disturb sediment surface.

Figure II-1. Methods of sample collection in water and sediment. Advantages and disadvantages[11]

As can be observed in Figure II-1, the choice between a sampling method is dependent on available equipment but also the objective of the work. Different types of sampling can be used but all of them present several disadvantages such as expensive equipment, large volumes of samples and laborious laboratory processing. Furthermore, most of the methods requires the use of a boat which can difficult the sampling process.

Finally, and to summarize and highlight the struggles originated due to the large rang of available sampling methodologies options a table is presented below. The sampling and sorting methods applied in recent studies are unequal in function of the objective of the investigation (see Table II-1).

Table II-1. Principal objectives of the examined studies on microplastics[10,12–14]

Objectives	sediment	sea surface	water column	total
Methodology	2	2	-	3
Presence/absence	4	2	1	5
Spatial distribution	25	22	5	45
Temporal variability	3	6	1	9
Dispersal processes	1	-	-	1
Physical properties and fragmentation processes	4	2	1	6
Contaminants	12	3	13	13

As can be seen in Table II-1, studies focused in different topics have been made. Different results in function of the location of the microplastics have been obtained, the methodology used

to collect them, the microplastics physical properties or the presence or not of pollutants adsorbed.

As can be concluded, the actual sampling methodologies for microplastics collection from seawater are still under development and have several weak aspects. Are quite simple, with non-robust instrumental and use volumes which makes the sample difficult to manipulate. These sampling methods complicate the procedure if several samples have to be taken or samples from different locations are needed to obtain a representative study. Moreover, until now, do not exist sampling methodologies which combine the collection of both, microplastics and pollutants suspended or solved in seawater. A new device capable to collect at the same time the pollutants dissolved in seawater in addition of microplastics and in consequence the pollutants adsorbed on their surface will enable a more representative and global study about the actual environmental concern. Thus, some improvement in terms of sampling have to be done.

Then, the main objective of this chapter is the development of a new methodology for microplastics sampling and retention of pollutants present in seawater. In this sense, we have collaborated with the Barcelona World Race (BWR) organization and the "Fundació de Navegació Oceànica de Barcelona" (FNOB). Throughout this collaboration, we have developed a device, named COA (Clean Ocean Analysis), installed with the collaboration of the Sailing Technologies Group in a racing boat which participates in the BWR 2015 which collects microplastics and pollutants on superficial seawater from the different locations of the world going through all the oceans.

In order to achieve this objective, the following tasks were proposed:

- Selection of the most suitable elements to create the COA device. First, new sampling method for collecting microparticles that improves the actual systems. Combination of microparticles collection with another element for the retention of pollutants solved in seawater.

- Concept laboratory tests for the validation of all the elements placed in the COA device and its installation in the BWR 2015 racing boat.

- Itinerary study and selection of the location where the samples need to be taken to obtain a representative study.

II.2 Material and Methods

II.2.1 Polymer samples

The microplastics used for all the experimental procedures were obtained from the trituration of commonly used plastic materials chosen according to the desired polymeric composition. As has been mention in the introductory section of this Chapter, the COA device collects superficial seawater samples so it makes sense to work with microplastic particles found floating in seawater. Thus, we chose to work with those most commonly used plastics. Table II-2 shows the origin and specifications of the plastics used.

Table II-2. Plastics used. Their origin and specifications

Polymer type		Origin	Specification
Polyethylene Terephthalate	PET	Water bottle	1.5 L Water bottle (Veri®)
Low Density Polyethylene	LDPE	Ring bag (six pack)	Coca-cola® Ring bag (Dia®)
Polypropylene	PP	Straws	Flexible straws (DonPalillo®)
Polystyrene	PS	Coffee spoon	Spoon (Papstar®)

The original plastics were cut in pieces of a size of the order of 1.0 or 1.5 cm. Then, they were introduced into a cryogenic mill (SPEX^{TM} 6770 SamplePrep). The samples were frozen with liquid N_2 and crushed through mechanical movements against a stainless-steel cylinder until the plastic was grinded to a microscopic scale sized particle.

The polymeric micro sized particles were sieved to group the mixture in three particle sizes, 500 μm, using sieves with a particle size of 700 μm and 490 μm, 50 μm, using a sieve with a particle size of 49 μm, and 5 μm, where the rest of the particles not retained in the other sieves.

II.2.2 Synthetic seawater

For the experimental procedure, 1 L of synthetic seawater was prepared using the compounds described in Table II-3 and adjusting the pH with hydrochloric acid (HCl, Panreac, 1 M) between 7.5 and 8.5.

Table II-3. Composition of synthetic seawater. Composition and product references[15]

Compounds	Concentration ($g \cdot L^{-1}$)	Product reference
NaCl	24	Panreac® 141659
$MgCl_2$	5.0	Sigma-Aldrich® M8266
Na_2SO_4	4.0	Panreac® 141716
$CaCl_2$	1.1	Sigma-Aldrich® 499609
KCl	0.70	Panreac® 141494
Na_2CO_3	0.20	Panreac® 141648
NaBr	0.096	Panreac® 141646
H_3BO_3	0.026	Panreac® 141015
$SrCl_2$	0.024	Sigma-Aldrich® 439665
NaF	0.0030	Sigma-Aldrich® 450022

II.2.3 Plasma treatment conditions

For the elimination of the electrostatic forces related to the microplastics used a plasma treatment has been applied. An oxygen (O_2) treatment at 40 W for 3 minutes are the conditions selected. A dutty cycle in a continuous mode has been used.

II.2.4 Filters

The election of the filters is based on the requirements raised. Long term filters are needed since the time of sampling is not known and the samples may take time until they arrive to the laboratory. The filters have to be resistant to seawater corrosive effects and strong. The filters that satisfy all these requirements are from Fisher Scientific company and their principal properties are listed below[16], also an image of the filters used aspect is presented (see Figure II-2):

- **Spectra/Mesh™ 5 µm Nylon (N) Woven Filters:**
    - *Properties: Strong, durable, hydrophilic, inert*
    - *Best Resistance to corrosion*
    - *Tolerance: 3-10 pH, up to 180°C*
    - *Sterilization: irradiation*
    - *Mesh opening size: 5 µm*
    - *Disc diameter size: 25 mm*

- *Thickness: 100 µm*

- **Spectra/Mesh™ 50 µm Nylon (N) Woven Filters:**
  - *Properties: Strong, durable, hydrophilic, inert*
  - *Best Resistance to corrosion*
  - *Tolerance: 3-10 pH, up to 180°C*
  - *Sterilization: irradiation*
  - *Mesh opening size: 53 µm*
  - *Disc diameter size: 25 mm*
  - *Thickness: 60 µm*

- **Spectra/Mesh™ Polypropylene (PP) Woven Filters:**
  - *Properties: Strong, durable, hydrophobic, inert*
  - *Best Resistance to corrosion, acids, alkalis and organics*
  - *Tolerance: 2-14 pH, up to 130°C*
  - *Sterilization: Autoclavable*
  - *Mesh opening size: 500 µm*
  - *Disc diameter size: 25 mm*
  - *Thickness: 610 µm*

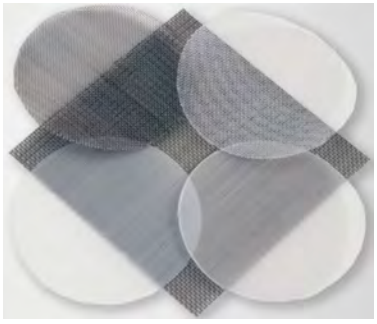

Figure II-2. Appearance of Fisher Scientific 50 and 5 filters µm installed in the COA device

As can be seen in the Figure II-2 and from the properties described before, the three filters accomplish the requirements established. Two of the filters are fabricated with nylon and the 500 µm one is fabricated using propylene. The filters are inert, strong and durable and have a small thickness.

II.2.5 Solid phase extraction

Oasis HLB is a universal sorbent for acidic, neutral and basic compounds. It has a hydrophilic-lipophilic water-wettable reversed-phase sorbent and is made from a balanced ration of two monomers, the hydrophilic N-vinylpyrrolidone and the lipophilic divinylbenzene (see Figure II-3).

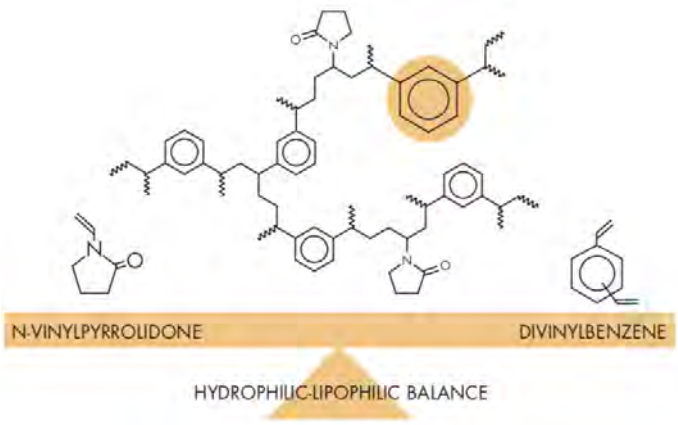

Figure II-3. Oasis HLB stationary phase polymer composition[17]

This balanced copolymer structure provides to the SPE cartridge a superior reversed-phase capacity with a special polar force for enhanced retention of polar analytes. The ability of this cartridge of adsorbing not only non-polar analytes as in most of the cases but also polar analytes.

This type of cartridge is widely used for agrochemical and environmental applications such as triazine or acid herbicides, metabolites, phenols or PAHs but is also used in pharmaceutical or food applications such as tetracyclines and basic drugs or tetracyclines and pesticides[18–22].

For all the mentioned previously, this type of SPE cartridge meets all the requirements established. Is versatile, universal and allows the detection of a huge amount of pollutant families.

II.3 Results and Discussion

As has been mentioned in the introductory section the sampling methods for seawater microplastics collection are diverse. For the development of a new sampling device, the type of samples to collect has to be established. Since the collaboration between the GEMAT group and the FNOB allows us to install the device created in one of the racing boats of the BWR 2015, it is decided that superficial seawater samples will be collected for the further research. Thus, the COA device have to be able to capture all the particles in suspension, as well as the pollutants dissolved or suspended in seawater along the oceans.

II.3.1 COA device elements

II.3.1.1 Superficial seawater microparticles retention

For the collection of microplastics, a methodology based on the existing methods has been raised. It is known, based on bibliography, that the most practical way to collect microplastics is by filtration methods. Filtering or sieving is the most frequent method in separation of microplastics from water samples and for the supernatant containing plastics from density separation. Filter's pore size mesh can vary greatly and determine the lower size of microplastics detected.

As has been stated, the use of a filtration system brings a lot of advantages regarding to the rest of the methodologies presented in the introductory section such as, easy collection of samples, known water volume samples and the possibility of selection the mesh sizes. But, by the development of this device, the disadvantages of the previous sampling methods have to be avoided. Some of this methodology disadvantages mentioned in several reviews are:

- Sampling of low volumes
- Transportation of water samples to the laboratory
- Potential contamination by the apparatus
- Time consuming depending on mesh size
- Variation with sampled area and depth

To do so, the collection of huge volumes of water samples have been simplified, an in-situ filtration system has been developed, the timing for sample processing has been minimized and a reproducible methodology has been developed.

A system of three filters connected in series has been proposed, filters of different pore sizes have been installed in the COA device. To cover the full range of sizes, filters of 500 μm, 50 μm and 5 μm of pore sizes have been selected, that is to say, microplastics of sizes higher than 500

µm to 5 µm microplastics will be retained. The filters selected specifications have been mentioned in the Material and Methods section of this chapter (II.2.4).

II.3.1.2 Solved and suspended pollutants retention

In addition to microplastics, in seawater can be also found suspended or dissolved other type of pollutants. Some families of pollutants, more precisely, the persistent organic pollutants, will be those that probably will be found adsorbed on the microplastic particles but solved in the seawater can be other families that could be interesting to analyze. In Chapter V an extensively discussion about this families of pollutants have been done.

Due to this interest, it is decided to install in the COA device, a system capable to collect these pollutants solved in seawater without interfering with the filtering system to collect microparticles. To do that, different options have been taken into account according to the studies found in bibliography[23–25]. The system has to follow the same requirements than the filters. It has to be robust, durable and resistant to seawater effects. Moreover, it has to be a system capable to work with various volumes of water and that do not require a complicated laboratory sample processing.

Solid Phase Extraction (SPE) is the technique which has been decided to use. A typical SPE device has 50 times more separation power than a simple, single liquid-liquid extraction. SPE is actually a column liquid-solid chromatography. A sample is introduced into a column or a cartridge device containing a bed of appropriate particles (stationary phase). Solvent (mobile phase) flows through the bed. By choosing an appropriate combination of mobile and stationary phases, sample components may pass directly through the column bed, or they may be selectively retained[26–30].

Compared to other sample preparation processes, SPE has some major benefits:

- Lower cost: lower solvent consumption, less apparatus
- Greater recoveries: minimal sample transfer
- Faster protocol: fewer steps
- Greater selectivity: no cross contamination
- No transporting of samples to laboratory: direct field sampling and minimal evaporation

Moreover, SPE allows the compound purification in a really complex matrix, as is our scenario with the analysis of pollutants in seawater. A complex sample could be broken into fractions to be able to analyze it more efficiently. Also, when dealing with samples where the analyte is too diluted to be analyzable, SPE can be used to concentrate the analyte to a quantifiable concentration[31–34].

As a result, solid phase extraction is the most adequate system to be installed in the COA device. The methodology to use has been selected but there are many different types of SPE in function of the matrix and the compounds to be analyzed. An Oasis HLB Plus SPE cartridge supplied by Waters has been chosed[35,36]. The SPE cartridge selected specifications have been mentioned in the Material and Methods section of this chapter (II.2.5)

II.3.2 COA device optimization

All the necessary elements for the COA device assembling have been sorted out. The most suitable filters for the microparticles collection and the SPE cartridge for the retention of the pollutants solved in seawater have been selected. Thus, it can be proceeded to the assembly of the COA. To do that, the three filters part of the microparticles sampling system are connected through PVC flexile pipes. At the same way, the filters are connected to the SPE cartridge using again PVC pipes. Figure II-4 shows a schematic representation of the appearance of the assemblage once all the elements are connected.

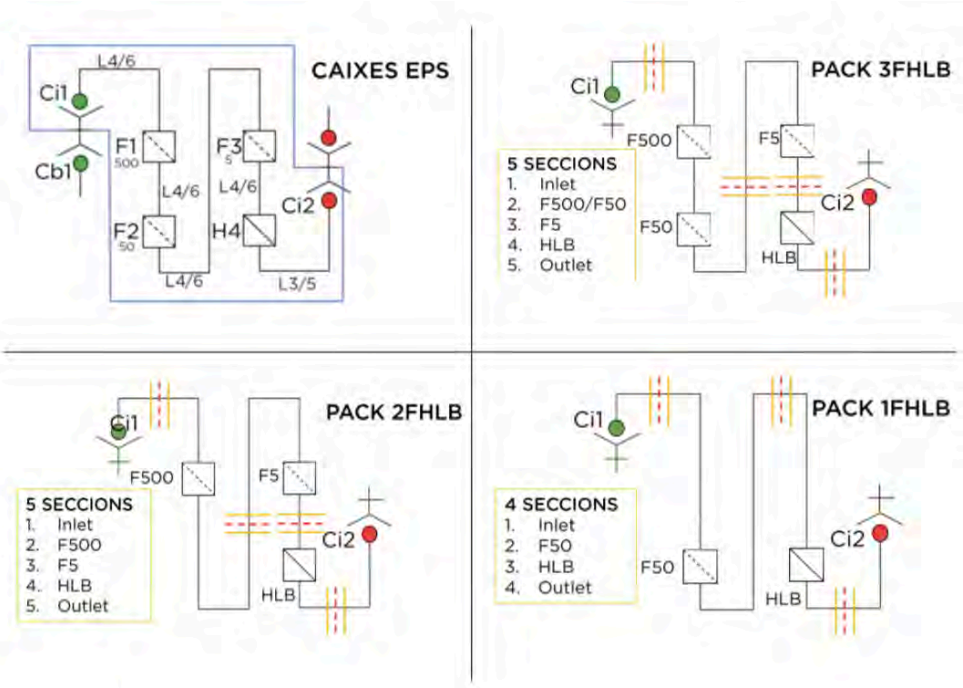

Figure II-4. Schematic representation of the COA device elements

Where F500, F50 and F5 correspond to the filters with a pore size of 500 µm 50 µm and 5 µm respectively. HLB corresponds to the solid phase extraction cartridge, the L4/6 connections corresponds to the PVC flexible pipes and Ci1 and Ci2 corresponds to the inlet and outlet of the COA device.

A peristaltic pump connected to the COA device (Ci1) in order to force the circulation of seawater through the system has been used. Furthermore, the peristaltic pump allows determining a constant flow rate since its intensity and voltage have been fixed. The use of a constant flow rate enables a homogeneous sampling.

As can be seen in Figure II-4, three different types of sampling devices have been developed. Pack 3FHLB have in the system three size filters (500, 50 and 5 μm) and the HLB cartridge, Pack 2FHLB have in the system two of the filters (500 and 5 μm) and the HLB cartridge and finally, Pack 1FHLB which have in the system one filter (50 μm) and the HLB cartridge.

Once the COA device has been assembled, the system suitability has been studied. To do that, different laboratory tests have been performed in order to test the COA device elements. The COA parameters studied are the following:

a) COA device flow rate optimization

b) Microplastic particles circulation through the COA device

c) Microplastic particles retention

d) Filters clean up

II.3.2.1 Flow rate optimization

In order to optimize the suitable flow, two main assumptions have been taken into account: an enough volume of seawater has to be collected to have representative samples and the flow rate cannot be higher than the break flow of the filters and HLB cartridges. The work conditions have been established as the collection of 1 litter of seawater for 23 hours, i.e., the device has to work at a flow rate of 0.73 $mL \cdot min^{-1}$.

As stated before, a peristaltic pump to circulate water through the system has been used. A specific voltage and intensity have to be selected in order to determine the flow rate of work defined. Thus, to establish the flow rate conditions, MilliQ water has been circulated through the COA filter (without the filters). In this first experience, the intensity of the system has been fixed at 60 mA while different voltages have been tested (20, 10 and 5 V). MilliQ water has been circulated through the system during 4 minutes in order to determine which is the voltage that results in a flow rate of approximately 0.73 $mL \cdot min^{-1}$. Table II-4 shows the results obtained for the described experience.

Table II-4. Flow rate conditions set up. Flow rate values obtained when the intensity is fixed at 60 mA and different voltages has been tested when MilliQ has been circulated through the COA device during 4 min

	Intensity (mA)	Voltage (V)	Time (min)	Volume (mL)	Flow rate (mL·min^{-1})
1	60	20	4	17	4.3
2	60	10	4	8	2.0
3	60	5	4	4	1.0

As can be seen in Table II-4, to maintain the flow rate at the conditions established, the intensity of the system has to be set at 60 mA with a voltage of 5 V.

Once the flow rate conditions has been established, the filters have been installed and the experience has been repeated to ensure that it remains constant over the time and does not change due to the presence of the filters. In this experience, as has been mentioned, the three filters have been installed in the device. Three replicates of this test have been done. The results of the experience are presented in Table II-5.

Table II-5. Flow rate values obtained when 3 mL of water during 4 min have been circulated through the COA device at 60 mA and 5 V. Three replicates have been done

	Intensity (mA)	Voltage (V)	Time (min)	Volume (mL)	Flow rate (mL·min^{-1})
1	60	5	4	3	0.75
2	60	5	4	3	0.76
3	60	5	4	3	0.75

As can be seen in Table II-5, the three replicates show the same results for the flow rate, 0.75 mL·min^{-1}, close to the theoretical value established at 0.73 mL·min^{-1}. The flow rate values obtained in this experience are fairly lower than the ones obtained in Table II-4 since a pressure loss has been experienced due to the incorporation of the filter into the system.

Then, the reproducibility along the time has been tested. The results obtained are presented in Table II-6.

Table II-6. Flow rate values obtained when 3 mL of water at different times have been circulated through the COA device at 60 mA and 5 V

	Intensity (mA)	Voltage (V)	Time (min)	Volume (mL)	Flow rate (mL·min^{-1})
1	60	5	4	3	0.75
2	60	5	8	6	0.75
3	60	5	24	17	0.73

The flow rate values obtained show that it remains constant at 0.74 mL·min^{-1} in coincidence with the theoretical value fixed.

Lastly, it has been verified that the flow remains constant in the established range when seawater circulates through the system. The following test experience has been performed by connecting the three filters circuit with 60 mA intensity and 5 V of voltage. Two different circulation times has been tested. Table II-7 shows the results obtained in this experience.

Table II-7. Flow rate values obtained when 3 mL of water at different times have been circulated through the COA device at 60 mA and 5 V

	Intensity (mA)	**Voltage (V)**	**Time (min)**	**Volume (mL)**	**Flow rate (mL·min^{-1})**
1	60	5	4	3	0.75
2	60	5	36	27	0.75

Table II-7 shows the results obtained when seawater has been circulated through the system. The flow rate achieved is 0.75 mL·min^{-1}, which is within the optimum range.

II.3.2.2 Microplastic particles circulation through the COA device

The next step in the COA device optimization is to ensure the correct microplastic particles circulation through the system.

The first two experiences (P1 and P2) have been based on the introduction of an aqueous solution with the microplastics through a peristaltic pump (to mimic the final procedure) in to the COA device to check the filters retention. Both procedures are described below:

P1 has been performed as follows: 4 mg of PET microparticles in a range between 430 µm and 50 µm have been introduced in a glass container with 50 mL of MilliQ water. The experience has been performed under the conditions of 60 mA and 5 V, and 1F (50 µm) installed in the COA device.

P2 has been performed as follows: 2 mg mixture of PET, PP, LDPE and PS microparticles in a range between 430 µm and 50 µm have been introduced in a glass container with 1 L of synthetic seawater. The experience has been performed under the conditions of 60 mA and 5 V, and 1F (50 µm) installed in the COA device.

During both of the experiences (P1 and P2) it has been observed that microplastic particles have not entered into the system since them have been adhered to the glass container walls and to the pipes used for the solution aspiration. Due to the accumulation the microplastics into the aspiration pipe, an obstruction has been formed blocking the entrance of the particles into the system. Consequently, no presence of microplastics has been detected in the sampling device or retained in the 50 µm filter. It is deduced that probably the microplastics electrostatic forces

have been caused the adhesion to the glass container and the obstruction. Below, a figure is presented (see Figure II-5) where the microplastic particles obstruction can be observed.

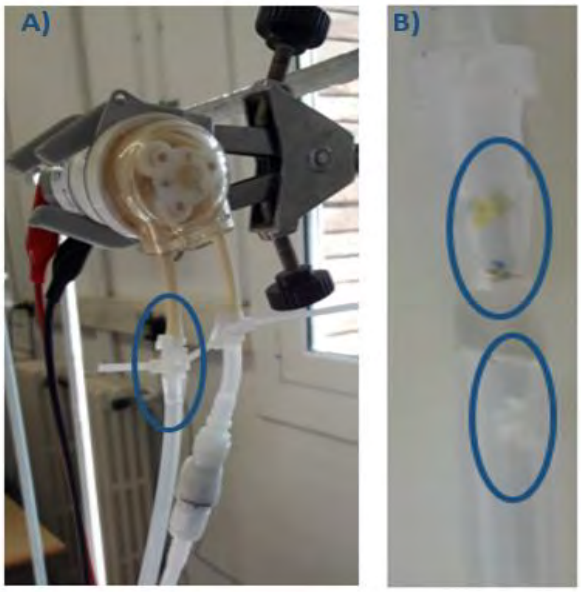

Figure II-5. A) Microplastics obturation in the COA aspiration pipe zone. **B)** Ampliation of an obturate COA device section

As has been mentioned in the introductory chapter (Chapter I), the majority of the microplastics detect in the oceans have been suspended in seawater for many decades. During this time, different physical and chemical processes such as oxidation/reduction process or a biological degradation due to the presence of microorganisms may have changed some of their physical properties and behavior such as their hydrophobicity strength. Thus, it is logical to think that the laboratory prepared microplastics will not have the same behavior than the microplastics collected from the oceans.

Since it has been not possible to reproduce the real environmental conditions (UV radiation, biological degradation or physical erosion) to which suspended microplastics have been exposed, different treatments have been applied to the laboratory prepared microplastics to change their properties and decrease their hydrophobicity and electrostatic forces.

The first treatment applied to the microplastics is the use of temperature. The microplastic particles have been brought under 70ºC in a stove for 48 h. The experiences P1 and P2 have been repeated after the temperature treatment applied and some differences in the microplastics behavior have been observed but not enough to obtain significative differences in their hydrophobic behavior. The results obtained have not changed, the microplastic particles do not reach the COA system since they have remained attached to the glass container walls and to the aspiration pipe.

So, a more aggressive treatment has been applied to the microplastics. A plasma treatment has been performed on the four types of microplastics (PET, PP, PS and LDPE). The plasma

treatment attacks the microplastics surface. It changes their hydrophobicity and decrease their electrostatic forces. The main advantage of the plasma treatment is the reduction of these charges and therefore the reduction of their attachment to the COA device pipes. The plasma treatment conditions have been described in the Material and Methods section.

Although the plasma treatment has considerably changed the laboratory microplastics behavior, the results obtained from the repetition of P1 and P2 experience demonstrate that it has not been possible to introduce the total amount of microplastics present in the aqueous solution into the COA device system.

It can be concluded from all the results obtained that although the laboratory microplastics behavior has considerably changed, the degradation conditions that seawater microplastics have suffered for decades are not feasible in the laboratory. This causes the microplastics prepared in the laboratory cannot be completely introduced into the COA device system.

It has to be highlighted that once the COA devices used during the BWR 2015 arrived at the laboratory it was no observed the presence of microplastics stoked on the plastic pipes or into the filter holders. So, the hypothesis of the differences of the laboratory prepared microplastics behavior has been confirmed.

In order to continue with the COA device optimization, it has been decided that the microplastics will be further introduced into de COA device by gravity and not by aspiration through a pipe.

The next step in the COA device optimization is the validation of the filter's retention.

II.3.2.3 Filters microplastic particles retention

This section of the COA optimization have been focused on the proper retention of the filters selected. As has been mentioned in the previous section, the microplastics could not be introduced into the system through the adsorption pipe, which is why for the following experiences the microplastics have been introduced into the system by gravity using a syringe as can be observed in the Figure II-6.

Figure II-6. Gravity method used for the introduction of microplastics aqueous dispersion into the COA device system

The experimental procedure (P3) followed for the filter's retention study is described down below:

a) 2.5 mg of microplastic particles are weight with the composition and size range selected for the experience. A 50 mL aqueous solution is prepared with the microplastics. The microplastics aqueous suspension is introduced into the COA system.

b) The filter (with the microplastics retained) is extracted from the filter holder. It is kept in a glass container and it is introduced into the stove at 40ºC during 1 h for the drying process. Once the filter with the microplastics is dried, a microbalance is used for the determination of its weight. The use of a microbalance has been necessary in this step of the procedure since the low amount of microplastics used and the accuracy needed to obtain satisfactory results.

c) The aqueous solution is collected at the end of the system after the circulation through the COA system to analyze the presence or not of microplastics.

Two experiences have been performed to validate the filters retention. The first one (P3_1*)* with a 500 µm pore sized filters and the second one (P3_2) with a 50 µm pore sized filter. Two of

the filters sizes have been used since the 5 µm filter has the same composition than the 50 µm, so it is deduced that the results have to be pretty similar.

For the experience performed with the 500 µm filter, PET microplastics have been used in a range of sizes between 750 µm and 430 µm. For the 50 µm filter study, PET microplastics in a range of sizes between 430 µm and 50 µm have been used.

The results for the following experiences have been expressed as the recovery values obtained for the microplastics retained in the filters, the microplastics attached on the filter holders and the microplastics remained in the aqueous solution. Although the microplastics used for the development of these experiences have been received a plasma treatment, as has been mentioned in the previous section, do not have completely lost their electrostatic forces and hydrophobicity. This is why, part of the microplastics have been attached to the filter holders. Thus, those microplastic particles found both in the filter and in the filter's holder have been considered as a positive results.

The results obtained for both experiences (P3_1 and P3_2) are presented in the following tables (see Table II-8 and Table II-9):

Table II-8. P3_1 procedure. Results obtained for the 500 µm retention study. 2.64 mg of PET microparticles (from 750 to 430 µm) have been used. Three replicates of this experience have been performed

	Microplastics (mg)	**Recovery (%)**
Filter holder	0.792 ± 0.2	30
Filter surface	1.540 ± 0.2	58
Filter retention	*2.332*	*88*
Aqueous phase	0.280 ± 0.01	10

Table II-9. P3_2 procedure. Results obtained for the 50 µm retention study. 2.69 mg of PET microparticles (from 430 to 50 µm) have been used. Three replicates of this experience have been performed

	Microplastics (mg)	**Recovery (%)**
Filter holder	0.285 ± 0.1	10
Filter surface	1.660 ± 0.1	62
Filter retention	*1.945*	*72*
Aqueous phase	0.145 ± 0.03	5

As can be observed in the results presented in both tables, Table II-8 and Table II-9, most of the microparticles introduced in the COA system have been retained in the filters.

Table II-8 shows that the 88% of the PET microparticles introduced into the COA system have been retained in the 500 µm filter, 30% attached into the filter holder and a 58% retained in

the filter surface. Only the 10% of the total amount of microplastics have been pass through the 500 µm filter. This could be due to the range of sizes selected. As stated before, the microparticles used are in a size range between 750 µm and 430 µm, since the filter has a pore size of 500 µm, those particles with a size between 500 µm and 430 µm have been pass through the filter system. Thus, it has been demonstrated that the 500 µm used in the COA system retains satisfactorily the microparticles.

The experience performed using 50 µm pore sized filters have also shown positive results. The 72% of the microparticles introduced into the COA system have been retained in the filter used. In this scenario, only the 5% of the microparticles have been passed through the system with the aqueous solution, this have sense since the size ranges is more delimitated. It can also be observed in the recovery results obtained that it has been recovered the 77% of the total amount of PET microplastics introduced. A 20% of the total has been lost during all the procedure. Several hypotheses such as the microplastics size or the use of a microbalance during the process has been raised. Microparticles comprised in a range between 430 µm and 50 µm are not easy to manipulate. In addition to that, as has been stated, the microparticles used has not completely lost their electrostatic forces and thus, some of them could be lost during this experience. Other possible reason for the loss of a 20% of the total has been the use of a microbalance. The difficult handling of this instrument and the challenge of weight microparticles of these sizes may lead to an error accumulation.

In these two experiences the filters selected retention has been validated. Both composition filters, 500 µm filter of polypropylene and the 50 µm filter of nylon have been capable of retaining at least the 70% of the microplastic particles introduced into the COA system. It is assumed that the 5 µm pore sized filters have the same capacity than the 50 µm filters.

To end with the COA device system suitability study, a filter's clean up procedure has been developed. Seawater contains a huge amount of sea salts, so it is possible that the water evaporation induces the formation of sea salt crystals which can cause interferences during the microplastics quantification.

II.3.2.4 Filters clean up

The last consideration to have into account is the filters cleaning after the retention to obtain a realistic estimation of the amount of microplastics collected with no interferences. The elimination (dissolution) of the sea salts crystalized on the filters surface allow to perform a proper microparticles characterization. The microplastics characterization has been developed in detail in Chapter III of this thesis.

One of the probable problems associated with the seawater sampling methods is the present of salts in the seawater. The most probable scenario is the crystallization of the sea salts on the filters, interfering with the microplastic particles detection. That is why a cleaning procedure has been established. The procedure followed is described below (P4):

A previously weight filter that contains a known amount of microplastics is inserted into the 1F sampling device. 1 L of synthetic seawater has circulated during 23 h through the COA system, procedure that mimics the one followed during the Barcelona World Race. The filter is left to stand inside the filter holder for 24 h to let the sea salts crystalize. After that, the filter is collected and placed in a glass support previously weight. It is introduced into the oven at 40ºC for 1 h, then into the desiccator for 3 h and the filter is weighed. Finally, the filter is observed under the microscope to detect the formation of sea salt crystals and the presence of microplastics (P4_a).

For the further filter cleaning and the elimination of sea salt crystals, the filter has been carefully deposited on vacuum filtration system. Two drops (~30 µL) of MilliQ water have been deposited on the surface of the filter as can be observed in the following figure (see Figure II-7). Its wait 30 seconds until the salt crystals have been dissolved. After that, the vacuum system is activated, and the drops are vacuum filtered. The filters have been let dry during 24 hours in a stove at 40ºC.

Figure II-7. Sea salt crystals elimination procedure

After the cleaning procedure the filter has been observed again under the microscope to confirm the elimination of the sea salt crystals. Finally, the filter is weighted in order to determine that the amount of microplastics introduced at the beginning of the process is remained constant (P4_b).

Next, a figure is presented (see Figure II-8) as an example where two optical microscope images can be observed with the before and after of the cleaning procedure developed (P4_a and P4_b).

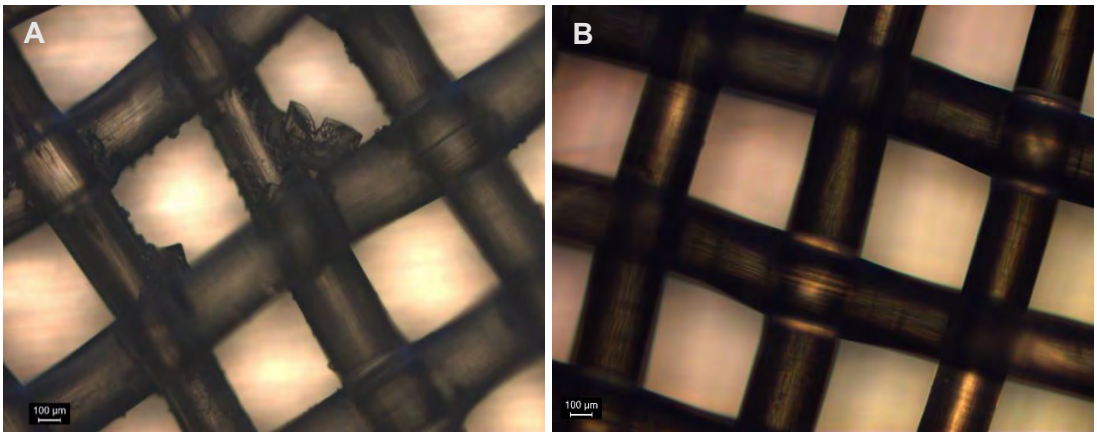

Figure II-8. A) 500 µm filter before the cleaning process with the presence of sea salt crystals, *P4_a*. **B)** 500 µm filter after the cleaning process, sea salt crystals elimination, *P4_b*

As can be seen in the Figure II-8, during the filters cleaning procedure the sea salt crystals generated due to the seawater evaporation have been eliminated. The crystals observed in the edges of the filter pores have been dissolved and cannot be observed in the "after" optical microscope image.

Finally, it has been checked that the amount of microplastics introduced into the system before the procedure has remained constant and have not been eliminated with the sea salts cleaning procedure. The results obtained for the determination of the amount of microplastics remained in the filters are shown hereunder. Two experiences have been performed, with a 500 µm (P4_1) and a 50 µm (P4_2) pore sized filters (see Table II-10 and Table II-11).

Table II-10. Results obtained from the cleaning study using a 500 µm filter, P4_1. 0.95 mg of PET microparticles (from 700 to 430 µm) have been used

	Weight (g)	**Sea salt increase (mg)**
Microplastics + filter	10.14130	-
Microplastics + filter + sea salts	10.14379	2.49
Cleaning process	*10.14131*	*0.01*

Table II-11. Results obtained from the cleaning study using a 50 µm filter, P4_2. 1.65 mg of PET microparticles (from 430 to 50 µm) have been used

	Weight (g)	**Sea salt increase (mg)**
Microplastics + filter	9.43681	-
Microplastics + filter + sea salts	9.43749	0.68
Cleaning process	*9.43685*	*0.04*

As can be seen from the results presented in both tables, Table II-10 and Table II-11, a maximum of 0.04 mg of sea salt crystals have been remained in the filters after the cleaning process. That is to say that with this cleaning process we have been able to eliminate almost all the interferences so a proper microplastics characterization can be performed.

During the laboratory experiences with the COA device the resistance and the durability of the filters have been studied. All the elements which make up the system have been passed the tests so the COA device is ready to be installed.

II.3.3 COA device installation

The COA device is placed inside a box to protect all the items from the environment as can be seen in Figure II-9. After that, the COA device is connected to a pump system that will circulate the water through all the system.

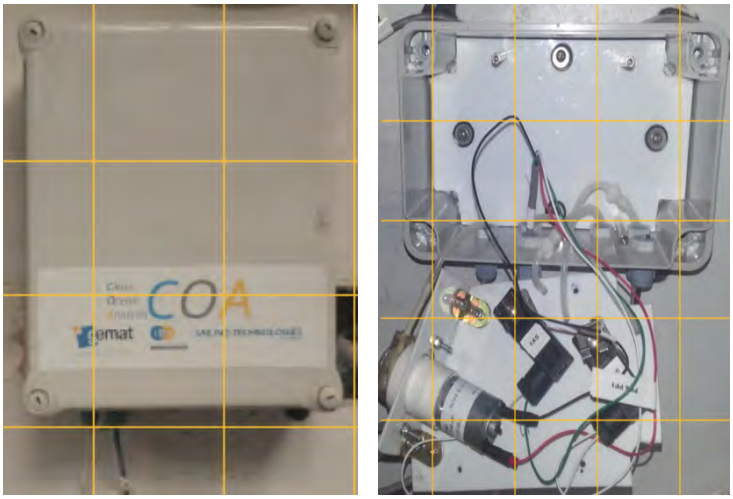

Figure II-9. System elements connection and COA device assemblage

Finally, the COA device developed has been installed in the racing boat, an IMOCA Open60 Kingfisher, that participated in the Barcelona World Race (BWR) 2015. The following Figure (see Figure II-10) shows how and in which position the elements were installed in the racing boat, the COA computer and the filters, HLB cartridges and pumping equipment.

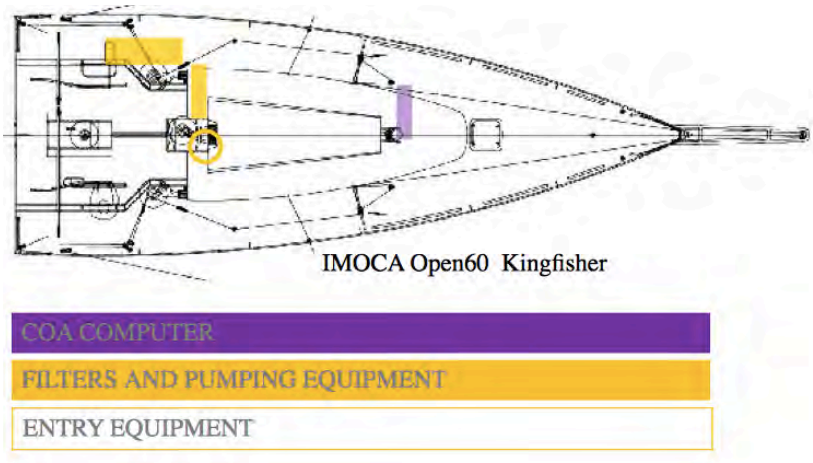

Figure II-10. Installation of the COA device in the racing boat

So, one COA device is connected to the pump system and 1 litter of seawater is circulated through for 23 hours. After that, the used device is disconnected, and stays saved until the laboratory processing. The old one is replaced for a new one with which the same procedure will be followed.

II.3.4 Barcelona World Race 2014-205 itinerary

Finally, the itinerary followed by the racing boat during the BWR 2015 have been studied to determine, with the help of the racing boat crew which will be the most interesting locations and at what time to do the sampling. Moreover, the distribution of the different Packs along the locations (1FHLB, 2FHLB and·3FHLB) have been established. Figure II-11shows the route that the racing boat was followed and where all the samples were collected.

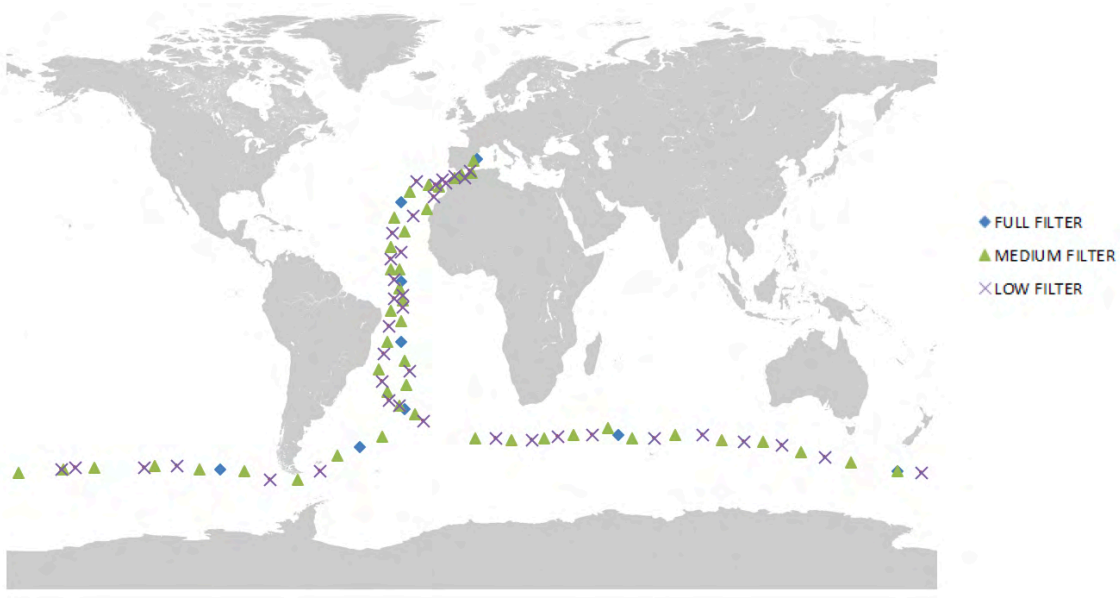

Figure II-11. BWR 2015 itinerary followed by the racing boat and locations where the samples were collected

Figure II-11 shows the itinerary followed by the racing boat and how the different Packs were distributed. In the designation of the figure full filter is referred as the Pack 3FHLB, medium filter as the Pack 2FHLB and low filter as the Pack 1FHLB. As can be seen, the three Packs are uniformly distributed along the oceans to obtain samples as much representative as possible.

The procedure followed by the racing boat crew was the following, every day at a stablished hour (every day the same) during the BWR 2015, 1L of superficial seawater was collected in a specific latitude and longitude which was recorded in order to know with exactitude the sampling points. The seawater liter was suctioned into a tank placed before the COA device and connected to it through a pipe. Then, the peristaltic pump was activated making the 1L seawater circulate through the system for 23h. When the tank was emptied, the COA device was disconnected, properly stocked and replaced for the one used the next day.

The COA device is capable to collect microplastics on superficial seawater from different locations of the world going through all the oceans. With this device, we have been capable of capture all the microparticles in suspension including both microplastics and microplankton, both microplastics and plankton particles as well as the pollutants dissolved in seawater along the oceans. As can be observed from Figure II-11, more than hundred samples from many locations have been collected so a representative study can be performed.

II.4 Concluding remarks

In this chapter, we have optimized a new device (COA device) capable to collect microparticles in suspension, both microplastics and microplankton particles, as well as the retention of pollutants present in seawater from different locations of the world going through all the oceans.

To reach this aim, the first step in the process have been the study of the needed improvements based on the actual sampling methodologies to develop a new device capable to cover all the requirements.

The filtration is the methodology selected to collect the microparticles from superficial seawater. The filters of choice are resistant to seawater effects, hydrophobic, strong and durable. Three different sized pore filters of 500 µm 50 µm and 5 µm have been chosen to cover the full range of sizes which microparticles can be found. The three filters have been installed in the device in serial and in a descendent order of pore sizes.

Another of the elements part of the COA device is the solid phase extraction cartridge. SPE is the methodology selected for the retention of the pollutants dissolved in seawater. The cartridge Oasis HLB Plus from Waters have been selected which is versatile and with a polymeric reversed phase capable to adsorb not only hydrophobic but also polar pollutants making this cartridge the most suitable for our type of matrix.

Three different types of COA device have been developed. The Pack 3FHLB which have the three different sized filters (500, 50 and 5 µm) and the Oasis HLB cartridge, the Pack 2FHLB which have two of the filters (500 and 5 µm) and the Oasis HLB cartridge and the Pack 1FHLB which have one filter (50 µm) and the Oasis HLB cartridge.

After the COA assembling, several laboratory tests have been performed in order to validate all the elements of the sampling device. The flow rate has been sated up, the microplastic particles circulation through the system and the different sizes filters retention has been confirmed and a filter's clean up method has been stablished.

The optimal flow rate has been sated up at the circulation of 1 litter of seawater for 23 hours, which means at a flow rate of 0.73 $mL \cdot min^{-1}$ or 60 mA and 5 V. Moreover, the resistance, the durability and their maximum capacity of both filters and the cartridge have been tested in the laboratory experiences.

During the introduction of the microplastic particles into the system, the plastics electrostatic forces have difficulted their circulation through the system. Temperature and plasma treatments have been applied to the microparticles reduce these forces and try to mimic the real environmental conditions which the microplastics have been exposed. Although the plasma

treatment has reduced the electrostatic forces of the microparticles it has not been enough for the introduction of the particles into the system through a peristaltic pump. Thus, the rest of the laboratory tests has been performed using the gravity for the introduction of the particles.

The three sizes filters retention has been validated and it has demonstrated that more than the 70% of the microparticles introduced into the COA device have been retained on the pertinent filter.

Finally, clean up procedure for the sea salt crystals elimination has been developed. Two drops of MilliQ water have been deposited on the surface of the filters. Its wait 30 seconds until the salt crystals have been dissolved and the drops are vacuum filtered. The filters have been let dry during 24 hours in a stove at 40ºC. The optical microscopic images and the use of a microbalance to check the weight increments have allowed to demonstrate the elimination of the sea salt crystals without the elimination of the microplastics retained in the filters.

The COA device have been installed in a racing boat, specifically an IMOCA Open60 Kingfisher, which was participated in the BWR 2015. The pumping system which will allow the seawater circulates through the device have been also installed making all the elements ready for the microparticles and pollutants sampling. The itinerary followed by the racing boat during the BWR 2015 have been studied to determine the most interesting locations to obtain samples as much representative as possible and the different Packs developed have been distributed around the locations.

Thus, we can conclude that, the sampling device developed have fulfilled all the requirements established. Is capable to collect microparticles in suspension (microplastics and microplankton) and pollutants solved in seawater. Moreover, through the collaboration of our research group, GEMAT, and the FNOB the device has been installed in one of the racing boats of the BWR 2015 so around hundred samples of different locations along all the oceans have been collected which makes the study way more representative. The locations, dates and timings of the samples collected will allow to extract further information during the subsequent analysis.

Once the development and installation of the COA device in the racing boat have been finished, the next chapter goes a step further studying the samples collected. In Chapter III the qualitative and quantitative analysis of the microparticles in suspension collected along the oceans have been performed.

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Chapter III.

Microplastics global distribution along the oceans

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Microplastics distribution along the oceans

The present chapter is focused in the characterization and distribution study of microplastics collected during the Barcelona World Race 2014-2015 from different locations along the oceans. The final consequences of having microplastics in the oceans are still unclear. In this chapter, the microplastics collected have been insulated and an analytical procedure for their characterization has been developed. Their morphology, composition and quantification have been studied. The differences between microplastics and microplankton have been established allowing to know the level of contamination. A global study about the actual state of our oceans regarding this type of particles have been developed.

III.1 Introduction

As previously discussed in Chapter II, a device installed in a racing boat of the BWR 2015 was developed. The device was capable of collecting microplastic particles and persistent organic pollutants (POPs) on superficial seawater from different locations of the world going through all the oceans. Collecting these particles will help to get a better idea of what is the state of plastic pollution in the marine environment and which are the global implications.

As stated before, microplastic is used as a collective term to describe a heterogeneous mixture of particles ranging in size from a few microns to several millimetres in diameter (5 mm maximum), including particles of various shapes from completely amorphous to elongated fibres[1]. It is necessary to insist again on that microplastic contamination has since been reported on a global scale from the poles to the equator and contaminates the water surface of the open ocean[2–8].

Thus, understanding the distribution of microplastics in the oceans, between different geographic regions, in open and enclosed seas or across surface, mid water and sediments is a requirement for assessing the potential impacts on the marine biota and the environment[9,10]. However, in general, it is extremely difficult to identify and point out the ultimate sources of microplastics due to the fragmentation and degradation nature of the debris occurring in small and heterogeneous assemblages. This is why in the second chapter a new sampling device capable to collect these microparticles has been developed.

Other point to take into account is that microplastics, due to their small size, can be ingested by several marine species from all oceanic regions, leading to direct physical damage and potential toxic effects. Since microplastics share the same size fraction as sediments and some plankton organisms, they are potentially bioavailable to a wide range of organisms[11]. Microplastics can be ingested by low trophic suspension organisms, filter and deposit feeders,

detritivores and planktivores[12,13]. Several studies have also reported the ingestion of microplastics by other species such as mussels[14,15], harbour seals[16] or wales[17] since they ingest accidentally or voluntary by feeding on lower trophic organisms that have themselves consumed microplastics. Therefore, they may accumulate within organisms, resulting in physical harm, such as internal abrasions and blockages.

In addition to the potential physical impacts of ingested microplastics, toxicity could also arise from leaching constituent contaminants such as monomers and plastic additives, capable of causing carcinogenesis and endocrine disruption[8,18]. The microplastics toxicity effects will be treated in more detail in Chapters IV and V.

Although first steps towards a standardization of methodologies used for the detection and identification of microplastics in environmental samples were made, the comparability of data on microplastics is currently hampered by a huge variety of different procedures, which result in the generation of data of extremely different quality and resolution[19–22].

In summary, there is a lack of information in many aspects related to microplastics, including their distribution, impact in the environment or the biota as well as how to analyse them. Over the past decade, interest in the topic has grown immensely and there are now over numerous publications and reviews spanning sources, occurrence, abundance, ingestion by biota and its consequences[23,24].

Thus, as has been mentioned, in Chapter II a new sampling device which satisfies all the criteria stablished to collect this type of microparticles has been developed. But, not only a collection system is needed, also new techniques have to be sorted out in order to analyse microplastics.

The two key characteristics in microplastic analysis are physical (size, shape and colour) and chemical (polymer type) features. Because it is difficult to obtain both types of characteristics using only one analytical technique, the combination of multiple methods is applicable. Figure III-1 shows a graphical representation of the most common used methods for water microplastics identification and quantification.

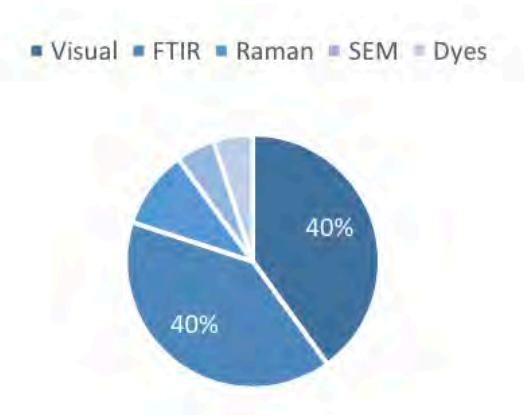

Figure III-1. Details from identification methods reviewed from the literature for microplastics in water (N=20, N= reviewed papers)[25]

As can be observed in Figure III-1, several techniques can be used for the identification and quantification of microplastics in water.

The use of staining dyes is a low-cost method to ease visual identification. Unsatisfactory results have been reported by several studies. Problems arise from the affinity of the dye for plastics and the confounding effect of staining biogenic material in the sample, requiring a through digestion step. Some types of plastics such as PC, PUR, PET and PVC have weak signals and fibers are difficult to stain.

The microscopy is the most commonly used technique for the analysis of microplastics. The microscopy is a widely used identification method for microplastics whose size falls between the tens and hundreds of micron range. Magnified images using microscopy provide detailed surface texture or structural information, which is essential for identifying ambiguous, plastic-like particles[26–29].

Visual inspection allows classification of particles as plastic based on physical characteristics. Visual identification is a fast, simple, and cheap technique that may be carried out in situ for sampling microplastics. However, this method is subjective, may produce wide variations between observers and is highly time consuming. Additionally, includes the inherent difficulty in distinguishing microplastics from other materials. Visual microplastics identification is also performed by using a stereoscope or an optical microscope. This is one of the most used and widely available methods of identification and quantification of plastic particles, even used as pre-selection when chemical characterization is performed.

Chemical characterization of potential microplastics by Fourier transform infrared spectroscopy (FTIR) is highly recommended. The MSFD[30] (Marine Strategy Framework Directive) technical subgroup recommends subjecting 10% of microplastics of sizes 100-5000 μm and all the suspected particles in the range of 20-100 μm to this method, but more may be required for the larger sizes due varying accuracy in visual identification[22,25].

Raman spectroscopy has also lately been used to identify microplastics since can provide physical and chemical information of microparticles. The laser beam falling on a particle results in different frequencies of back-scattered light depending on the molecular structure and atoms that present, which produce a unique spectrum for each polymer. Raman analysis not only identifies plastics, but also provides profiles of the polymer composition. Moreover, the non-contact analysis of Raman spectroscopy offers the benefit that the microplastic samples remain intact for possible further analysis[31,32].

Both spectroscopy methods are non-destructible, highly accurate and complementary, producing a spectrum based on the interaction of light with molecules: FTIR produces an infrared spectrum resulting from the change in dipole moment, whereas Raman provides a molecular fingerprint spectrum based on the polarizability of chemical bonds. Diverse FTIR techniques have been used in the characterization of microplastics, such as attenuated total reflection (ATR-FTIR) that improves the information on irregular microplastics, which in contrast to transmission FTIR that produces a high-resolution map of the sample. Raman microscopy allows the characterization of microplastics <20 μm, but may be limited by weak signals, overcome by increasing measurement duration, and fluorescence interference, dependent on material characteristics such as colour, biofouling and degradation.

Microscopic techniques such as the scanning electron microscopy (SEM) can provide extremely clear and high-magnification images of plastic microparticles. The high-resolution images of the particles surface facilitate the discrimination of microplastics from other organic particles. SEM could also have some limitations. However, SEM is expensive with laborious sample preparation steps, as well as time-consuming for an adequate examination of all samples, hence limiting the number of particles that may be analysed in a given timeframe. Additionally, the colours of the particles cannot be used as identifiers in SEM analyses[28].

In addition of the techniques mentioned above, further analysis with energy-dispersive X-ray spectroscopy (EDS) provide the elemental composition of the same particle. The elemental composition of particles is useful for identifying carbon-dominant plastics from inorganic particles[33]. When is coupled to SEM (SEM-EDS), the elemental composition of plastic particles is obtained, thus discerning carbon-dominant plastics from inorganic particles.

The thermo-analytical technique, which measures changes in the physical and chemical properties of polymers depending on their thermal stability, has been recently tested for microplastic identification. Differential scanning calorimetry (DSC) can be a useful method for studying the thermal properties of polymeric materials[33].

In pyrolysis - gas chromatography - mass spectrometry (Pyro-GC-MS), microplastics are thermally decomposed (pyrolyzed) under inert conditions and the gas formed is trapped in the head of the column and separated on a chromatographic column, identified by mass spectrometry. This method can provide the chemical characterization of a single microplastic or

of a bulk sample, but it is destructive and provides no information regarding number of microparticles, size or shape. Thermo extraction and desorption coupled with gas chromatography-mass spectroscopy (TED-GC-MS) combines a thermogravimetric analysis (TGA) for thermal degradation (100-600ºC) and solid phase extraction of plastic degradation products, further analysed by thermal desorption in GC-MS[34].

A number of the previously mentioned techniques are used for the analysis of the microparticles retained in the filters as will be shown in this chapter.

Then, the main objective of this chapter is to characterize the microparticles retained in the three different sizes filters disposed in the device designed and installed in a race boat from the BWR 2015. The analysis of their morphology, composition and distribution will allow us to know better the level of pollution of the marine environment and which is the global impact of having that particles in the oceans. As have been previously mentioned, the characterization of the microparticles collected will provide a global approach about the actual state of the oceans, study that does not exist until today.

In order to achieve this objective, the following tasks were proposed:

- Retained microparticles initial study.
- Global characterization of the microparticles. Morphology and composition study of several microparticles retained in the filters from specific locations using microscopic techniques such as IR or SEM microscopy.
- Raman spectroscopy of several microparticles retained in the filters.
- Filter retained microparticles quantification. The previous morphological and composition analysis will allow to do a quantification in function of the particle's origin.

III.2 Material and Methods

III.2.1 Salinity elimination

For the elimination of sea salt crystals from the filters before starts all the analysis two drops (~30 µL) of MilliQ water were deposited on the surface of the filters. Its wait 30 seconds until the salt crystals were dissolved. The drops were vacuum filtered, and the filters were let dry during 24 hours in a stove at 40ºC.

III.2.2 Composition study by IR Microscopy

For the determination of the composition of microplastic particles an Infrared Microscope was used. A Thermo Scientific Nicolet iN10 MX instrument in a transmission mode and with a diamond cell and MCT detector has been used. The overture was of 100 microns, an accumulation of 64 scans and a spectral range from 4000 to 674 cm^{-1}. The reflection mode was also used with an overture of 100 microns, an accumulation of 64 scans and a spectral range from 4000 to 674 cm^{1}.

To apply the transmission mode when the IR analysis is being done it is necessary to have a very planar surface. In the case of the filters of study the surface has been affected due to the time that them have remained in the case. Thus, the reflexion mode has been applied in the majority of the cases. The particles were extracted one by one from the filter and analysed.

III.2.3 Morphology study by SEM Microscopy

For the morphological study of the microparticles retained in the filters a Scanning Electronic Microscopy (SEM) was used. A Jeol JSM-5310 in combination with the Oxford INCA Energy software was the model of the microscope. The SEM was set up at the conditions of 20 kV of acceleration voltage, a spot size of 11 nm and a working distance of 20 mm.

In order to obtain better conditions for the image's quality, the filters were covered with a gold layer. The samples were gold coated using the low vacuum gold sputtering method. The analysis process starts outside the microscope, cooling the sample at the maximum speed by liquid nitrogen. Then goes to the cryopreservation system, where it is cover with gold for the observation and the analysis.

III.2.4 Quantification of microparticles trapped in the filters

For the quantification of microparticles retained in the filters collected, an Optical Microscope was used. The microparticles were observed and counted manually. To make the quantification as homogeneous as possible a transparent template was created. This template consists in the division into eight exactly equal parts of a circumference (see Figure III-2) that allows the observation of the filter through them. The delimitation of the filter in zones with the same surface allows a quantification to always be carried out in the same way.

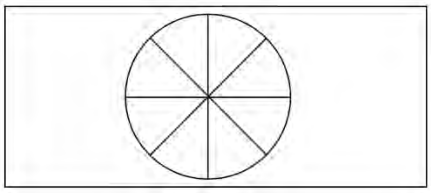

Figure III-2. Template for the optical microscopy microparticles quantification

The Optical Microscopy allows to observe microparticles until 5 μm and differentiate between and in combination with the composition study if this microparticles corresponds to microplastics or microplankton.

III.2.5 Raman spectroscopy analysis

For the Raman spectroscopy analysis, a Thermofisher DXR 2 instrument were used. The analyses were carried out using the green laser (532 nm) and Omnic Spectra software, with spectra covering a wavelength range between 200 and 3000 cm^{-1}. The work spectral conditions were fixed at 60 seconds of registration time and 5 kW.

Reference libraries of Raman spectra for different polymers and natural fibers were collected from several bibliographic sources such as, Aldrich ™ Raman Condensed Phase Spectral Library from Thermo Fisher, Infrared and Raman Databases of Reference Spectra from ACD/Labs or Nicolet Standard Collection of Raman Spectra from Thermo[35–37].

III.3 Results and Discussion

In this section of the present chapter the analysis of the microparticles collected with the COA device will be performed.

As stated before, the two key characteristics in microplastic analysis are physical (size, shape and colour) and chemical (polymer type) features. Since there is not a unique technique capable to analyse all these properties at the same time, several microscopic techniques mentioned in the introductory section have been used.

The Optical Microscopy and SEM microscopy have been the two techniques selected for the morphological analysis, determining the size, shape and colour of the microparticles. Moreover, the infrared microscopy has been the technique used for the chemical microparticles analysis in order to determine the polymer type.

To end the analysis, the Raman microscopy has been used in some of the filters to perform the microplastics analysis. Raman microscopy is a microscopic technique capable to perform the morphological and chemical analysis of the microparticles retained in the filters at the same time.

Thus, in this section several microparticles collect from different filters have been analysed to have a global idea about the state of the oceans regarding these microparticles.

For the initial analysis, where the morphology and the composition of several microparticles have been studied, a strategic number of samples have been selected. The samples are selected so that the information extracted of them be as representative as possible. Samples from the beginning, the midway and the end of each ocean have been selected. Next, a table (see Table III-1) is presented with relevant information about the different filters analysed throughout the section.

Table III-1. References, locations (latitude and longitude) and pore size of the filters analysed

Reference	Location	Latitude	Longitude	Pore size (µm)
1	Mediterranean Sea	41.1720	2.1482	500
2	Mediterranean Sea	37.1097	0.1629	5
8	Atlantic Ocean	26.0628	-16.8955	500
9	Atlantic Ocean	24.1227	-22.3008	50
11	Atlantic Ocean	13.0470	-27.0662	50
13	Atlantic Ocean	4.1802	-27.0257	5
22	Atlantic Ocean	-29.3632	-32.5878	500
26	Atlantic Ocean	-36.2800	-21.9015	500
30	Atlantic Ocean	-44.3158	15.9515	500
32	Indian Ocean	-43.5422	28.4488	500
36	Indian Ocean	-40.4578	52.5985	500
37	Indian Ocean	-42.5977	57.1142	500
39	Indian Ocean	-43.6772	70.6037	50
48	Pacific Ocean	-51.0833	146.6571	500
51	Pacific Ocean	-54.0331	174.1315	50
55	Pacific Ocean	-52.4606	-152.6948	5
61	Pacific Ocean	-52.7821	-96.7240	500
96	Mediterranean Sea	40.6725	1.2221	5

As can be observed in the table presented above (Table III-1), eighteen samples have been chosen to perform the microparticles initial study. Three samples from the Mediterranean Sea, seven samples from the Atlantic Ocean, four samples from the Indian Ocean and four samples from the Pacific Ocean have been selected. The strategy followed for the selection of this specific samples has been:

- Samples from each ocean have to be analysed.
- For each ocean, samples from coastal locations and from the middle of the ocean have been selected.
- Samples at locations where two oceans are in contact.
- Samples that allows the analysis of microparticles retained in the different pore sizes filters (500, 50 and 5 µm).

III.3.1 Preliminary remark

The first step in the analysis is a preliminary observation of the different types of microparticles retained in the filters.

The observation of the retained microparticles has been studied using an Optical Microscope. A template to restrict the area of observation and to ease a homogeneous count has been used. Images of different types of microparticles observed in some sizes filter are shown in Figure III-3.

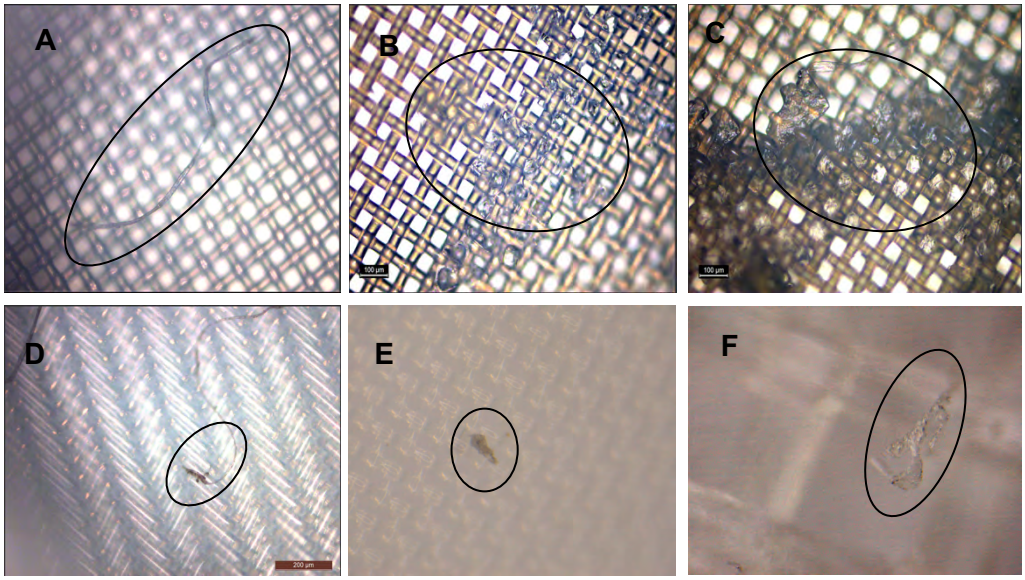

Figure III-3. Optical microscopy images of different types of microparticles found in the filters. **A)** Sample 9 (50 µm) from the Atlantic Ocean. **B** and **C)** Sample 39 (50 µm) from the Indian Ocean. **D** and **E)** Sample 2 (5 µm) from the Mediterranean Sea**. F)** Sample 22 (500 µm) from the Atlantic Ocean

Observing some filters in the optical microscope, differences in terms of colour between the microparticles have been detected: brown coloured particles (E) and translucid particles (F). Geometrical differences have been also observed. A relatively high number of particles with a structural or angular shape have been noticed (C). Those particles correspond to the crystallization of seawater salts. To confirm the presence of sea salt crystals, the filters have been also observed with the scanning electronic Microscope since the images gives a more detailed idea of the particle morphology. Because the crystals can interfere with the following analysis, these particles have been eliminated.

To eliminate the crystals, the procedure detailed in Chapter II has been applied. Several drops of MilliQ water were deposited on the filters surface allowing the sea salt crystals solubilization. In this way the salt crystals were not confused with the microparticles during the quantification.

Figure III-4 shows optical microscope images where can be observed how the sea salt crystals has been detected causing problems at the moment of quantification. To observe more clearly the elimination of the sea salt crystals from the filters is also presented, in Figure III-5, some Electronic Microscopy images. Both figures show the before and after of crystals elimination process.

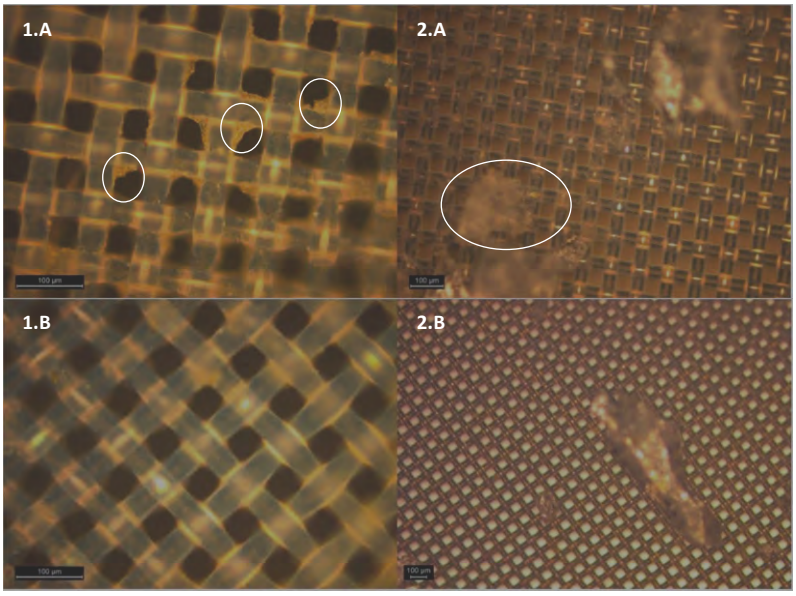

Figure III-4. Optical images of a 50 μm filter before and after the sea salt crystals cleaning process. **1A** and **2A)** Images corresponding to filters with sea salt crystals. **1B** and **2B)** Images of the same filters after the cleaning. Samples 9 and 39 from the Atlantic and Indian Ocean respectively

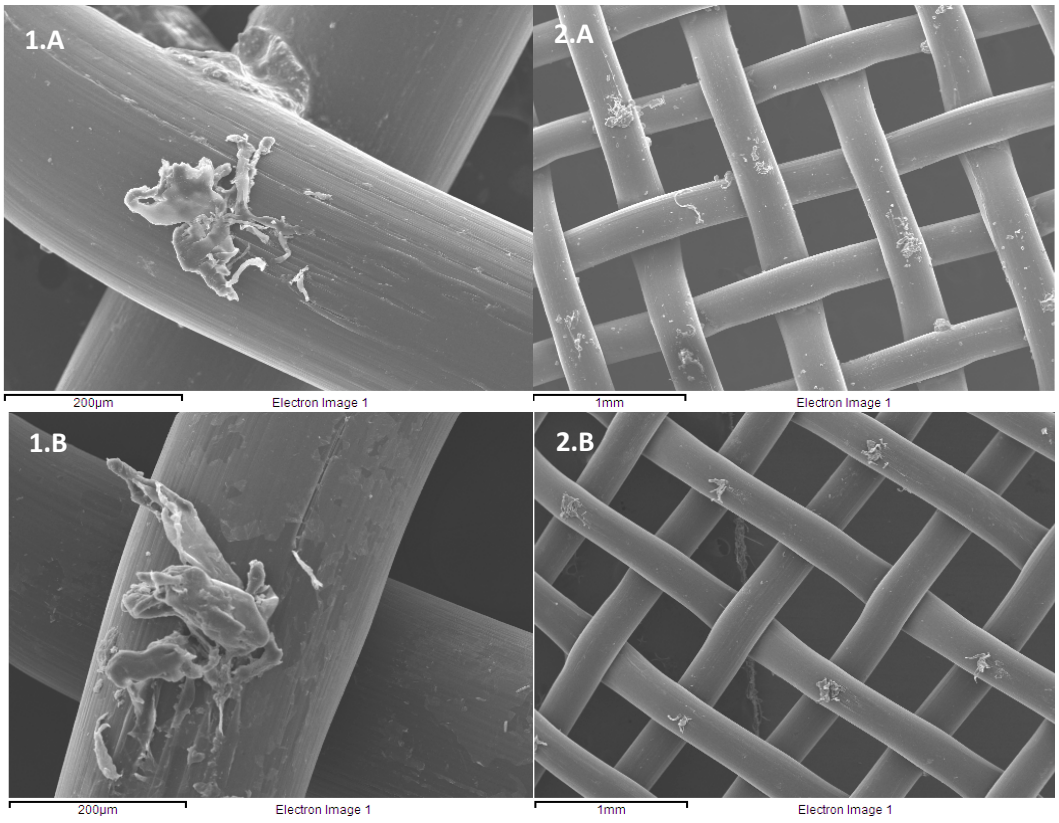

Figure III-5. Before and after sea salt crystals cleaning process electronic microscopy images. **1A** and **2A)** Images corresponding to filters with sea salt crystals. **1B** and **2B)** Images of the same filters after the cleaning. Samples 22 (500 µm) and 26 (500 µm) from the Atlantic Ocean

The elimination of sea salts was confirmed by the observation of before and after. The disappearance of angular shape particles (corresponding to sea salt crystals) have been detected. Thus, it can proceed to perform the entire study.

III.3.2 Initial analysis

Once the elimination of the sea salt crystals has been finished, it can proceed with the proper analysis. To do that, the morphology and the composition of several microparticles retained in the filters presented in the Table III-1 have been sorted out. Microparticles from the different locations and with different sizes have been studied in order to has a global idea of the state of the oceans.

As has been mentioned, in the preliminary analysis different shape and colour microparticles has been observed. Thus, to analyse in more detail these particles, their morphology and composition have been studied using IR, SEM Microscopy and Raman Spectroscopy.

The determination of the morphology of the particles has been carried out using a Scanning Electronic Microscope. The use of this technique has allowed to determine which is the shape of the microparticles and be able to differentiate between all of them. Below, several electronic

microscopy images are presented where differences in the microparticles shape can be observed (see Figure III-6).

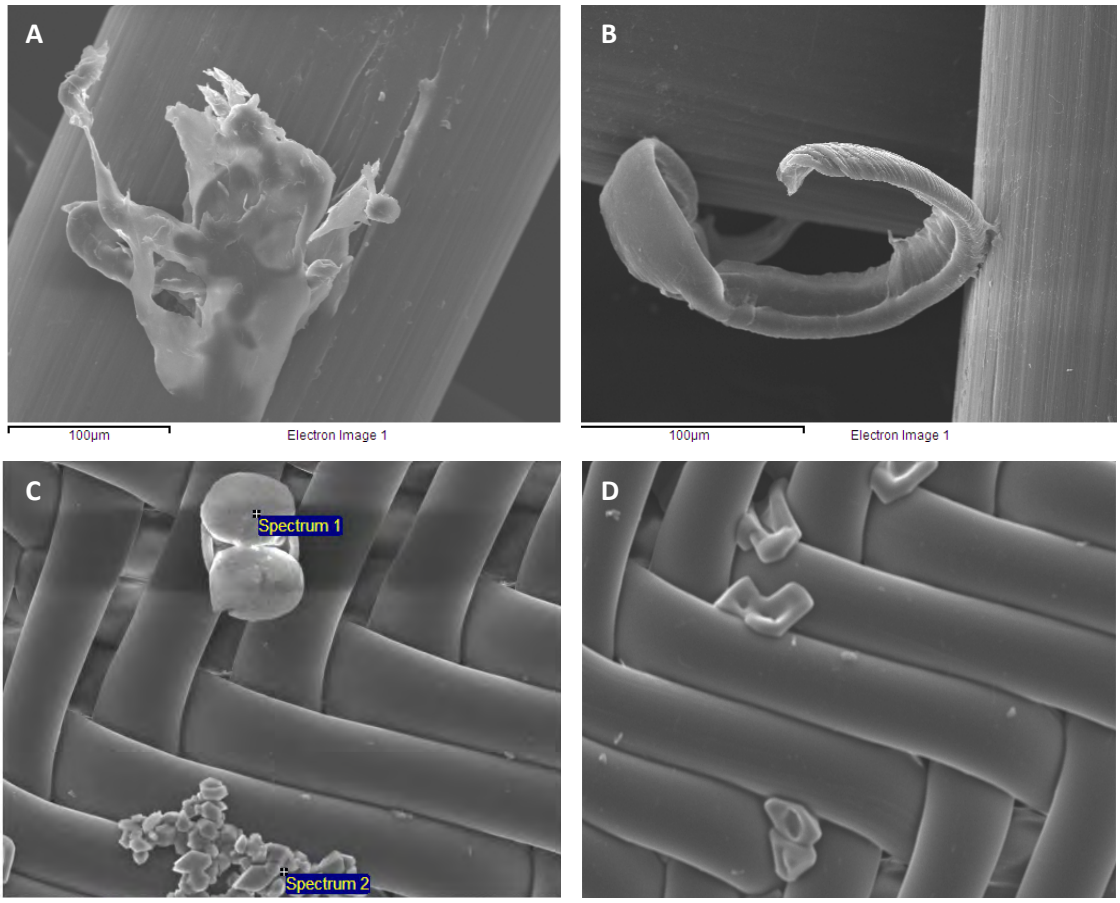

Figure III-6. Scanning electronic microscopy images. **A)** Mediterranean Sea microparticle retained in a 500 µm pore size filter (sample 1). **B)** Indian Ocean microparticle retained in a 500 µm pore size filter (sample 32). **C)** Pacific Ocean microparticle retained in a 5 µm pore size filter (sample 55). **D)** Atlantic Ocean microparticle retained in a 5 µm pore size filter (sample 13).

Figure III-6 shows images of different types of microparticles retained in the filters. Microparticles shown in the images A, B and D correspond to microplastics. The microparticles shown in the example C image corresponds to a microplankton particles.

Evident differences are shown in the Figure III-6 between all the images presented. As it has previously mentioned, the majority of microplastics in seawater are the result of the degradation of first-generation plastics that due to de pass of the years and external factors have been disintegrated. The plastic particles can be break down in smaller microplastics as a result of erosion processes. Those polymeric particles with a high strength and rigidity give rise microplastics with a certain structured shape as can be observed in the microplastics of the example D. They can also be disintegrated and fragmented giving rise to a smaller amorphous microparticles as the ones shown in the example A. In addition to these types of microplastics,

microfibers have been also found as a result of the disintegration of those polymeric particles with a more elastic nature as in the example B.

It is known by searching in bibliography, on the other hand, that microplankton usually shows a homogeneous shape, more specifically, spherical shape as can be observed in the example C. Differences can be observed between the example C and D where two microparticles retained in a 5 µm filter are shown. In the first image (C), a perfectly spherical microparticle is presented. On the other hand, in the second image (D), the particles presented have a more irregular structure.

The SEM microscopy technique has allowed us to do a first distinction between the microparticles retained based on their morphology. Microplastic and microplankton particles have been observed retained in filters of different sizes and locations.

With the objective to determine at which polymer type corresponds the microparticles found the composition analysis has been performed. Knowing the composition of the microparticles could give an idea of its origin. Besides this, have the information about the location where those were collected could give us the probable source of the particles. To determine the microparticles composition an Infrared Microscopy was used. Figure III-7 shows an example of IR spectra of some microparticles analysed found in the sea water. The conditions used for the IR analysis are described in the Material and Methods section of this chapter.

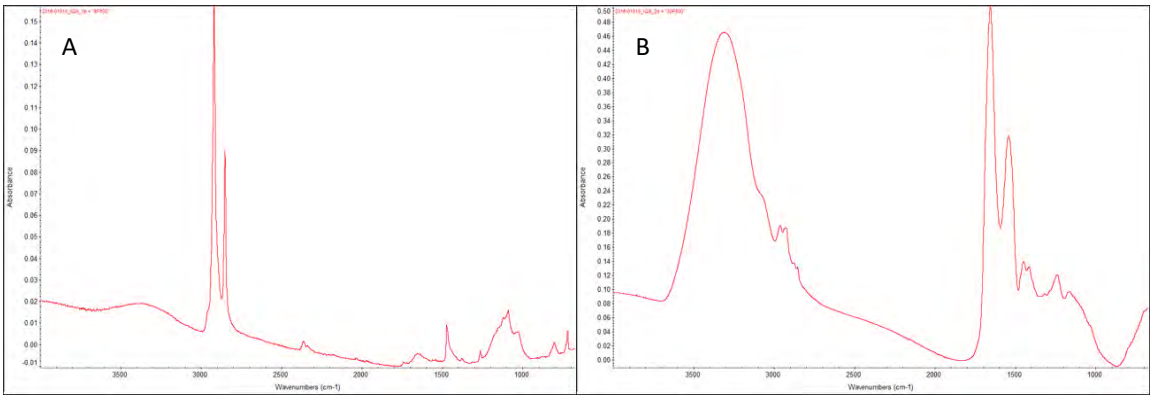

Figure III-7. A) Image corresponding to an IR spectra of a polyethylene microplastic (Sample 8 from the Atlantic Ocean). **B)** Image corresponding to an IR spectra of a protein microparticle (Sample 11 from the Atlantic Ocean)

Figure III-7 shows an example of the IR spectra of two types of microparticles that have been found retained in the filters. Both cases correspond on microparticles found in the Atlantic Ocean. Example A shows the spectra of a polyethylene microparticle found in a 500 µm filter, which just confirm the presence of microplastics in seawater. On the other hand, example B shows the spectra of a protein found in a 500 µm filter confirming that other organic microparticles have been also retained in the filters.

In addition to the previous IR microparticles composition examples, the composition of other microparticles retained in different pore sized filters from several locations have been analysed.

In the Mediterranean Sea samples analysed (samples 1, 2 and 96) polymeric microparticles identified as polypropylene and polyethylene or polyacetal have been found.

In the Atlantic Ocean filters analysed, which corresponds to the samples 8, 11, 22, 26 and 30, polymeric microparticles such as polyethylene, polyethylene terephthalate and polypropylene have been found. Also, several microparticles corresponding to polyacetal microplastics have been identified retained the most of them in the 500 μm filter of sample 8. During the analysis other composition particles have been found corresponding to organic and inorganic particles such as cellulose, protein microparticles or sulphate salts.

From the analysis of the filters of the Indian Ocean (samples 32, 36 and 37), microparticles corresponding to microplastics (for example polypropylene or cellulose microplastics) but also microplankton or microparticles corresponding to proteins have been identified. Finally, several microparticles found in three filters from the Pacific Ocean have been analysed (samples 55, 58, 51 and 61). The majority of the microparticles from the Pacific Ocean analysed, corresponds to microplankton particles. In addition to that, microparticles corresponding to microfibers have been found, more precisely microparticles retained in the 500 μm filter of the sample 61 identified as indigo cellulose fibers. This type of polymeric composition is commonly used for the “jeans” fabrication.

Several conclusions from the IR analysis performed have been extracted. At first place, IR analysis has corroborated that microplastics contamination is a global concern since in all the oceans studied microplastic particles have been found. In addition of that, a contrast between the Pacific Ocean and the rest of the oceans studied has been observed. In the Pacific Ocean a high concentration of microparticles corresponding to microplankton have been identified unlike the other oceans where high amount of polymeric microparticles have been found. Thus, different types of microparticles such as microplastics, microplankton or inorganic particles such as sea salty crystals have been retained in all the filters analysed.

Finally, differences in terms of microparticles sizes have been studied. In any of the filters analysed, 500 μm, 50 μm and 5 μm pore size filters, microplastics, microplankton and also inorganic particles have been found interchangeably of the filter size.

The IR analysis confirms the conclusions extracted during the physical analysis where the microparticles found have been assigned to microplastics and microplankton.

To end with the initial study, some microparticles retained in the filters of the Mediterranean Sea have been analysed by Raman microscopy. The possibilities that Raman offers in terms of microplastics identification and quantification have been demonstrated.

As has stated in the introductory section, Raman spectroscopy has lately been used to identify microplastics since can provide physical and chemical information of microparticles. Raman analysis not only identifies plastics, but also provides profiles of the polymer composition.

Two filters have been analysed by Raman spectroscopy in order to localize and identify several microparticles retained. The filters analysed (samples 96 and 2) were used for the microparticles collection from the Mediterranean Sea and both of them are of 5 μm since this type of analysis is commonly used for maximum 10 μm microparticles.

The first filter analysed has been the related to the sample 96 from the Mediterranean Sea. Several microparticles distributed along the filter have been found. Next, Figure III-8 shows a spectroscopic image of one of the microparticles found.

Figure III-8. 10x Raman microscopy image found in a 5 μm filter (sample 96), scale specified in nanometers

As can be seen in the Figure III-8, a fibrous shape of approximately 50 μm microparticle has been found retained in this 5 μm filter. After the localization of one of the microparticles retained in the filter, its composition analysis has been performed. The Raman spectra of the microparticle obtained has been compared with bibliographic spectra libraries to found coincidences. In addition to the spectra of the microparticle, the spectra of the nylon filter to ensure that it not corresponds to a piece of the filter has been performed.

A superposition of the three Raman spectres extracted are presented in the following figure (see Figure III-9).

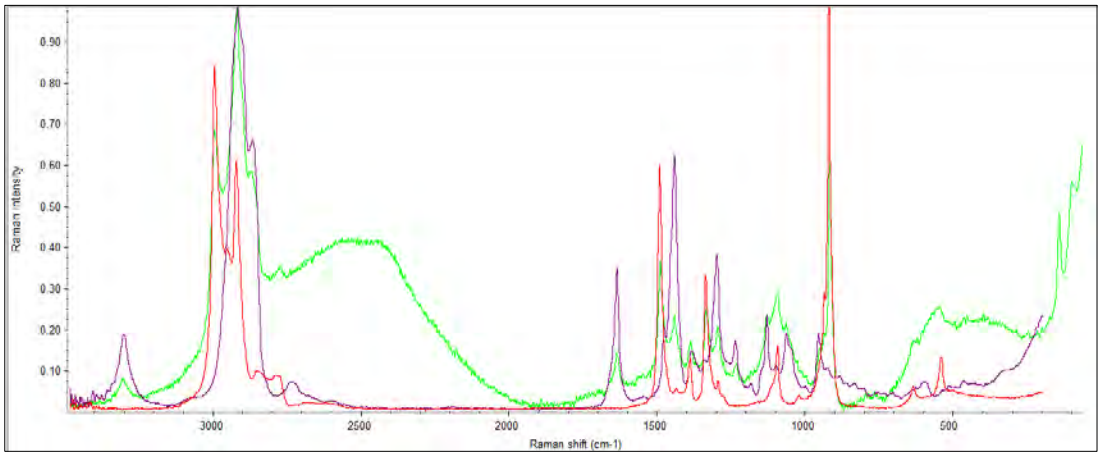

Figure III-9. Raman spectroscopy spectres extracted from the sample 96. Microparticle spectra presented in green, bibliographic spectra presented in purple and nylon filter spectra presented in red

As has been mentioned, the superposition of three spectres are presented above (Figure III-9). Based on the comparison of both spectres shown (green and purple), the microparticle and the bibliographic library spectres has been determined that the microparticle found corresponds to a polyacetal fiber microplastic.

Another 5 μm filter from the Mediterranean Sea has been analysed by Raman spectroscopy. From the mosaic extracted of the filter several images showing the retention of microparticles have been obtained. The following figure (see Figure III-10) shows the image of one of these microparticles.

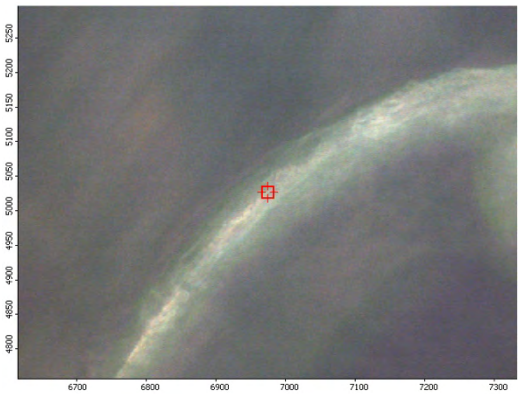

Figure III-10. 20x Raman microscopy image found in a 5 μm filter (sample 2), scale specified in nanometers

After the location of the microparticle, the Raman spectra has been performed in order to determine its composition. The following figure (see Figure III-11) shows the superposition of two Raman spectres corresponding to the microparticle and its matching spectra found in bibliographic libraries.

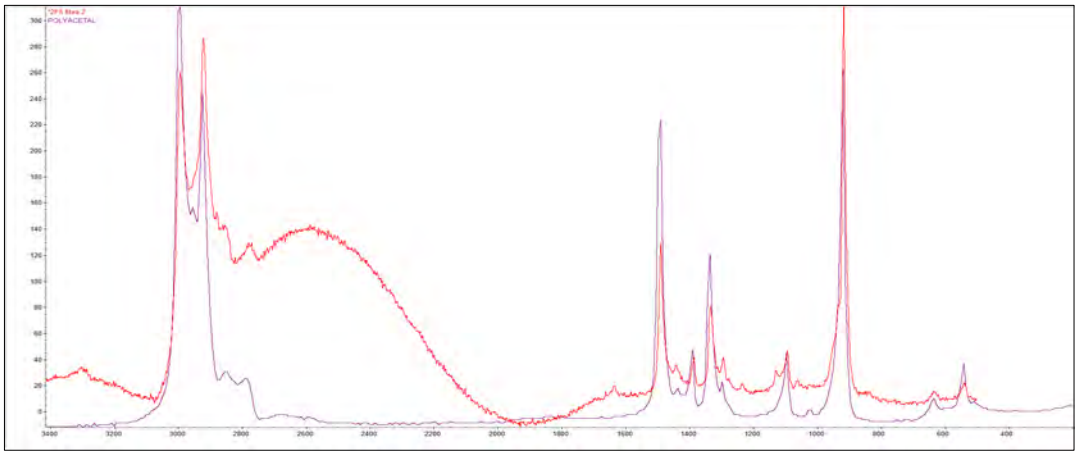

Figure III-11. Raman spectroscopy spectres extracted from the sample 2. Microparticle spectra presented in red and bibliographic spectra presented in purple

As can be observed in the Figure III-11, a concordance with a spectrum of one bibliographic library has been obtained. The microparticle found retained in the filter corresponds to a polyacetal microplastic.

In both filters analysed polyacetal microplastic particles have been found. Polyacetal is an engineering thermoplastic used in precision parts requiring high stiffness, low friction, and high dimensional stability. It is characterized by its high strength, hardness and rigidity. The typical applications for polyacetal include high-performance engineering components such as small gear wheels, eyeglass frames, ski bindings, knife handles, among others. Due to the rigidity and the strength of this polymer, it is logical to think that its breakdown has given rise small structured pieces (microplastics) of sever sizes as the ones seen in the previous images. In contrast with that, exist other modulable polymers which due to the past of the years and the exposition to external factors have been decomposed in fibers or microparticles which probably will be found in the 500 or 50 μm pore size filters.

The microscopic image presented in the following figure (see Figure III-12) shows the presence of another microparticle retained in the 5 μm filter.

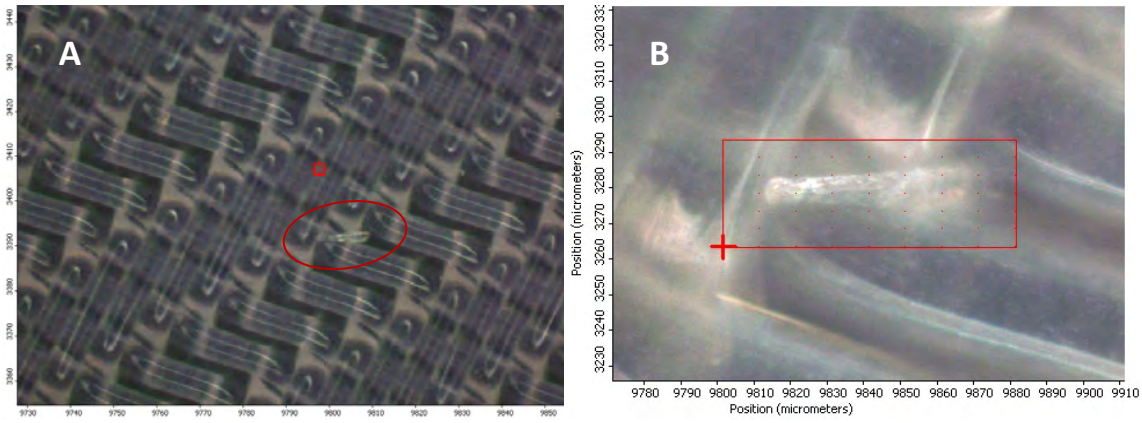

Figure III-12. Raman microscopy image found in a 5 μm filter (sample 2), scale specified in nanometers. **A)** x10 Raman spectroscopy image. **B)** x20 Raman spectroscopy image

In this case, as can be seen in Figure III-12, a microparticle retained is shown using two objective augments (x10 and x20). A fibrous microparticle retained has been found.

As stated before, Raman spectroscopy is a microscopic technique that allows a mapping creation. With this, the differences in terms of composition between materials can be sorted out. Thus, a map of the filter section where the microparticle was found has been created. The mapping will allow us to distinguish between the filter and the microparticle composition and so, confirm the retention of microparticles.

Next, a figure is presented (see Figure III-13) where the generated map can be observed. The delimitated zone selected is shown in the image B of Figure III-12.

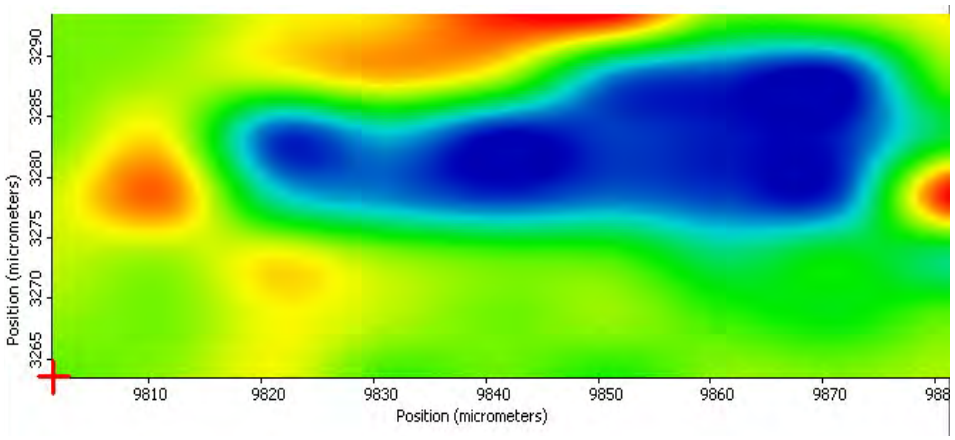

Figure III-13. Mapping image extracted from the one microparticle retained in a 5 μm filter (Figure III-12). The different colours represent differences in the materials composition

Differences in terms of composition from the mapping generated of the microparticle image have been observed. The composition differences are presented in the map as changes of colours due to variations of the intensity detected by the Raman laser. The coloration profile of the mapping is pretty similar than the microscopic image of the microparticle. Two colours are the

predominant, blue and green. Comparing both figures (Figure III-12 and Figure III-13) can be observed that the blue colour coincides with the location in the filter where the microparticle has been retained and the green colour could correspond to the filter material.

To corroborate the differences in terms of the polymeric composition between both surfaces, their Raman spectra have been performed. Figure III-14 is presented below with the spectres.

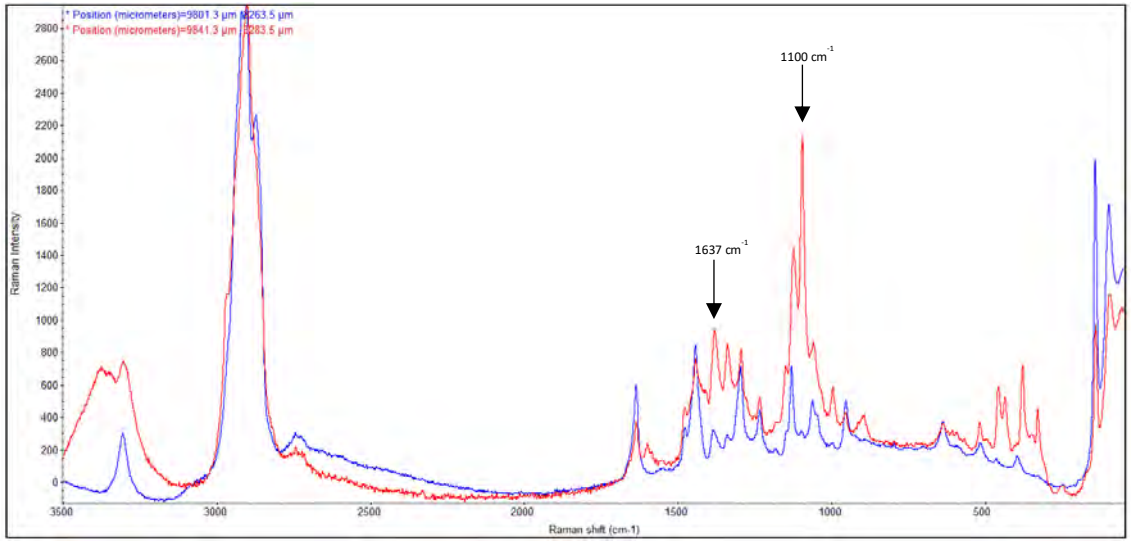

Figure III-14. Raman spectroscopy spectres extracted from the sample 2. Microparticle spectra presented in red and nylon filter spectra presented in blue

The Raman spectra of the fibrous microparticle, in red, and the spectra of the filter (nylon spectra), in blue, have been overlaid and differences in both profiles can be observed. As has been pointed in the previous figure (Figure III-14), two the key signals allow to differentiate between both materials. In the red spectra two signals can be observed at 1637 cm^{-1} and 1100 cm^{-1} that do not appear in the other spectra.

The fibrous microparticle spectra and the filter spectra have been compared with bibliographic libraries to determine the composition of both materials. Figure III-15 shows the superposition of the specters with their most probable bibliographic coincidence.

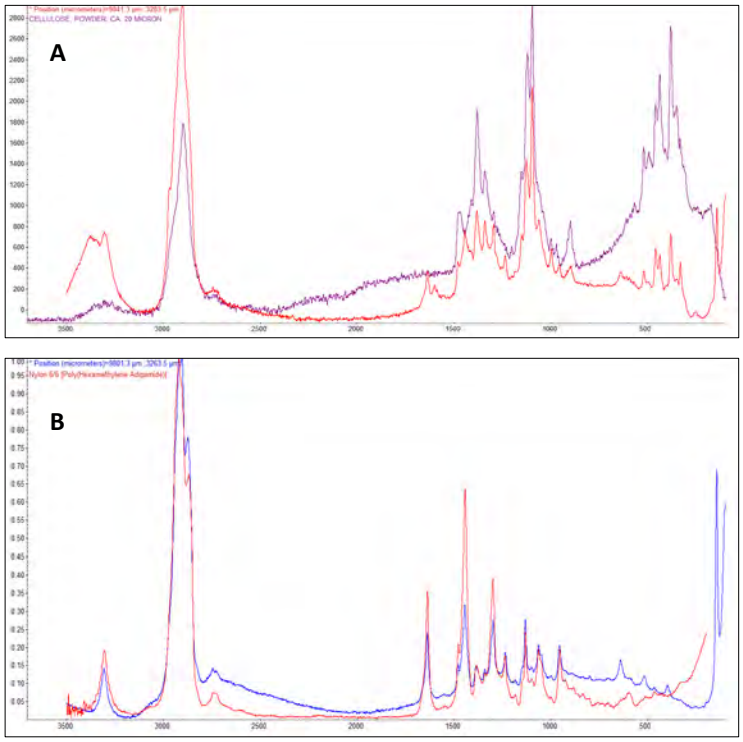

Figure III-15. Raman spectroscopy spectres extracted from the sample 2 and bibliographic libraries comparison. **A)** Microparticle spectra presented in purple and bibliographic spectra presented in red. **B)** Microparticle spectra presented in blue and bibliographic spectra presented in red

The Raman spectra presented in Figure III-15 A have shown a coincidence with a bibliographic spectra related to a cellulose microfibre in contrast with the Raman spectra of Figure III-15 B, where a coincidence with nylon has been obtained.

Cellulose is an organic polymer mainly used to produce paperboard and paper. Cellulose has no taste, is odourless and insoluble in water and most organic solvents. Its huge production and hydrophobicity increase the chance of found this type of polymer suspended in seawater. Thus, the retention of cellulose microparticles in any of the filters installed in the COA device can be expected.

The identification and the determination of the morphology of the microparticles retained in the filters has been obtained using Raman Microscopy. It would be a useful technique to perform a complete quantification and identification of the microparticles of all the filters.

III.3.3 Microplastics quantification

As have been mentioned before, important differences between the particles retained in the filters have been detected:

a. The IR analysis has shown differences in terms of composition. Microparticles corresponding to microplastics or other organic compounds such as proteins, amines, etc. (organic compounds) have been found. Also, inorganic compounds corresponding to sea salts have been detected.

b. The Electronic Microscope analysis has shown differences in terms of particles morphology. Particles correspondent to microplastics show an amorphous shape, those particles correspondent to microplankton show a spherical shape and finally, those particles correspondent to sea salt crystals show an angular morphology.

Those previous specifications along with the quantification of all microparticles retained allow a complete characterization. The quantification of microparticles of the filters has been carried out once filters are clean and dry and using an Optical Microscope. Differences in the coloration of the microparticles have been observed during the analysis. It has been found a correlation between the brown coloured particles and those particles assigned as microplankton during the IR and SEM analysis (initial study). Another correlation between translucid particles and microplastics have been determined. Thus, the combination of the three analysis (IR, Electronic Microscope and Optical Microscope analysis) have allowed to differentiate between microplastics, microplankton and sea salt crystals.

Thus, and using the Optical Microscopy technique, the quantification of the microparticles retained in all the samples (97) collected during the BWR 2015 has been performed. The exact location, the hours and days when the samples were collected has been presented in the Table of the Annexes of this thesis. Moreover, as has been mentioned before, a template has been used for a homogeneous quantification.

Based on the total number of microparticles in each filter and the proportion between microplankton and microplastics, several hypotheses have been made. Figure III-16 shows a global quantification of the total number of microparticles found in each ocean. To obtain a more representative results, the number of microplastics found have been normalized to the number of samples taken in each ocean.

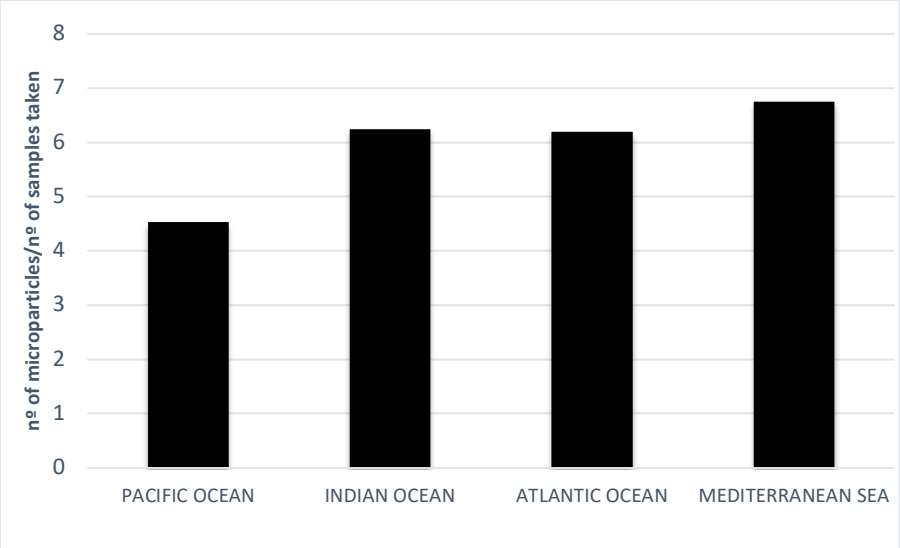

Figure III-16. Graphical representation of the total amount of microparticles normalized to the number of samples taken from each ocean

As can be seen in Figure III-16, the differences between the oceans in term of number of microparticles is not so evident. The quantification confirms then the conclusions extracted from the initial analysis (morphology and composition analysis). The microplastics contamination has become a global concern since all the oceans present this type of microparticles.

Mediterranean Sea is where the highest amount of microparticles have been found. Followed by the Indian Ocean and Atlantic Ocean with similar amount of microparticles. Finally, the Pacific Ocean is the ocean where the lowest number of microparticles have been detected.

But, as have been explained in the introductory section of this chapter, the problem associated of having microplastics in seawater is that the aquatic animals do not distinguish between if they are eating microplastics or microplankton. Thus, the interesting data to analyse is the existent relation between microplastics and microplankton to know the scope of the problem. Next, a graphic is presented (see Figure III-17) where this relation is shown.

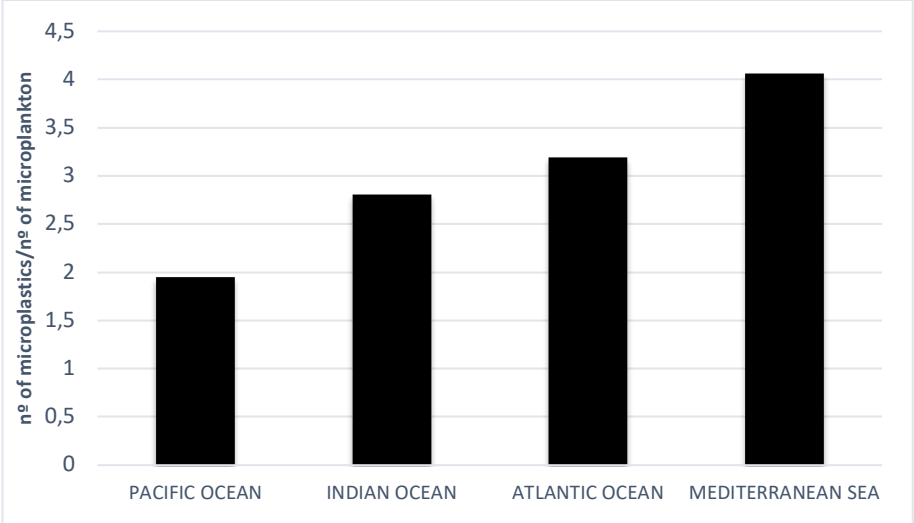

Figure III-17. Graphical representation of the amount of microplastics related with the amount of microplankton found in all the filters from each ocean

In this Figure (Figure III-17) it can be seen that is in the Mediterranean Sea where the proportion of microplastics related on microplankton found is the highest. The number of microplastics found is four times bigger than the amount of microplankton particles retained. Unlike the Pacific Ocean which is the less polluted ocean in terms of microplastics although the amount of microplastics found doubles the amount of microplankton particles. Two hypotheses have been raised to explain these differences:

a. During the BWR, the location points where the samples were collected when the racing boat was sailing through the Mediterranean Sea were relatively near to the coast. So, the probability of found plastic particles coming from the industries or due to the human activities is higher than if the samples are taken in remote locations of the oceans.

b. In the Pacific Ocean the proportion is less than half in relation with the amount found in the Mediterranean Sea. This fact can be correlated with the currents generated in the locations where the racing boat was sail through during the race when the samples were collected in this ocean. The racing boat was sailing very close to the Western Boundary Currents (WBC) during the Pacific Ocean sampling. Those are defined as ocean currents with dynamics determined by the presence of a coastline, are warm, deep, narrow, and fast flowing currents that form on the west side of ocean basins due to western intensification. WBC probably were causing the movement of plastic particles towards areas further away from the location points. Moreover, the locations where the samples were collected in the Pacific Ocean the industry density is pretty low since the points are relatively near of the Antarctic Ocean.

These hypothesis can explain why the Pacific Ocean has been established as the lees polluted ocean in terms of microplastics (see Figure III-17). In the previous studies, in the Pacific, microparticles such as cellulose, amines or sulphates have been found contrary to the Atlantic Ocean where a higher presence of microplastics have been determined since particles such as polyethylene or polypropylene have been found. Thus, the quantification analysis sustains the composition and morphology study.

These results show the approximate amount of microplastics pollution depending the ocean. New information can be extracted analysing the size of the microplastics collected. As have been mentioned, the COA device have three different pore sized filters (500 μm, 50 μm and 5 μm) which allows to study the distribution of microplastics sizes. The number of microplastics in function of their size and ocean location is represented in Figure III-18. As in the previous quantification figures, the results represented in the graphic are normalized to the number of samples in each ocean.

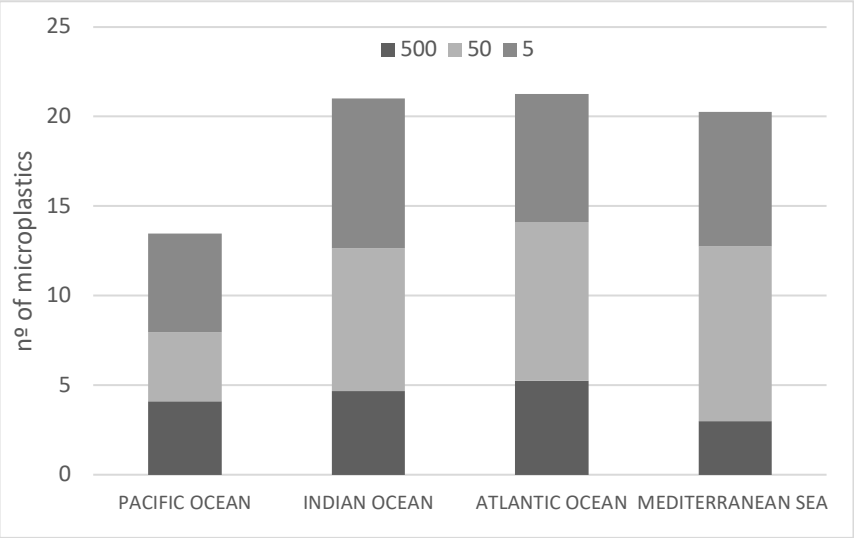

Figure III-18. Graphical representation of the distribution of microplastics in function of their sizes found in all the filters from each ocean

Figure III-18 shows that the highest amount of microplastics are found in the 50 μm pore sized filters. Thus, plastic particles of sizes between 499 μm and 50 μm are the most abundant followed by particles with a diameter higher than 500 μm. This information is relevant because the main concern, as has been mentioned previously, is the confusion of the aquatic animals between the microplankton and microplastics. Microplankton is defined as microorganisms with a size comprised between 20-200 μm which coincides with the range of sizes where the majority of the microplastics that have been trapped in the filters. This fact confirms the problem previously assumed that those plastic particles are able to be introduced in the trophic chain reaching the human organisms.

III.4 Concluding remarks

In this chapter, we have detected the presence of suspended microparticles in seawater. The filters installed in the COA device, with pore diameters of 500 µm, 50 µm and 5 µm, have retained a certain amount of microparticles.

To reach this aim, qualitative and quantitative analysis of the microparticles retained have been done. First, the qualitative analysis has been performed. An infrared microscopy has been used to determine the composition of the microparticles. The SEM microscopy technique has applied to determine the morphology of the microparticles localized in the filters. Also, the Raman microscopy has been performed to identify and determine the composition of the microparticles retained in the filters. After that, the quantitative analysis has been performed. The quantification has done using an optical microscope with the help of a self-created template which delimitates different zones of the filter with the same surface allowing the quantification to always be carried out in the same way.

The application of these techniques in combination have allowed us to differentiate between three types of particles. Sea salt crystals, which shows a symmetric geometry, have been detected. Microplankton particles have also been observed, the composition analysis and their spherical shape leads us to think that it is this kind of particles. Finally, microplastic particles have also found retained in the filters, again with the determination of their composition and the amorphous morphology induced by itself degradation due to external factors allows us the identification. The Raman microscopy analysis has confirmed the conclusions extracted from the previous microscopic analysis since different polymeric microparticles have identified retained in several filters.

The differentiation between the particles allow to have a greater idea of the level of seawater pollution due to microplastics. It is determined that Mediterranean Sea is the most polluted in terms of microplastics. The amount of microplastics related with the amount of microplankton found in this sea is four times greater. Mediterranean Sea is followed by the Atlantic Ocean, the Indian Ocean. Last but not least, the Pacific Ocean is the lest polluted ocean although the amount of microplastics doubles the amount of microplankton. Moreover, in the IR microscopy analysis organic composition particles that do not correspond to microplastics have been detected. We considered that this distribution of microparticles is due to the different oceanic currents.

Furthermore, having in the COA device three pore diameter filters allows to study the distribution of microplastics sizes. The high amount of microplastics found are those retained in the 50 µm pore sized filters for all the oceans and where the samples were collected. Thus, those plastic particles of sizes between 499 µm and 50 µm are the most abundant. As have been mentioned in this chapter the principal concern is, the confusion by the aquatic animals between

the microplankton, with a size is comprised between 20 and 200 µm, and microplastics. So, the majority of microplastics retained in the filters have the same size of microplankton.

Thus, we can conclude that, the amount of microplastic particles in all of the oceans overcome the presence of microplankton. Moreover, microplastics found are in a size range that coincides with microplankton size. This fact increases the risk of introducing this type of plastic particles by aquatic organisms to the trophic chain due to the confusion of them with microplankton. In addition to that, it has to be mentioned that no previous studies have been found where global analysis like this has been performed. New and relevant data has been extracted from the microparticles retained in the filter's analysis.

Once the qualitative and quantitative analysis have been performed and the distribution of the microparticles have been studied, the next chapter goes a step further exploring the microplastics capacity of adsorbing organic pollutants suspended in seawater as well as their concentration effect. Two different families of pollutants were selected for the study, polycyclic aromatic hydrocarbons (PAHs) and polybromodiphenyl ethers (PBDEs). Both pollutants were selected since their hydrophobic character makes them potential candidates for the adsorption on microplastic particles and also because IQS has a wide experience in their chromatographic analysis.

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Chapter IV.
Microplastics as concentrators of POPs

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Microplastics as concentrators of POPs

Microplastics represent a growing environmental concern for the oceans due to their potential of adsorbing persistent organic pollutants (POPs) suspended in water. They are ingested by various species introducing both microplastics and organic pollutants to the trophic chain potentially affecting human health. In this chapter, we analyse the adsorption and release process of two families of pollutants on polyethylene terephthalate, polypropylene, low density polyethylene and polystyrene microplastic particles. We demonstrate the importance of the combination solvent-polymer for an optimal desorption.

IV.1 Introduction

The presence of microplastics in the oceans have two main consequences. The first one, as already discussed in chapter III, microplastics occupy the same size fraction as sediments and some planktonic organisms, they are potentially bioavailable to a wide range of aquatic animals. The second one, and even more dangerous, is the possible presence of environmental contaminants adsorbed on the microplastics surface[1–3].

Besides the adverse physiological effects to marine organisms that arise from ingestion of micropieces of plastic, microplastics in the marine environment may also pose an additional chemical hazard, especially those containing known or suspected endocrine disrupting chemicals. Microplastics can have in their surface chemical compounds used as additives or can adsorb those compounds from the sea water[4]. In the first case, the plastic additives are incorporated into polymers during manufacturing processes to improve their properties or extend resistance to heat (e.g. by using polybrominated diphenyl ethers), oxidative damage and microbial degradation (with triclosan). These additives are an environmental concern since they can increase the degradation time of plastic but are also desorbed from the polymer at rates depending on the pore size of the synthetic matrix, the amount and typology of the additive, and various environmental factors[5]. Furthermore, can be found in microplastic litter unintentional chemicals coming from the production processes (e.g. vinyl chloride, BPA, etc.) normally present in traces (ppm) or chemicals coming from the recycling of plastic waste[6–8].

In addition to the leaching of additives, chemical risk of microplastics derives also from the adsorption of a wide range of organic and inorganic contaminants on these particles. Microplastics are liable to concentrate hydrophobic persistent organic pollutants (POPs) coming from wastewaters due to a bad management or the human activity and which accumulates in sea water[8–11]. Those POPs have a greater affinity for the hydrophobic surface of plastic compared to seawater. Due to their large surface area to volume ratio, microplastics can become heavily contaminated with waterborne POPs (see Figure IV-1).

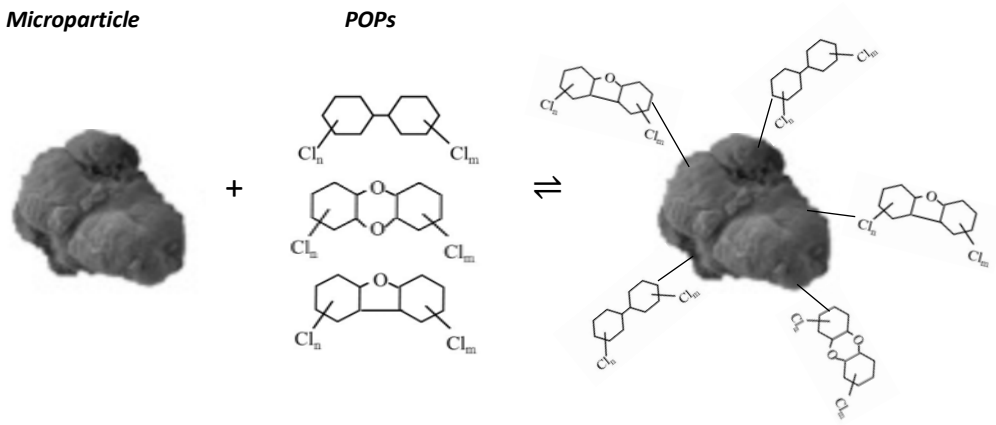

Figure IV-1. Schematic representation of POPs adsorption on microplastic particle surface

Plastics contaminated by persistent organic pollutants are found globally, from coastal areas to the remote habitats[12–16]. Microplastics are capable of concentrating contaminants, increasing their concentration even up to the order of 10^6. But adsorption is not only a kind of physical behaviour but also a kind of chemical behaviour. Both the sorbent and the sorbate properties can influence the adsorption extent significantly. The adsorption primarily depends on the great specific surface area and Van der Waals'force, but also due to greater affinity of organic pollutants of the hydrophobic surface of plastic compared to seawater. The physical and chemical properties of sorbent including surface area or diffusivity influence in the sorption of chemicals[17]. Furthermore, adsorption or desorption of organic pollutants from microparticles could varied in the seawater and in the freshwater, which may be due to the impact of salinity. In general, the combination of physical, chemical and biological factors allows concentrations of chemical pollutants to increase over time via sorption by particles and accumulation by biofilms.

Despite the risk that means that microplastics can adsorb, desorb and transport POPs, they can also represent a source of chemical exposure within marine food webs. The ingestion of microplastics by biota, could highlight an additional concern for their potential toxicological effects to the organisms (see Figure IV-2). Some studies suggested that both adsorbed pollutants and chemical additives of plastics might be released to organisms after ingestion. Therefore, they introduce both microplastics and organic pollutants to the trophic chain potentially affecting human health.

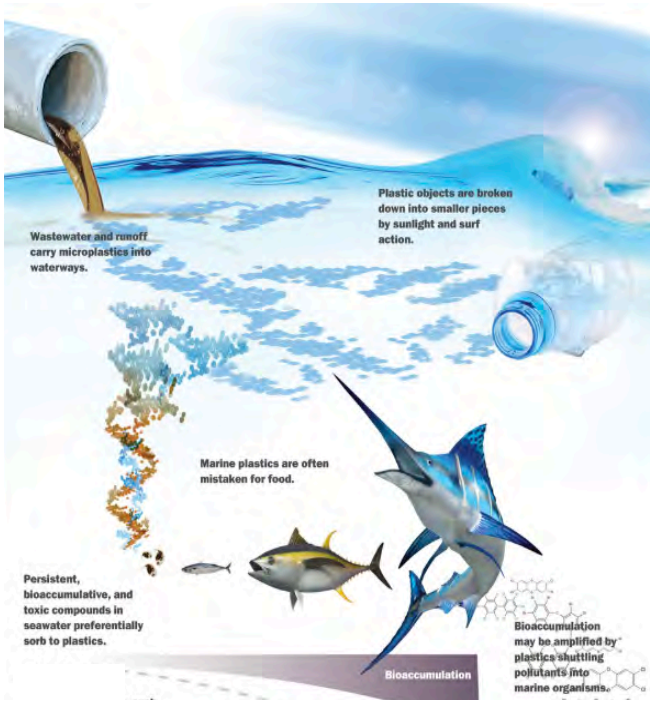

Figure IV-2. Main consequences of the adsorption of POPs on microplastics surface[18]

Commonly used POPs, such as phthalates, bisphenol A, alkylphenols, polybrominated diphenyl ethers (PBDEs) or polycyclic aromatic hydrocarbons (PAHs) are hazardous to biota acting as endocrine-disrupting chemicals that can mimic, compete with, or disrupt the synthesis of endogenous hormones. Different researches have demonstrated the presence of specific POPs present in plastic in pelagic fishes, confirming the presence of those pollutants in both the fatty tissues of the birds and in the plastics found in their stomachs[19–24].

The analytical methodologies used until the moment to demonstrate the microplastics concentration effect have been usually based on the hydrophobicity of the pollutants and their affinity for the polymeric particles compared with their affinity for seawater. The release process of POPs from microplastics so, in the same way is based on the extraction of the pollutants using different organic solvents. But, has not yet been established a reproducible methodology that can be used for any combination polymer-pollutant.

In other words, the understanding of the factors influencing sorption/desorption is still poor. Thus, further studies are actually needed to better elucidate the magnitude of chemical load on environmental microplastics and the real potential to transfer such compounds to marine biota[25–29]. Then, the main objective of this chapter is to demonstrate the microplastics concentration effect and develop new strategies to extract and quantify the POPs adsorbed.

In order to achieve this objective, the following tasks were proposed:

- Adsorption study of two families of pollutants on four most commonly used types of polymers.
- Development of new strategies for the controlled release of pollutants from microplastic particles.
- Study of the most influencing factors on POPs release.
- Release and quantification of persistent organic pollutants adsorbed on microplastic particles collected during the BWR 2015 from the different oceans.

IV.2 Material and Methods

As has been mentioned in the introduction section, PBDEs and PAHs have been the pollutants selected for the development of the following experiences. The selection has been done according the institution extensive experience in the analysis of these pollutants. Some of them has been analysed in the IQS for many years[30,31].

IV.2.1 Reagents and reference substances

IV.2.1.1 PBDEs

The following compounds coming from different technical mixtures were selected: Pentabromodiphenylether (PeBDPE) at 1000 μg/mL level, Octabromodiphenylether (OcBDPE) at 1000 μg/mL level and Decabromodiphenylether (DeBDPE) at 100 μg/mL level. A detailed table (see Table IV-1) is presented below with the main congeners present in the three technical mixtures with their abbreviations, the number of bromine atoms, the chemical name and their solubility in water at 25ºC. BDE congeners in the table are presented following their ascending bromine atoms number and their chromatographic elution.

Table IV-1. PBDE congeners in the technical mixtures[32]

Nº. IUPAC	Nº. Br	Name	S at 25ºC (μg/mL)
BDE-28	Tri	2,4,4'-Tribromodiphenyl ether	0.133
BDE-47	Tetra	2,2',4,4'-Tetrabromodiphenyl ether	0.133
BDE-100	Penta	2,2',4,4',6-Pentabromodiphenyl ether	0.133
BDE-99	Penta	2,2',4,4',5-Pentabromodiphenyl ether	0.133
BDE-154	Hexa	2,2',4,4',5,6'-Hexabromodiphenyl ether	< 0.001
BDE-153	Hexa	2,2',4,4',5,5'-Hexabromodiphenyl ether	< 0.001
BDE-183	Hepta	2,2',3,4,4',5',6-Heptabromodiphenyl ether	< 0.001
BDE-197	Octa	2,2',3,3',4,4',6,6'-Octabromodiphenyl ether	< 0.001
BDE-196	Octa	2,2',3,3',4,4',5,6'-Octabromodiphenyl ether	< 0.001
BDE-207	Nona	2,2',3,3',4,4',5,5',6-Nonabromodiphenyl ether	< 0.001
BDE-206	Nona	2,2',3,3',4,4',5,5',6-Nonabromodiphenyl ether	< 0.001
BDE-209	Deca	2,2',3,3',4,4',5,5',6,6'-Decabromodiphenyl ether	< 0.001

Two stock solution mixtures containing all PBDEs were prepared by dilution of the technical mixtures at concentrations of 1 μg/mL for each congener. The first one dissolved in acetonitrile and another one in hexane and stored at -22°C. Working standard solution mixtures containing all PBDEs at different concentrations were prepared by dilution of the stock solutions with the appropriate volume of acetonitrile or hexane and stored at -22°C.

For the analysis by GC-HRMS the $^{13}C_{12}$-BDE-139 congener (a hexabromodiphenyl ether) was used as an internal standard.

IV.2.1.2 PAHs

The PAH MIX 9 from Dr. Ehrenstofer™ (Reference Standards for Residue Analysis) with the reference XA20950009AL was selected which contains the 16 EPA-PAHs at 100 mg/L each in acetonitrile. A detailed table (see Table IV-2) is presented below with the name of the compounds of the mixture, their abbreviations, their molecular weight (MW), their solubility (S) in water at 25ºC and the coefficient octanol-water ($logK_{ow}$, pollutants affinity relation octanol vs water). PAHs in the table are presented following their molecular weight and their chromatographic elution.

Table IV-2. PAHs compounds and properties in the standard mixture[33,34]

Abbreviation	Name	MW	S at 25ºC (mg/L)	$logK_{ow}$
Na	Naphtalene	128	31.7	3.5
Acy	Acenaphthylene	152	3.93	4.0
Acp	Anaphtene	154	3.93	4.33
Flu	Fluorene	166	1.98	4.18
Phen	Phenanthrene	178	1.29	4.5
Ant	Anthracene	178	0.045	4.5
Flt	Fluoranthene	202	0.26	5.1
Pyr	Pyrene	202	0.135	4.9
BaA	Benz[a]anthracene	228	0.0057	5.6
Cry	Crysene	228	0.0018	1.64
BbF	Benzo[b]fluoranthene	252	0.0043	6.06
BkF	Benzo[k]fluoranthene	252	0.0038	6.0
BaP	Benzo[a]pyrene	252	0.014	6.06
BghiP	Benzo[ghi]perylene	276	$5.3 \cdot 10^{-4}$	6.40
DiBahA	Dibenzo[a,h]anthracene	278	$2.6 \cdot 10^{-4}$	6.50
IP	Indene[1,2,3-cd]pyrene	276	0.0006	6.75

Two stock solutions mixtures containing all PAHs at 1 μg/mL were prepared. The first one dissolved in acetonitrile and another one in hexane and stored at -22°C. Working standard solutions mixtures containing all PAHs at different concentrations were prepared by dilution of the stock solutions with the appropriate volume of acetonitrile or hexane and stored at -22°C.

IV.2.1.3 Synthetic seawater

For the experimental procedure, 1 L of synthetic sea water was prepared using the compounds described in Table II-3 and adjusting the pH with hydrochloric acid (HCl, Panreac, 1 M) between 7.5 and 8.5.

Table IV-3. Composition of synthetic sea water. Composition and product references[35]

Compounds	Concentration ($g \cdot L^{-1}$)	Product reference
NaCl	24	Panreac® 141659
$MgCl_2$	5.0	Sigma-Aldrich® M8266
Na_2SO_4	4.0	Panreac® 141716
$CaCl_2$	1.1	Sigma-Aldrich® 499609
KCl	0.70	Panreac® 141494
Na_2CO_3	0.20	Panreac® 141648
NaBr	0.096	Panreac® 141646
H_3BO_3	0.026	Panreac® 141015
$SrCl_2$	0.024	Sigma-Aldrich® 439665
NaF	0.0030	Sigma-Aldrich® 450022

IV.2.1.4 Solvents

Acetonitrile (ACN), HPLC LC-MS grade, HiPerSolv Chromanorm, acetone, for pesticide residue analysis, Pestinorm and n-hexane for pesticide residue analysis, Pestinorm were purchased from VWR Chemicals; methanol (MeOH), LC-MS grade, was supplied by Fisher Scientific; ethyl acetate for pesticide residue analysis was obtained from Panreac; toluene, Chromasolv, for pesticide residue analysis was purchased from Honeywell.

IV.2.1.5 Polymer samples

The microplastics used for all the experimental procedures were obtained from the trituration of commonly used plastic materials chosen according to the desired polymeric composition. As has been mention in Chapter II the COA device collects superficial seawater samples so it makes sense to work with microplastic particles found floating in seawater. Thus, we chose to work with those most commonly used plastics. Table II-2 shows the origin and specifications of the plastics used.

Table IV-4. Plastics used. Their origin and specifications

Polymer type		Origin	Specification
Polyethylene Terephthalate	PET	Water bottle	1.5 L Water bottle (Veri®)
Low Density Polyethylene	LDPE	Ring bag (six pack)	Coca-cola® Ring bag (Dia®)
Polypropylene	PP	Straws	Flexible straws (DonPalillo®)
Polystyrene	PS	Coffee spoon	Spoon (Papstar®)

The original plastics were cut in pieces of a size of the order of 1.0 or 1.5 cm. Then, they were introduced into a cryogenic mill (SPEX^{TM} 6770 SamplePrep). The samples were frozen with

liquid N_2 and crushed through mechanical movements against a stainless-steel cylinder until the plastic was grinded to a microscopic scale sized particle.

The polymeric micro sized particles were sieved to group the mixture in three particle sizes, 500 μm, using sieves with a particle size of 700 μm and 490 μm, 50 μm, using a sieve with a particle size of 49 μm, and 5 μm, where the rest of the particles not retained in the other sieves. During the following analysis presented in this chapter, microparticles of 500 μm have been used. The rest of the microplastics sizes sieved have been used for other purposes.

IV.2.2 Methods

IV.2.2.1 Analytical GC-ECD system for PBDEs analysis

GC-ECD analysis were performed using an Agilent HP 6890N Network Gas Chromatograph coupled to an electron capture detector (ECD). The suitable separation of the analytes was achieved connecting a 5% phenyl, 95% dimethyl siloxane bonded fused silica capillary column (Rtx-5MS, 5 m x 0.25 mm x 0.5 μm film thickness from Restek GC Columns). Helium was employed as carrier gas at a flow of 1 mL/min. The column temperature was initially set at 140°C and held for 3 min, then increased to 210°C at a rate of 25°C/min and held for 3 min, after increased to 280°C at a rate of 2.5°C/min and finally increased to 300°C at a rate of 20°C/min and held for 4.2 min. The total run time was 42 min. Two microliters of a sample solution were injected in the splitless mode (1 min) at 250°C. The electron capture detector temperature was set at 330°C and with makeup gas (N_2) at 59 mL/min.

IV.2.2.2 Analytical GC-HRMS system for PBDEs analysis

GC-HRMS analysis were performed using an Agilent HP 6890N Network Gas Chromatograph coupled to a high-resolution mass spectrometry detector (HRMS) AutoSpec Ultima. HRMS incorporates a tri-sector and double-focusing geometry with a wide gap magnet and a resolution up to 100000 units. The suitable separation of the analytes was achieved connecting a 5% phenyl, 95% dimethyl siloxane bonded fused silica capillary column (Rtx-5MS, 5 m x 0.25 mm x 0.5 μm film thickness from Restek GC Columns). Helium was employed as carrier gas at a flow of 1 mL/min. The column temperature was initially set at 140°C and held for 1 min, then increased to 300°C at a rate of 10°C/min and held for 3 min. The total run time was 20 min. Two microliters of a sample solution were injected in the splitless mode (1 min) at 270°C. Conditions of the MS were the following: capillary temperature at 320°C, EI voltage in positive mode with three time windows, from 3.00 min to 6.15 min analyzing the m/z from 405.80 to 497.75 for Tri to Tetra PBDE congeners, from 6.15 min to 9.73 min analyzing the m/z from 511.34 to 655.57 for Penta to Hexa PBDE congeners and from 9.73 min to 20.00 min analyzing the m/z from 721.44 to 815.39 for Hepta to Deca PBDE congeners.

For the quantification of PBDEs with GC-HRMS the isotope dilution method was used.

IV.2.2.3 Analytical GC-MS system for PAHs analysis

GC-MS analysis was performed using an Agilent 6890GC Network Gas Chromatograph coupled to an Agilent 5975B inert XL mass spectrometry detector (MS). The suitable separation of the analytes was achieved connecting a 50% phenyl, 50% dimethylpolysiloxane bonded fused silica capillary column (VF-17ms, 20 m x 0.15 mm x 0.05 µm film thickness from Agilent). Helium was employed as a carrier gas at a flow of 1.5 mL/min. The column temperature was initially set at 60ºC and held for 3.1 min, then increased to 195ºC at a rate of 30ºC/min, then increased to 205ºC at a rate of 10ºC/min, after increased to 250ºC at a rate of 20ºC/min and finally increased to 310ºC at a rate of 5ºC/min and held for 5 min. The total run time was 27.85 min. one microliter of sample solutions were injected in split mode (ratio 5:1) at 300ºC and 40 psi. Conditions of the MS were the following: capillary temperature at 300ºC, EI voltage in positive mode with seven time windows, from 3.00 min to 7.00 min analyzing the m/z of 128 for Na, from 7.00 min to 8.50 min analyzing the m/z of 152, 153 and 166 for Acy, Acp and Flu respectively, from 8.50 min to 10.00 min analyzing the m/z from of 178 for Phen and Ant, from 10.00 min to 12.00 min analyzing the m/z of 202 for Flt and Pyr, from 12.00 min to 14.50 min analyzing the m/z from of 228 for BaA and Cry, from 14.50 min to 18.00 min analyzing the m/z from of 252 for BbF, BkF and BaP and from 18.00 min to 27.00 min analyzing the m/z from of 276 for BghiP, DiBahA and IP.

IV.2.3 Experimental procedure

To demonstrate the microplastics adsorption proposed theory a laboratory scale procedure was carried out. 200 µl of PAHs/PBDEs stock solution of 1 µg/mL in acetonitrile was dissolved in 15 mL of MilliQ® water (six replicates). Then, 10 mg of a specific composition microplastics were added to the solution. The mixture was heated at 40°C to achieve a higher adsorption during 2 hours with continuous agitation and allowed to stand for 24 hours. After that, the microplastics were separated of the aqueous phase. Finally, 2 mL of six different extraction solvents (ACN, MeOH, acetone, ethyl acetate, toluene, n-hexane) were added to each of microplastics phase. The mixture was allowed to stand for 48 hours. The organic phase (2 mL of the organic solvent) was evaporated to dryness with a stream of nitrogen, reconstituted with 2 mL of n-hexane and injected in the GC-ECD for the analysis of PBDEs and in the GC-MS for the analysis of PAHs.

The procedure was repeated for a different composition microplastics (LDPE, PET, PP and PS). In all the experiments 500 µm size particles were used. A PBDEs/PAHs working standard solution of 0.1 µg/mL in hexane was prepared by dilution of stock solution to calculate the recoveries (“reference standard”). In all cases, the amount of PBDEs/PAHs remained in glass container and in aqueous phase were determined by the addition of 2 mL of hexane to achieve the extraction.

For the study of the adsorptions and release of PBDEs, the methodology was also done using 15 mL of synthetic seawater instead of 15 mL of MilliQ® water using again all the different composition microplastics in each replicated.

The results obtained injecting in GC-ECD were confirmed injecting in a GC-HRMS in the case where PBDEs were used.

IV.2.4 Solubility parameter effect

The following section pretends to explain the theory and equations in what we based our approach. The solubility values of both solvent and polymer are essential parameters for the understanding of this approach.

Dissolution of polymers is a two-step process, first the polymer swells, and the swollen polymer then dissolves. If there are too many cross-links or hydrogen bonds in the polymer, which cannot be broken by the solvent, the process stops in the swelling stage. It is well known that in processes occurring spontaneously enthalpy must decrease. So, solubility occurs only if ∆G (see Equation 1), the free energy of dissolution is negative[36].

$$\Delta \boldsymbol{G} = \Delta \boldsymbol{H} - \boldsymbol{T}\Delta \boldsymbol{S}$$

Equation 1

Where G is the free energy, H is enthalpy, T is absolute temperature and S is the entropy.

∆S, the entropy of dissolution is normally positive. The sign of ∆G is therefore usually determined by ∆H, the enthalpy mixing. If there exists an exothermic interaction between liquid and polymer, ∆H is negative, the system heats up, and the polymer dissolves. If the chemical effect is endothermic; ∆H is positive, and the system cools down. In such cases the magnitude of ∆H determines whether the polymer dissolves or not.

As has already been mentioned, the dissolution of the polymer is possible only if the free energy of the solution decreases with respect to the sum of the pure components. It has also been established that if the polymer-solvent interaction is strong (∆H null), dissolution is always possible but if there are only dispersion interactions between the components ∆H is usually positive, and its magnitude decides whether the material is soluble or not. According to Hildebrand and Scott (1950), the mixing heat per unit volume can be expressed as[36]:

$$\Delta \boldsymbol{H} = \boldsymbol{\nu_1 \nu_2} (\boldsymbol{\partial_1} - \boldsymbol{\partial_2})^2$$

Equation 2

Where ΔH is enthalpy, ν is the volume fraction, indices 1 and 2 refer to the solvent and the polymer respectively, and ∂ is the solubility parameter, which is the square root of the cohesion energy density (CED).

When a polymer is in contact with a solvent, the enthalpy of mixing (ΔH, Equation 2) should be very low or zero to have a good solubility. The more similar the solubility parameters are, the more near zero is ΔH and thus more soluble is the polymer in the solvent.

We hypothesize (Equation 2) that the solvent selected can affect dramatically to the pollutant extraction from the microplastic matrix. If the solvent achieves that the polymer structure moves to a state of less rigidity, the extraction of the analytes from its inside will be maximized. The parameter that can be directly related with the ability of a solvent to extract the pollutants from the plastics is the solubility parameter ∂, as stated before, the square root of the cohesion energy density (CED).

The tables presented next (see Table IV-5 and Table IV-6) show the solubility parameters for both solvents and polymers used in the following experiments.

Table IV-5. Solubility parameters of the chosen solvents for the desorption process[36]

	MeOH	ACN	Acetone	Toluene	Ethyl Acetate	Hexane
Solubility parameter (∂) $(J/cm^3)^{1/2}$	29.7	24.4	20.0	18.8	18.1	14.8

Table IV-6. Solubility parameters of the chosen polymers for the desorption process[36]

	PET	PS	PP	LDPE
Solubility parameter (∂) $(J/cm^3)^{1/2}$	21.9	18.9	17.2	16.2

IV.3 Results and Discussion

The analysis of all the results has been divided in two sections: The adsorption or not of the pollutants on a different composition microplastics and then, the release process study. The pollutants tested are, as has been mentioned, PBDEs and PAHs.

These pollutant families have been widely studied and analysed for many years due to their toxic effects to the environment. Moreover, as has stated before, both the IQS Chromatography Section, and the Environmental Laboratory, have a broad experience in their analysis and quantification. This is why, they are the pollutants families chosen.

IV.3.1 Adsorption and release of PBDEs

The following procedure has been applied during the adsorption and release PBDEs experiences: at first place the interferences analysis has been performed. Then, the PBDEs adsorption and release study on four different types of polymeric microparticles has been performed. All the analysis performed using PBDEs as pollutants have been performed by GC-ECD as stated in the experimental procedure section. The GC-HRMS has been used to validate the results obtained and for the analysis of several samples collected during the BWR 2015.

First, blanks have been checked and no interference problems have been detected during the analysis. The chromatograms pertaining to the solvents analysed do not present any peaks that could cause interferences during the analysis. In addition to these, the rest of solvents and the glass containers used during the experiences that have been done do not show interferences with the analytes (PBDEs) used.

The adsorption process has been studied by putting the different type microplastics in contact with an aqueous solution containing 0.013 µg/mL of each congener, which is close to the solubility limit of the heaviest congeners according to [37]. All the congeners concentration has been maintained fixed to mimic the real environmental situation where the concentration of each of them could not be chosen. The solubility in water of PBDE congeners studied at this concentration changes with the number of bromine atoms. The congeners from *tri-* to *penta-* bromine atoms have a solubility of 0.013 µg/mL, ergo, soluble at the level at which we have worked. Conversely, the PBDE congeners that have more than five bromine atoms have a solubility in water below 0.001 µg/mL. Knowing that this parameter could influence the adsorption process, we decided to maintain the concentration equal for all the congeners to mimic as much as possible the environmental real situation.

Then, the retention times of the BDE congeners analysed are presented in the following table (see Table IV-7). The assignation of the peaks in the chromatogram corresponds with the elution order presented in the Table IV-1.

Table IV-7. BDE congeners retention times, elution order

Nº. IUPAC	Retention time (min)
BDE-28	7.2
BDE-47	9.1
BDE-100	9.9
BDE-99	12.3
BDE-154	13.7
BDE-153	18.2
BDE-183	24.0
BDE-197	24.9
BDE-196	25.3
BDE-207	30.9
BDE-206	32.1
BDE-209	37.5

In Table IV-7 can be observed the assignation of peaks correspondent to the twelve congeners of BDE analysed during all the experiences as well as their retention times.

The distribution of the pollutant among the different sources has been analysed. Thus, for each type of studied polymer (PET, PS, PP and LDPE), the percentage of PBDEs present in the aqueous phase of the initial mixture solution, in the glass container (where the mixture solution has been prepared) and in the microplastic particles has been quantified. First of all, to asses if the adsorption process has taken place, the amount of PBDEs in both aqueous phase and glass container have been analysed. (see Table IV-8).

Table IV-8. Recovery values of PBDE congeners in the aqueous phase and the glass container for each type of microplastics using hexane as organic solvent to extract the pollutants

PBDEs	R% PET glass	R% PET H_2O	R% PS glass	R% PS H_2O	R% PP glass	R% PP H_2O	R% LDPE glass	R% LDPE H_2O
BDE-28	10	1	2	1	9	1	10	1
BDE-47	9	5	2	3	8	4	10	9
BDE-100	10	1	2	1	9	1	11	1
BDE-99	9	1	1	1	9	3	10	1
BDE-154	10	1	1	1	9	3	10	1
BDE-153	10	1	1	1	9	1	11	1
BDE-183	10	1	1	1	10	1	12	1
BDE-197	7	1	1	1	8	1	9	1
BDE-196	9	1	1	1	9	1	11	1
BDE-207	10	1	1	1	10	1	13	1
BDE-206	10	1	1	1	10	0	13	1
BDE-209	8	1	1	1	8	1	10	1

In the aqueous phase the concentration of all congeners detected is in a range from 1% to 5% in almost all of the cases (detection limit 0.001µg/mL). From this recovery values, it can conclude that all the congeners, independently of their solubility in water, have migrated to the plastic particles or glass container. It can be said that the solubility parameter does not affect the conclusions extracted from the adsorption and release experiments.

If the amount of PBDEs adsorbed on the glass container is observed, in all cases the adsorption profile is practically the same. The congeners have been adsorbed on the glass without exceeding around a 13% of the total. The exception is the experiment carried out with PS where the proportion of PBDEs adsorbed on the glass is below the 2%. This result indicates that PS is a type of polymer that allows the highest adsorption of these analytes compared with the other polymers tested, as it could predict looking at their structure.

Taking into account that for a specific microplastic, the amount of PBDEs in the aqueous phase is under a 5% and the quantity found in the glass container is lower than a 10%, it can be concluded that the remaining amount of PBDEs, (almost all), has been adsorbed on the microplastics, equalizing the mass balance. It can be said that in these conditions microplastics studied are acting as PBDEs concentrators.

Therefore, the extraction of the analytes from the four different composition microplastics was carried out. The final goal was to demonstrate that the optimal extraction will be achieved by choosing the correct combination of solvent-polymer based on their solubility parameters (solubility parameter approach).

The PBDEs absorbed on the different plastics have been analysed by GC-ECD. Six extraction solvents (MeOH, ACN, acetone, toluene, ethyl acetate and n-hexane) have been used to explore a large range of solubility parameter values. The idea is to determine the most suitable PBDEs solvent for each kind of microplastic.

First of all, the extraction method for each PBDE congener on PET has been studied (see Table IV-9).

Table IV-9. Recovery results of PBDE congeners from PET microparticles using the six solvents of choice. Three replicates of each experience have been done

	R% MeOH	***R% ACN***	***R% Acetone***	***R% Toluene***	***R% Ethyl Acetate***	***R% Hexane***
BDE-28	71.3 ± 6	77.7 ± 8	71.1 ± 3	53.4 ± 1	67.0 ± 9	37.1 ± 7
BDE-47	74.3 ± 8	77.3 ± 7	72.8 ± 4	56.8 ± 3	69.5 ± 9	40.9 ± 7
BDE-100	72.3 ± 8	75.4 ± 6	72.0 ± 3	55.3 ± 1	69.8 ± 7	38.7 ± 8
BDE-99	63.7 ± 5	63.6 ± 3	64.8 ± 3	47.4 ± 3	60.6 ± 7	36.3 ± 4
BDE-154	47.2 ± 4	48.5 ± 2	47.1 ± 3	35.4 ± 3	43.7 ± 8	25.5 ± 3
BDE-153	42.3 ± 3	40.6 ± 9	41.3 ± 2	32.2 ± 5	39.6 ± 4	24.2 ± 9
BDE-183	42.9 ± 2	39.8 ± 4	41.9 ± 2	32.0 ± 4	39.2 ± 6	24.6 ± 5
BDE-197	40.9 ± 5	35.7 ± 3	39.4 ± 2	31.4 ± 2	38.1 ± 10	23.9 ± 1
BDE-196	42.4 ± 5	37.7 ± 3	41.6 ± 3	32.9 ± 2	39.4 ± 12	24.9 ± 1
BDE-207	44.8 ± 9	40.3 ± 2	41.3 ± 1	31.3 ± 2	36.7 ± 4	26.5 ± 12
BDE-206	63.7 ± 7	51.4 ± 4	54.3 ± 1	45.0 ± 1	46.7 ± 4	35.6 ± 5
BDE-209	58.7 ± 7	54.2 ± 3	56.0 ± 1	44.5 ± 3	53.4 ± 8	38.1 ± 9
$(\partial_1 - \partial_2)^2$	61	6	4	10	14	50

The results have been analysed from two points of view:

- How the use of different solvents influences the yield of the PBDEs extraction from the microplastics.

- Which are the differences of the congener's behaviour regarding the number of bromine atoms in their structure.

On one hand, as it can be seen in Table IV-9, the recovery obtained is dramatically influenced by the solvent chosen. Solvents like MeOH, ACN or acetone followed by ethyl acetate allow the highest extractions for all the congeners in general. This profile in the recoveries can be justified using as a reference the Hildebrand and Scott equation, presented in the experimental part of this chapter. As stated before, the partial or total dissolution of the polymers is achieved

when the ΔH is zero or minimum. At that point, the polymer chains trap the solvent, allowing the PBDE congeners be extracted from the microparticles structure.

If ∂ for PET, 21.9 is looked (Table IV-6), and compared with the ∂ of the different solvents described in Table IV-5, it can be seen that, for this polymer, the solvents with a similar ∂ are ACN and ethyl acetate that results in a minimum ΔH. These two solvents are followed by the acetone, with the closer ∂ to PET which results in a ΔH practically zero. Comparing the recoveries obtained with these three solvents with the ones obtained using n-hexane, the hypothesis is validated again since hexane with a ∂ of 14.8 is far to be an ideal solvent for PET.

On the other hand, it should be noted, observing Table IV-9, that for those congeners that have in their structure between three and five bromine atoms (BDE-28 to BDE-99) the recovery is relatively higher than in those congeners with a higher number of bromine atoms (BDE-153 to BDE-209) for some of the solvents used. This fact could be explained considering the differences in solubility of each congener in the solvents used. The PBDEs solubility is higher in a more nonpolar solvents than in solvents such as MeOH or ACN. This fact can be explained using as an example the release using ACN and toluene. In the case of ACN, could be seen that the difference in terms of desorption between the lightest congener (BDE-28: 77.7%) and the heaviest congener (BDE-209: 54.2%) is approximately a 25% in the recovery. Otherwise, observing the behaviour of the congeners using toluene as a solvent the difference in the recoveries between the lightest (BDE-28: 53.4%) and the heaviest congener (BDE-209: 44.5%) is only 9%. Thus, in those solvents where the PBDEs have a higher solubility such as toluene or hexane the differences in terms of extraction between all the congeners are not so dramatic.

After study the release of PBDE congeners using PET as microplastic and the demonstration that it is possible to choose the optimal extraction solvent applying the “solubility parameter” approach before start the analysis, we have tried to apply this approach to any polymeric microparticles with different compositions and see if it also works.

In the case of PP, LDPE and PS, the other microplastics tested, again the same six solvents have been used.

Table IV-10 shows the recovery values obtained from the extraction of PBDEs using PP as microplastic and six different solvents.

Table IV-10. Recovery results of PBDEs congeners from PP microparticles using the six solvents of choice. Three replicates of each experience have been done

	R% MeOH	***R% ACN***	***R% Acetone***	***R% Toluene***	***R% Ethyl Acetate***	***R% Hexane***
BDE-28	54.9 ± 5	12.8 ± 3	77.0 ± 3	138.4 ± 2	68.3 ± 2	96.1 ± 1
BDE-47	52.1 ± 3	21.2 ± 4	77.0 ± 3	138.4 ± 1	68.6 ± 3	96.9 ± 4
BDE-100	52.4 ± 7	12.5 ± 1	76.1 ± 4	138.2 ± 6	70.2 ± 2	95.7 ± 2
BDE-99	41.4 ± 6	40.9 ± 6	61.9 ± 5	115.1 ± 3	57.6 ± 1	80.1 ± 2
BDE-154	32.1 ± 9	13.2 ± 6	49.0 ± 5	90.6 ± 1	44.2 ± 6	64.7 ± 3
BDE-153	27.2 ± 5	11.1 ± 5	42.2 ± 4	79.4 ± 6	40.1 ± 6	55.7 ± 5
BDE-183	26.2 ± 9	65.4 ± 4	40.0 ± 7	81.7 ± 8	39.8 ±8	56.8 ± 7
BDE-197	25.6 ± 2	65.5 ± 9	38.7 ± 6	81.1 ± 9	37.4 ±9	56.8 ± 4
BDE-196	36.5 ± 4	19.4 ± 6	39.2 ± 8	81.6 ± 7	39.1 ± 6	57.1 ± 3
BDE-207	26.9 ± 8	17.4 ± 7	37.4 ± 5	86.8 ± 6	40.8 ±4	59.3 ± 6
BDE-206	34.8 ± 9	34.2 ± 7	49.5 ± 5	129.3 ± 9	47.0 ±3	82.1 ± 3
BDE-209	35.0 ± 8	38.1 ± 8	50.7 ± 4	117.5 ± 7	55.5 ± 5	82.2 ± 2
$(\partial_1 - \partial_2)^2$	156	52	8	3	1	6

In Table IV-10, the difference in recoveries using the six solvents is presented. Observing in more detail the columns of two solvents with different solubility parameter values such as MeOH, with recoveries not exceeding 55%, and toluene with recoveries between 80 and 120% evident differences can clearly see. If the ∂ for PP is taken (Table IV-6) and is compared with the ∂ of the different solvents described in Table IV-5 it can be seen that, for this polymer, the solvent with the closest ∂ is toluene. On the other hand, methanol with a ∂ of 29.7 cannot be a good election for the extraction of this analytes from PP microplastics since the ΔH in this case is far from be zero. Thus, the approach has been confirmed for the PP.

LDPE is another example of polymer that demonstrates the compliance of the solubility parameter approach based on the Hildebrand and Scott equation. Figure IV-3 shows a schematic representation of the results for the extraction in this type of polymer.

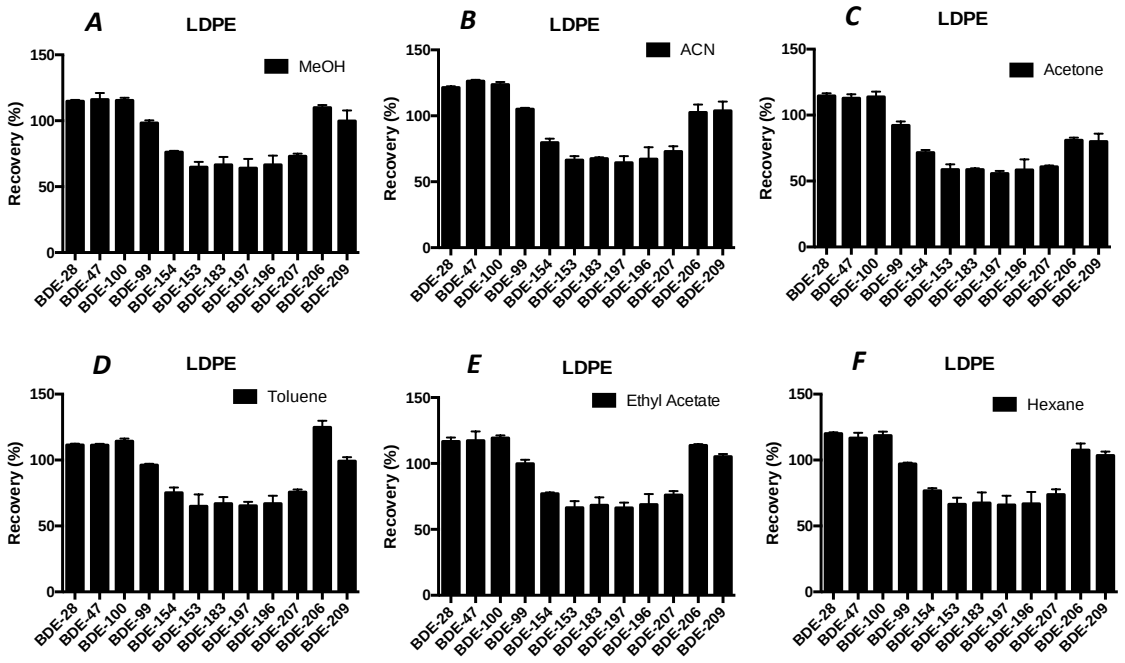

Figure IV-3. Recovery results of PBDEs congeners from LDPE microparticles. Individual graphical representation is shown for each solvent used. Three replicates of each experience have been done

LDPE is the polymer with the lowest ∂ (16.2) compared with the other polymers tested. So, those solvents with a lower ∂ such as acetone, ethyl acetate, toluene or hexane, should have the capacity to extract better the PBDE congeners. In contrast with that, acetonitrile or methanol, are solvents that do not follow the rule; thus, a lower recovery can be expected. However, analysing the recovery values presented, it can be seen that LDPE is a polymer, due to its structure, that shows good recoveries for any of the solvents used. This means therefore that although the solubility parameter approach is complied, all the solvents are suitable for the release of PBDEs from LDPE.

PS has a similar ∂ that PP (17.2). As expected, the extraction efficiency of the different solvents shows the same trend that for PP. This confirms the idea that ∂ can be a good parameter to choose the solvent. The behaviour observed coincide when a non-polar solvent is used, higher extraction is achieved for both PP and PS (see Table IV-11).

Table IV-11. Recovery results of PBDEs congeners from PS microparticles using the six solvents of choice. Three replicates of each experience have been done

	R% MeOH	***R% ACN***	***R% Acetone***	***R% Toluene***	***R% Ethyl Acetate***	***R% Hexane***
BDE-28	2.1 ± 6	33.5 ± 3	33.3 ± 5	65.6 ± 4	71.4 ± 6	53.7 ± 3
BDE-47	1.3 ± 5	25.5 ± 4	27.4 ± 3	63.5 ± 5	68.0 ± 5	49.3 ± 3
BDE-100	1.5 ± 3	27.3 ± 3	27.5 ± 2	65.2 ± 2	71.6 ± 3	50.0 ± 1
BDE-99	1.3 ± 7	18.1 ± 2	21.7 ± 7	52.4 ± 7	57.3 ± 2	42.8 ± 4
BDE-154	0.7 ± 1	15.1 ± 5	16.0 ± 5	41.7 ± 9	44.7 ± 3	33.2 ± 5
BDE-153	0.5 ± 1	11.4 ± 5	13.8 ± 3	35.6 ± 7	40.0 ± 5	26.8 ± 4
BDE-183	0.5 ± 5	8.1 ± 6	10.6 ± 1	34.5 ± 6	39.6 ± 6	21.0 ± 6
BDE-197	0.4 ± 8	10.0 ± 4	13.3 ± 1	33.9 ± 6	38.7 ± 2	24.7 ± 5
BDE-196	0.4 ± 4	9.8 ± 7	13.0 ± 5	34.7 ± 3	39.3 ± 4	23.3 ± 6
BDE-207	0.5 ± 3	7.5 ± 3	10.4 ± 3	35.5 ± 4	39.9 ± 1	19.2 ± 7
BDE-206	0.5 ± 2	11.9 ± 4	17.7 ± 2	52.3 ± 2	57.3 ± 1	29.3 ± 7
BDE-209	0.4 ± 5	8.4 ± 2	12.5 ± 4	45.5 ± 4	51.9 ± 3	20.4 ± 3
$(\partial_1 - \partial_2)^2$	117	30	1	0	1	17

Although nonpolar solvents are the appropriated for the extraction, PS case is different. The recoveries obtained for the desorption of almost all the PBDE congeners are lower than 50% (see Table IV-11). This is due to the fact that when the PS microparticles come into contact with any of the solvents used, they form an aggregate making difficult for the solvent to enter in its structure and preventing the optimal desorption of the pollutant whatever the congener analysed is.

Taking all those appreciations into account, for the analysis of pollutants adsorbed in any composition of polymer, specifically PBDEs in this study, the choose of the right solvent is key in relation with the microplastic where it is adsorbed. The solubility parameter approach could be used to choose the appropriate solvent before initiating the extraction procedure and the analysis.

One fact that is repeated in some of the experiences analysed above is that for those PBDE congeners that have in their structure between six and nine bromine atoms, the recovery values always descend not matching the mass balance regard the other congeners (three to five and ten bromine atoms). In fact, a U shape is clearly identifiable in Figure IV-3, showing those congeners (between six and nine bromine atoms) the lowest recoveries. These results cannot be explained taking in account their hydrophobicity, chemical structure or conformational state. One possible explanation, already suggested in literature[38], is that these congeners can suffer isomerization under the influence of typical fluorescence lights.

BDE-209, on the other hand, has a behaviour that does not follow the expected trend in contrast to the other congeners. This fact can be explained due to low diffusion coefficient of PDBE 209 in microplastics. At the high concentration of PBDEs used in the experiment (regarding the PBDE solubility in water) congener 209 is not able to diffuse completely through the microplastic, remained partially absorbed on the microplastic surface, facilitating its extraction[39].

Figure IV-4 shows a schematic representation of the optimal couple solvent-polymer for the extraction of the analytes.

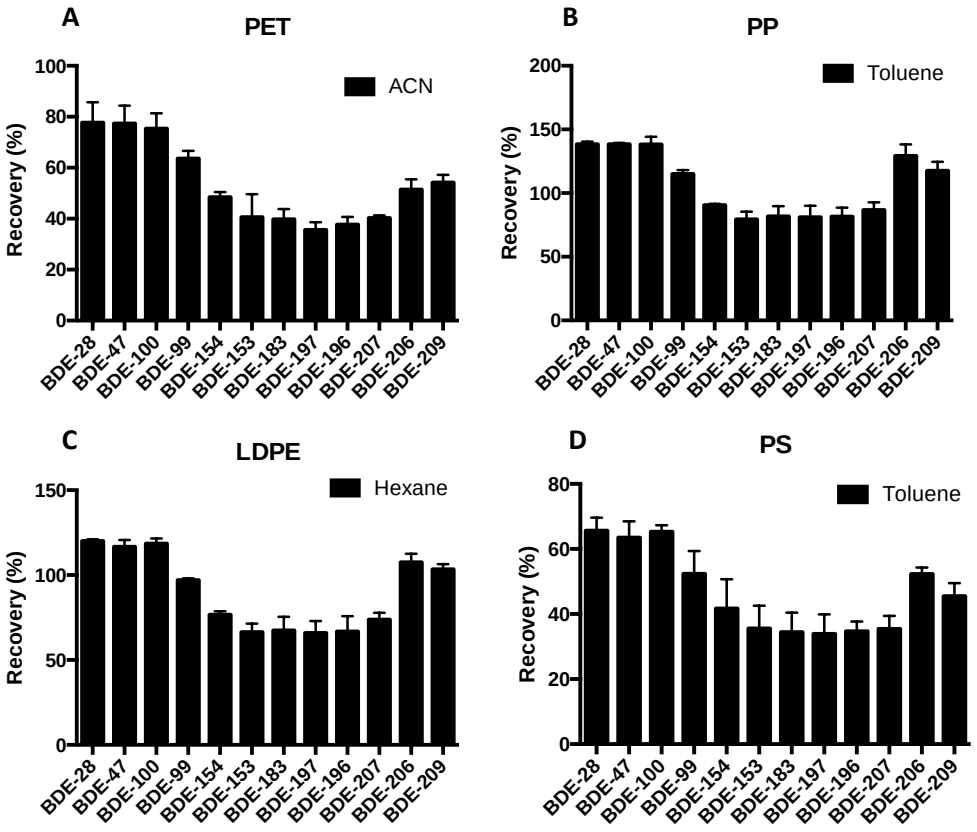

Figure IV-4. Schematic representation of the optimal solvent-polymer couple for the analyte's extraction

Figure IV-4 shows the optimal couple polymer-solvent to work with for the extraction of this family of pollutants, PBDEs. In the case of have PET as microplastic, ACN should be used; for PP and PS microparticles, toluene should be used, and n-hexane is the solvent to use when the microplastics are LDPE. The working methodology will be specific for each type of microplastic and pollutant to be desorbed. The solubility parameter ∂ for both the plastic and the solvent could be a good parameter for helping in the extraction procedure.

All the previous experiences have shown that microplastics act as concentrators of PBDEs and their extraction and quantification is also possible. Until this moment, the approach has been developed using as aqueous phase MilliQ® water. But it would be interesting to study how the presence of sea salts can affect to the adsorption or extraction process since during the analysis

of real samples the matrix (seawater) will have a certain amount of salts that could generate interferences or changes in the behaviour of the analytes. To this end, we have repeated the experiments carried out using synthetic seawater (see Section IV.2.1.3). Figure IV-5 shows a graph with the recoveries for each one of the congeners of PBDEs dissolved in synthetic seawater solution and adsorbed on microplastic particles of PET. The extraction solvent used for the extraction is ACN since it has been demonstrated previously that is the best solvent for this composition polymer. We show the comparison of the recovery values adding PBDEs on synthetic seawater vs adding them on MilliQ® water (Figure IV-5).

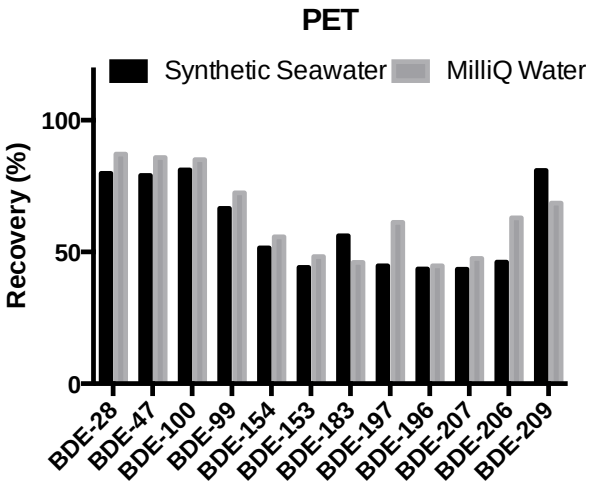

Figure IV-5. Comparison of the recovery values of PBDE congeners on PET microparticles in synthetic seawater vs MilliQ® water using GC-ECD

It is known that the presence of salts using synthetic seawater implies a decrease in the PBDEs solubility in the aqueous phase compared with the use of MilliQ® water. However, the amount of PBDEs found in the glass container and in the aqueous phase in this experience coincides with the results presented in the Table IV-8 (MilliQ® water). Thus, the analysis show that the adsorption of PBDEs in PET when these are dissolved in synthetic seawater is complete. Almost the 100% of the pollutant introduced is adsorbed. In Figure IV-5 can also be observed that the recovery results during the extraction process using synthetic seawater show recoveries pretty similar that when MilliQ® water is used. The results demonstrate that the differences in solubility of PBDEs in MilliQ® water or in synthetic seawater do not affect in their adsorption and release from microplastics. In nature the adsorption of PBDEs on microplastics suspended in the oceans will be complete and the extraction process takes place in the same way.

The analysis during the development of the approach have been carried out, as already mentioned above, using the GC-ECD, which is a useful instrument for a methodology development. One it has been demonstrated that the results obtained meets with the theory raised, some of the experiments have been repeated using GC-HRMS. The higher selectivity and sensibility of the GC-HRMS will allow us to validate the method previously developed.

Furthermore, during the analysis of the real samples the presence of interferences could take place, and this is why the use of an instrument with a higher selectivity will be much useful. Furthermore, the use of GC-HRMS will allows us to validate the approach raised before about the U shape in all the experiences presented due to the isomerization of some PBDEs.

The analysis of the samples using GC-HRMS showed in the chromatograms the loss of degrees in concentration in some congeners as can be observed the following figure (see Figure IV-6):

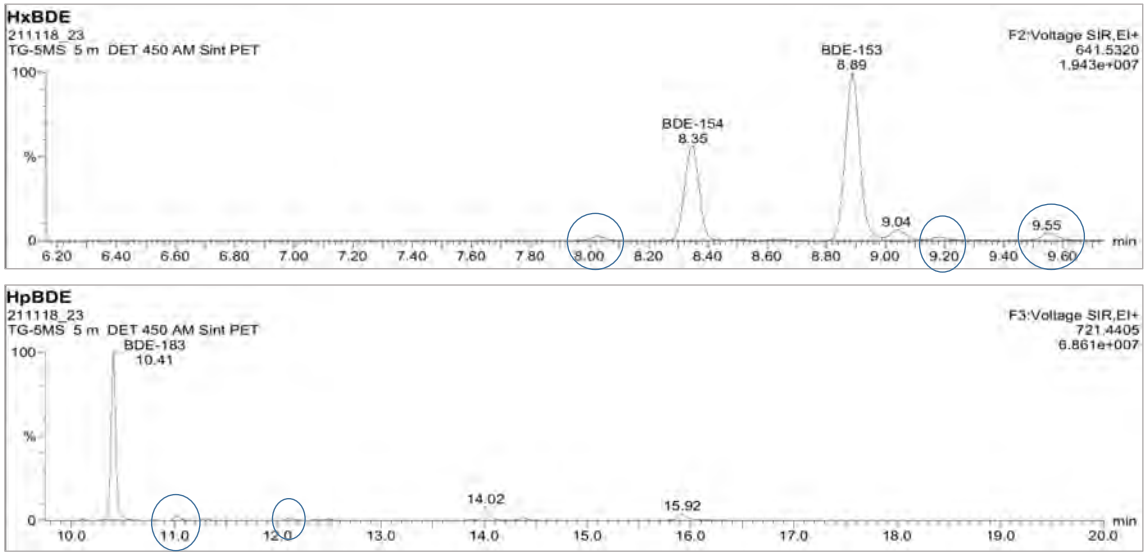

Figure IV-6. Mass extraction of HxBDE and HpBDE from a chromatogram of one sample injected in the GC-HRMS. Marked in blue the signals correspondent to possible BDE isomerizations. Analysis performed by GC-HRMS

Figure IV-6 shows two chromatograms with the mass extraction of two congeners of PBDEs present in a sample where the release of this pollutants from PET microparticles was studied. The pics shown in the figure pertains to a hexa-BDE (BDE-153) and hepta-BDE (BDE-183) since are some of the congeners that have a lost in their concentration during the experiences. In the figure can be observed the appearance of small peaks (signalled in blue) in the baseline that suggest on the basis of the proximity to existing PBDEs in the standard, the presence of BDE isomers. This fact corroborates the approach previously raised about the U shape that is observed in some figures (see Figure IV-3 and Figure IV-4) since the isomerization results in a diminution in the area values of the signals.

Finally, the validity of the theory at any concentration level has been demonstrated. The adsorption and release of PBDEs on different types of microplastics have been repeated adding the congeners into the aqueous solution in a lower concentration (60 times lower). In the new experiences presented below, the concentration of PBDEs in the aqueous solution is of 0.0002 µg/mL. As it has been mentioned at the beginning of the analysis, the solubility limit of the heaviest PBDEs (Hexa- to Deca-) in aqueous solutions is of 0.001 µg/mL. Thus, all BDE congeners are dissolved solution in the new experiences and any undissolved BDE congener

solid particles can be found. In this sense, several articles references described BDE congeners at similar concentrations in the aqueous media (0.0002 µg/mL), this is why this level has been chosen[40–42].

In this experience, the two polymers with the higher Tg: PET (~ 70ºC) and PS (~ 80ºC) have been used. Also, two of the previously used solvents, methanol and hexane, with the higher difference in terms of solubility parameters values, have been selected. These experiments have been performed using GC-HRMS, following the conditions described in the experimental section. The results obtained are presented in Figure IV-7.

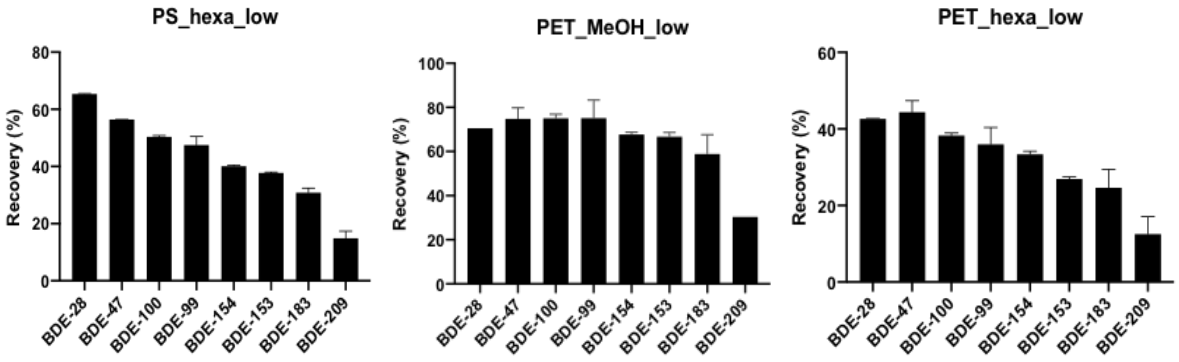

Figure IV-7. Recovery results of PBDEs congeners from PS and PET microparticles. Individual graphical representation is shown for each solvent used. Each BDE congener at 0.0002 µg/mL in the aqueous solution. Three replicates of each experience have been done

As can be observed in Figure IV-7, PET extracted with MeOH, where PBDEs are at 0.0002 µg/mL in the aqueous solution give rise pretty similar results than the ones obtained using the PBDEs in higher concentrations (Table IV-9). Differences of only a 10% have been found in terms of recovery values in almost all of the results, validating the results previously presented. The same happens when n-hexane has been used.

The experiences have been also repeated using PS microplastics and PBDEs in lower concentrations, as can be observed using hexane as extracting solvent, the same results than the previous ones have been obtained, excepting congener 209. When methanol is used for PBDEs extraction from PS microparticles a higher variability of the results between the two concentrations tested has been obtained (not presented in Figure IV-7). As it has been stated in the manuscript, PS microparticles show a different scenario since they tend to aggregate during the experimental procedure when methanol is used.

It has been confirmed that the results presented in the previous experiences are independent form the congener concentration used, except from congener 209. In this sense, the new results obtained demonstrates the validity of all the experiences presented in the chapter.

Specific comment has to be addressed for congener 209. It is possible that due to its low diffusion rate in the microplastics, as described in [39], when the congener concentration is high (as the one used in when BDE congeners are added at a higher concentration), congener 209 is not able to diffuse completely through the microplastic, remain in part absorbed on the surface, and facilitating its extraction.

IV.3.2 Adsorption and release of PAHs

The concentration effect of microplastics using PAHs as pollutants has been also studied. With that, is wanting to demonstrate that it's not only true with PBDEs but also with other families of pollutants. As has stated before, the IQS Chromatography Section has a broad experience in their analysis and quantification of PAHs[31]. This is why, it is the pollutants family chosen.

As in the PBDEs experiences, the procedure will be, at first place the interferences analysis and then the PAHs adsorption and release study on four different types of polymeric microparticles. All the analysis performed using PAHs as pollutants have been done by GC-MS as has been mentioned in the experimental procedure section. The GC-MS is a technique with a high sensibility and selectivity, and it has been used for the PAHs analysis in recent years.

So, the analysis of blanks has been performed to determine the presence or not of interferences. The pure solvents, the glass containers and the MilliQ® water used have been analysed in pursuit of traces of PAHs which may affect the results.

Figure IV-8 shows an example of a chromatogram obtained for the analysis interferences with one of the solvents.

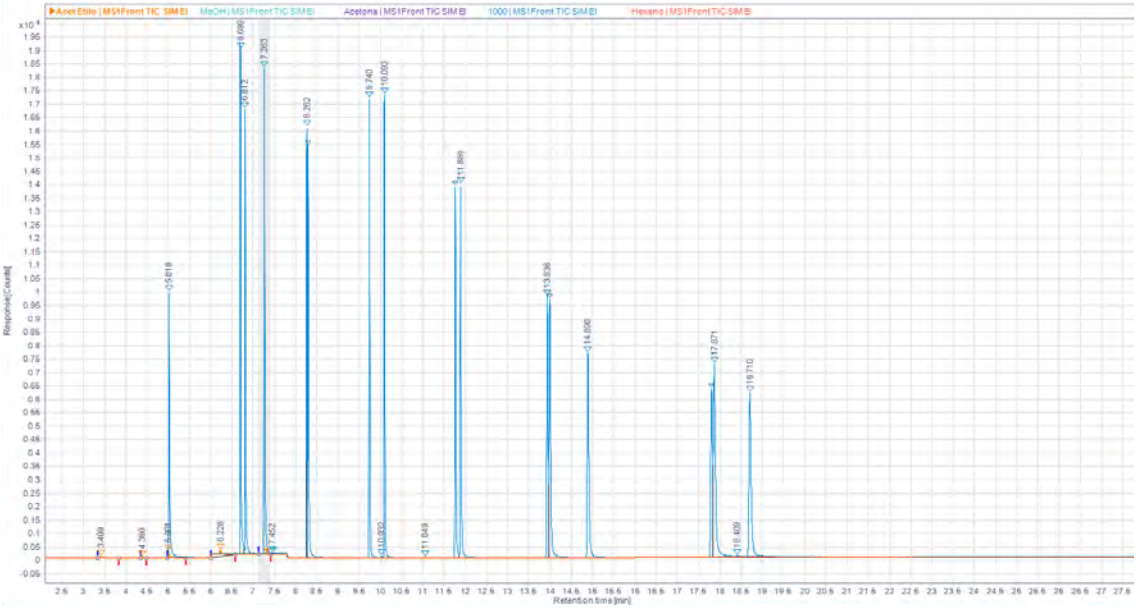

Figure IV-8. Superposition of chromatograms corresponding, in red, green, orange and purple to solvent's blank analysis and in blue to a 0.5 µg/mL standard of PAHs. Analysis performed by GC-MS

No interferences in any case have been found. Thus, it can proceed to the proper analysis.

The adsorption and extraction of PAHs on four types of microplastics have been studied. The concentration of work for PAHs is 0.013 µg/mL in the aqueous phase for each congener. The selection of this work concentration has been done based on the facility of the methodology development. At this concentrations, the heaviest congeners, i.e., from Benz[a]anthracene to

Indene[1,2,3-cd]pyrene are not soluble in water (see Table IV-2)[33]. As has been mentioned in the PBDEs analysis, the solubility of the pollutants in water is not a huge issue since the conditions are maintained as similar as how we will find the analytes in the environment where their concentrations cannot be chosen. So, we decided to maintain all the congeners at the same concentration and ignore the solubility variable during all the analysis.

Furthermore, as it has already demonstrated in the PBDEs analysis, the results obtained are independent from the analyte concentration used. The lower concentration results obtained for PBDEs experiences demonstrates the validity of the following results obtained for PAHs at the work concentration.

Then, an example of a chromatogram obtained for the analysis of a PAHs standard of 0.05 µg/mL is shown (see Figure IV-9). The numeration order presented in the following chromatogram corresponds with the followed in Table IV-2.

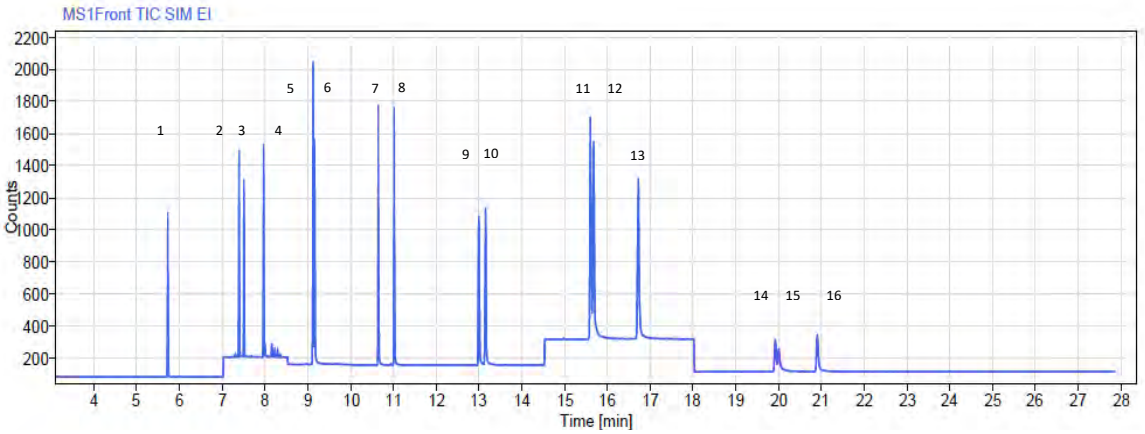

Figure IV-9. PAHs congeners working standard solution chromatogram. Concentration of 0.05 µg/mL. Analysis performed by GC-MS

First, the adsorption process has been studied. To do that, the amount of PAHs dissolved in the aqueous phase and the amount remained in the glass container have been quantified. Table IV-12 shows the recovery values obtained for all the plastic particles tested.

Table IV-12. Recovery values of PAHs in the aqueous phase and the glass container for each type of microplastic using hexane as organic solvent. Three replicates of each experience have been done

PAHs	PET glass	PET H_2O	PS glass	PS H_2O	PP glass	PP H_2O	LDPE glass	LDPE H_2O
Na	0	53±1	1±0	54±3	1±2	56±2	0	50±1
Acy	0	38±2	0	38±2	1±1	42±4	0	39±1
Acp	0	22±1	0	20±4	0	23±5	0	22±5
Flu	0	20±1	0	19±1	1±1	22±1	0	22±3
Phen	0	12±1	1±0	11±3	1±3	13±1	0	12±2
Ant	0	8±1	1±1	7±5	2±1	9±2	0	8±5
Flt	0	5±1	0	3±1	1±2	3±4	0	3±4
Pyr	0	5±1	1±1	3±1	2±2	2±3	0	3±2
BaA	0	3±1	1±0	1±3	0±1	1±1	0	1±2
Cry	0	6±3	0	1±2	0±1	2±2	0	2±2
BbF	1±0	2±1	1±1	1±6	1±2	0±7	1±2	1±1
BkF	0	16±3	1±1	1±8	1±2	1±8	1±3	2±5
BaP	0	8±4	0	1±9	1±1	0±3	1±1	7±7
BghiP	1±0	19±1	0	0	3±3	1±2	0	10±3
DiBahA	1±0	14±3	2±1	15±3	1±2	12±2	1±3	13±1
IP	0	28±1	4±2	11±8	2±5	11±1	1±4	10±1

As can be seen in the Table IV-12, in the glass container, the recoveries for the majority of the analytes are between 0.5% and 2% and in any case exceed the 5%. These values states that almost the totality of the analytes will be found dissolved in the aqueous phase or adsorbed in the microplastic particles.

Analysing the recovery values for the congeners found in the aqueous phase, it can be observed that the highest values are obtained for the four first congeners (the lightest ones) reaching the 50%. It also can be seen that the values diminish until reach the heaviest congeners where the recoveries increase to a 10% approximately. Figure IV-10 shows an example of the tendency that the values follow for all the microplastics.

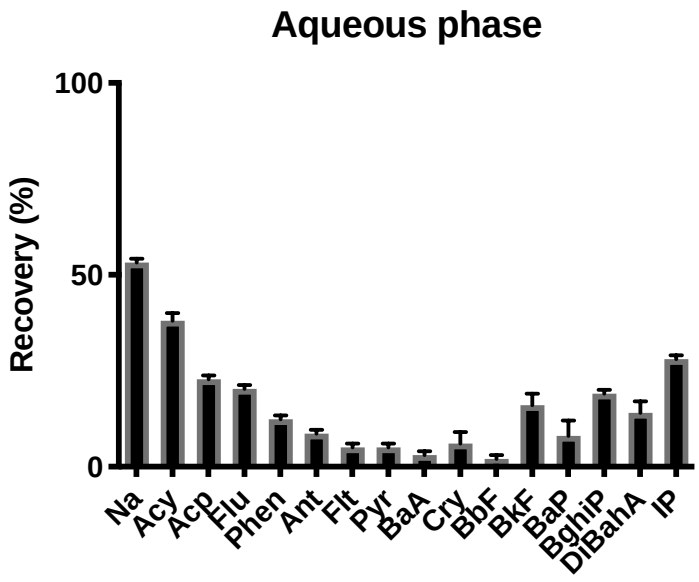

Figure IV-10. Recovery values of PAHs in the aqueous phase. PET analysis recovery values are used as an example

From the recovery values obtained, as already mentioned above, it can be concluded that for the two lightest PAHs almost the 50% and around 20% for the third one, have been adsorbed on the microplastics. It is possible that the solubility in water of the three first analytes (the lightest ones) hinders the transfer of them from the aqueous phase to the microplastic particles causing that part of them remained solved in water. For the rest of the analytes, the remaining amount (almost all), has been adsorbed on the microplastics, equalizing the mass balance. This values also demonstrate that the solubility in water of almost all of PAHs do not affect to their adsorption since the majority of them are not found in the aqueous phase. It can be said that microplastics are acting as PAHs concentrators.

Therefore, it proceeds to the release of the analytes previously adsorbed on the four different composition microplastics. In these experiences, three extraction solvents have been used: ACN, acetone, and hexane. From the study previously done using PBDEs it has concluded that these three solvents are the most representative and the ones with solubility parameter values further away from each other. We try to demonstrate that the "solubility parameter" approach is not only true for the PBDEs but also for other families of pollutants.

First, the extraction process of PAHs from PP microplastics has been studied. The following figure (see Figure IV-11) summarizes the recovery values for the extraction.

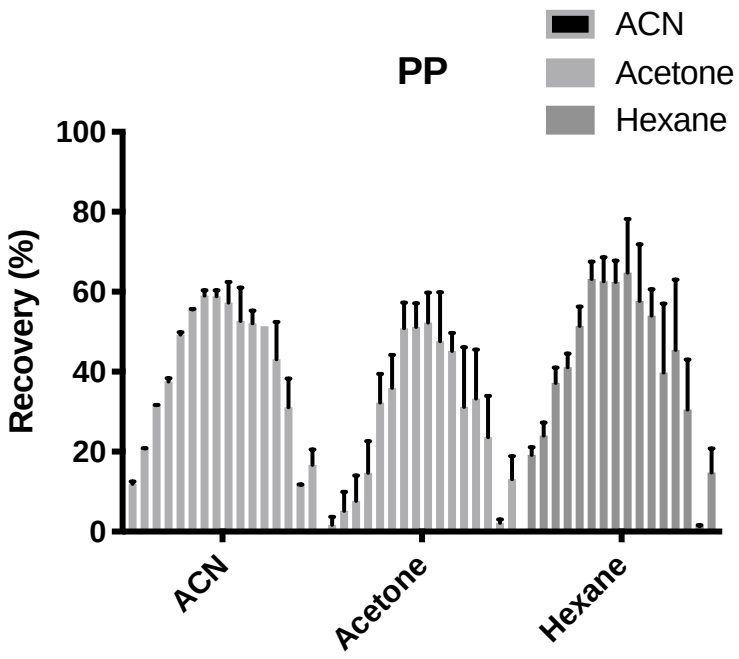

Figure IV-11. Recovery results for the extraction of PAHs from PP microplastics using the three solvents of choice. Three replicates of each experience have been done

The values presented in the Figure IV-11 of the extraction of PAHs from PP microparticles can be analysed having into account two parameters:

- The solubility parameter approach described in the Material and Methods section (see IV.2.4 section).

- The differences in terms of solubility of the analytes in the organic solvents used (see Table IV-2).

First, it should be noted in the previous Figure (see Figure IV-11) that the profile obtained for any solvent used is the same, the lightest analytes such as naphthalene, acenaphthylene or acenaphthene shows low recoveries in the same way that the ones obtained for DiBahA or IP where the recoveries descends again which corresponds with the inverse of the profile obtained in the aqueous phase analysis.

Hexane is the solvent with which the highest values have been obtained, reaching the 70-80% for the central analytes shown in the figure. ACN and acetone gives recoveries between 40% and 60% in some of the analytes. The differences obtained with the three solvents used can be justified using as a reference the Hildebrand and Scott equation. Looking the ∂ for PP, 17.2 (Table IV-6), and comparing it with the ∂ of the different solvents (see Table IV-5) we can see that for this polymer, the solvent with a similar ∂ is hexane (14.8) followed by the acetone (20.0) and finally the ACN (24.4). It can be concluded that the optimal solvent to use with this polymeric microparticles is the hexane. The tendency can be confirmed, and the recovery values obtained

corroborates the approach using this type of microparticles. Thus, the solubility approach theory in this scenario has been validated.

LDPE is another example of microplastic composition used for the adsorption and release experiences. See the recovery values obtained after the release in Figure IV-12.

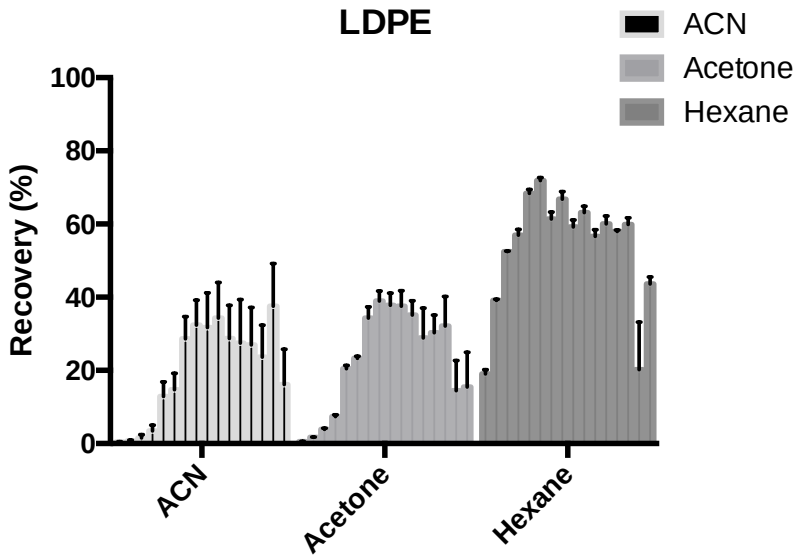

Figure IV-12. Recovery results for the extraction of PAHs from LDPE microplastics using the three solvents of choice. Three replicates of each experience have been done

Figure IV-12 shows that the optimal solvent for the extraction of this pollutants from LDPE microparticles is hexane, with a recovery values around 60-80% for almost all the congeners except again the first three and the last two where the recoveries are around a 40%. Comparing these results with the ones obtained analysing the PAHs found in the aqueous phase (see Figure IV-10) where the 50% of the total for the lightest and 20% for the heaviest congeners have remained solved in water, it can be said that the mass balance have been fulfilled. Using acetone or ACN as extraction solvent, recoveries of the order of 35% have been obtained. This differences in the recoveries can be explained using the "solubility parameter" approach, as closer are the values of ∂ between the solvent and the polymer, a minimalization of enthalpy will be achieved and therefore, less rigid the chains of the polymer will be allowing the solvent to extract the pollutant from inside the structure. In the Table IV-6 can be observed that LDPE is the polymer, of all that have been tested, with the lowest ∂ (16.2) so the optimal solvent will be also one with a low ∂. From all the solvents tested, hexane is the one that is in line with this requirement, with a ∂ of 14.8, making ΔH value minimal. Acetone and ACN with a ∂ values of 20.0 and 24.4 respectively are solvents less suitable for this composition polymer. Again, when LDPE microparticles are used, the solubility approach theory has been validated. For this type of microplastics, the optimal solvent to use is the hexane as in the PP microplastics case.

After the study of the release of PAHs from PP and LDPE, the same procedure to other composition microplastics has been applied. The recovery values obtained for PET are shown in the following figure (see Figure IV-13). The same three solvents, ACN, acetone and hexane, have been used.

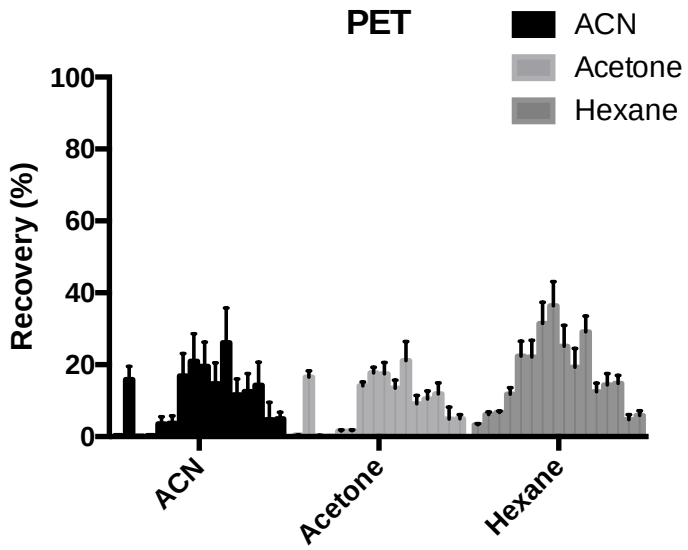

Figure IV-13. Recovery results for the extraction of PAHs from PET microplastics using the three solvents of choice. Three replicates of each experience have been done

Observing the tendency of the results (Figure IV-13), it can be observed that the profile, for any of the solvents used, is as in the previous cases the inverse of the profile obtained during the analysis of the aqueous phase. As have been mention before, a fraction of the lightest and the approximately the 10% heaviest PAHs have remained solved in the water.

Analysing the results from the point of view of the solubility parameter approach, the most suitable solvents for the release in this composition microparticles are acetone (20.0) and ACN (24.4) since the ∂ parameters of these two solvents are the closest to the PET one (∂, 21.9), achieving a minimum ΔH, in contrast to hexane with a ∂ value (14.8) far to be the appropriate for this type of polymer. But, observing the differences in the recovery values between the three extraction solvents used during the process, it can be seen that pretty similar recovery values have been obtained using the three solvents. Hexane, in any case, exceeding the 50% and ACN or acetone with recoveries with maximums of 30% and 40% respectively.

For the three solvents the recovery values obtained are pretty similar and quite low so appreciate differences in terms of the solubility parameter approach is difficult since almost half of the pollutant is still adsorbed inside the microplastic structure. It can be said that, although for this type of polymer the optimal solvent to use should be acetone or ACN, a partial extraction will be achieved with any of the three solvents tested, ACN, acetone or hexane.

The last type of microplastic particles tested are polystyrene microparticles. Figure IV-14 shows the recovery values obtained for the extraction in this case. The same behaviour detected using PET microparticles has been observed when PP microparticles have been used.

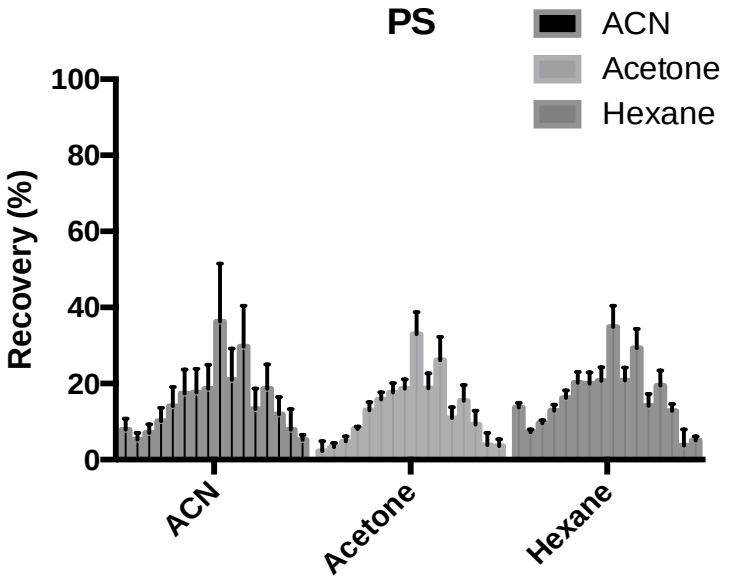

Figure IV-14. Recovery results for the extraction of PAHs from PS microplastics using the three solvents of choice. Three replicates of each experience have been done

The case of PS should be pretty similar to PP since both have similar ∂. Thus, hexane seems to be the most suitable solvent for this type of polymer but the results presented in Figure IV-14 show that the extraction efficiency of the different solvents shows the same trend. Although hexane should be the optimal solvent having into account the solubility parameter approach, for PS the three solvents shows same recoveries, so with any of them the release of PAHs shows pretty low recovery values.

As can be seen, the recoveries obtained for the desorption of all the PAH congeners are lower than 40% (see Figure IV-14). One of the reasons for these low recoveries is due to the fact that, just as using PBDEs as pollutants, when the PS microparticles come into contact with any of the solvents used, they form an aggregate making difficult for the solvent to enter in its structure and avoiding an optimal release of the pollutant whatever the congener analysed is.

As has been stated before, for both PET and PS experiences, pretty low recovery values have been obtained although the PAHs mass adsorbed on the microparticles is approximately the same as in the other polymeric microparticles tested, according to the mass balance. The hypothesis proposed for the results obtained is therefore, the differences in terms of structures between the solvents used and these two polymers.

As it is known PET and PS are two polymers that have in their structure aromatic rings in contrast with PP and LDPE which structures are lineal hydrocarbon chains. Thus, a solvent with

an aromatic ring in its structure, such as toluene, would give rise a higher recovery values since a higher compatibility between polymer and solvent structures had taken place. Although the hypothesis suggested the use of toluene for obtaining higher PAHs releases, it is known that the use of toluene is problematic and not recommended.

Finally, to corroborate the hypothesis raised about the incompatibility between structures, a second extraction with the most suitable solvent according to the approach has been performed. With this last experiment incompatibility of the polymer and the analyte has been demonstrated. As has been stated before, this occurs in the experiences using PET and PS as microplastics where only the 50% of the total at most have been recovered and the rest have been remained inside the polymer structure. To do that, a second extraction using the optimal solvent have been performed. Another extraction will allow to determine if we are able to continue recovering the rest of the pollutant or if we have to assume that a certain amount of pollutant will remain trapped inside of the polymeric structure avoiding the use of toluene.

This second extraction analysis is applied in two different experiences, the first one using the combination PP-hexane, which resulted in good recoveries (maximums around 70%) in previous experiences and a second one using the combination PET-ACN, with previous maximum recoveries of 30%-40%.

Figure IV-15 shows the recovery results obtained for both of the experiences.

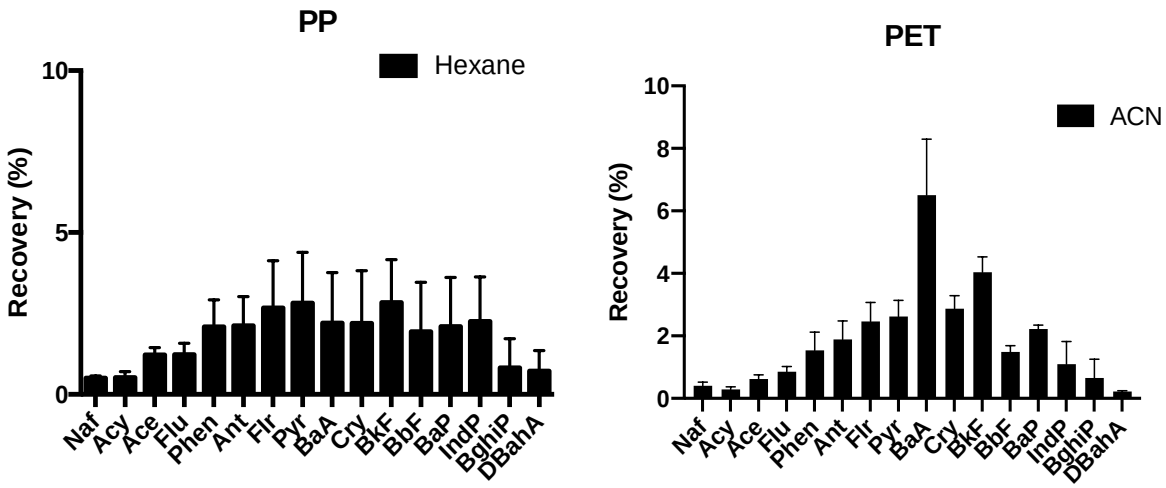

Figure IV-15. Recovery values obtained for a second extraction with hexane using PP and PET as microparticles

As can be observed in Figure IV-15 the recoveries obtained for both experiences do not exceed the 7%. The three lightest analytes and the last three show lower recoveries than the rest probably due to the first have remained solved in the water phase and the last three have a high hydrophobic character.

In the case where PP microparticles have been used, the mass balance is achieved for almost all the congeners since the first extraction show values around 80%, 10% of the total have been found in the glass container and the aqueous phase and a 5% have been recovered during the second extraction. In the experience using PET microparticles also around a 5% have been recovered during the second extraction, so around a 40% have been remained trapped inside the polymeric structure. Probably the amount of PAHs recovered in the analysis corresponds to residual analytes ubicated in the surface of the polymeric microparticles since both of the graphs show a pretty similar appearance although the amount of pollutant to be extracted was not the same. This fact, comparing the results obtained using PP or PET, corroborates that the solvent, whatever the volume used, do not have the capacity of extract the pollutant from PET structure. Thus, it is demonstrated the approach that the conformation of PAHs in combination with the structure of PET makes difficult the release of this pollutant.

It is assumed that when PET microparticles are used, part of the pollutant, PAHs, will be remained inside the structure.

IV.3.3 Concentration effect of microplastics collected during the BWR 2015

Finally, and to validate the solubility parameter approach, some of the filters with the microplastics collect during the Barcelona World Race 2014-2015 have been analysed. The presence of PBDEs and PAHs have been analysed by GC-MS. Of all the samples available, and having into account that 1 L of seawater has been circulated through each device installed in the race boat, the pollutants are analysed using the same procedure followed in all the previous experiences: a filter with 500 μm pore diameter from each ocean has been selected and 2 mL of the optimal organic solvent for each microplastic composition have been added to extract the pollutants of interest from the microplastics retained in the filters[43]. A table (see Table IV-13) with the relevant information about the samples used for the following analysis is presented.

Table IV-13. Information regarding the BWR samples analysed. Reference, latitude and longitude.

Ocean	Reference	Latitude	Longitude
Mediterranean	1	41.1720	2.1482
Atlantic	28	-43.5988	1.5920
Indian	36	-40.4578	52.5985
Pacific	52	-53.9912	-174.7275

Prior to the addition of the solvent to for the release of POPs from microplastics, and to be able to apply the approach developed, the chemical composition of the plastics retained have been determined through infrared microscopy (IR) coupled with microscopy technique. The fact of knowing the composition of the particles allow us to choose which is the most suitable solvent for the extraction of the two families of pollutants.

First, the quantification of PBDEs adsorbed on the microplastics retained have been performed. Prior to the analysis, the infrared microscopy analysis has been performed to know the composition of the particles. In the Atlantic Ocean filter PET microplastics has been found, so the extraction of PBDEs from these filters has been done with ACN. In the case of the Indian Ocean and Mediterranean Sea, the microparticles found and analysed corresponds to PP, so the extraction is achieved with the addition of toluene. Finally, in the filter relevant to the Pacific Ocean no microplastic particles have found, but we determined the presence of microparticles corresponding to microplankton.

Table IV-14 shows the concentrations of the PBDE congeners found in the filters from each location.

Table IV-14. Results of PBDEs analysed extracted from microplastics collected in the BWR 2014. Microplastics from the four oceans have been used (where d.l. corresponds to experimental quantification limit, < 0.9 µg/Kg). First column results expressed as µg of pollutant/Kg of microplastics per filter (assuming that 1 mg of microplastics have been retained in the filter). Second column results expressed as ng of pollutant/L of seawater through the filter (1L)

	Mediterranean Sea		***Atlantic Ocean***		***Pacific Ocean***		***Indian Ocean***	
	PBDEs (µg/Kg)	***PBDEs (ng/L)***	***PBDEs (µg/Kg)***	***PBDEs (ng/L)***	***PBDEs (µg/Kg)***	***PBDEs (ng/L)***	***PBDEs (µg/Kg)***	***PBDEs (ng/L)***
BDE-28	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.
BDE-47	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.	0.2	2
BDE-100	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.
BDE-99	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.
BDE-154	11	106	3	60	< d.l.	< d.l.	< d.l.	< d.l.
BDE-153	23	219	5	113	< d.l.	< d.l.	< d.l.	< d.l.
BDE-183	27	258	6	132	0.3	4	2	22
BDE-197	27	249	6	137	< d.l.	< d.l.	1	8
BDE-196	24	226	5	115	0.4	6	< d.l.	< d.l.
BDE-207	23	215	4	101	< d.l.	< d.l.	< d.l.	< d.l.
BDE-206	23	214	5	108	< d.l.	< d.l.	< d.l.	< d.l.
BDE-209	19	178	4	84	< d.l.	< d.l.	< d.l.	< d.l.

The results presented in Table IV-14 show the presence of the heaviest congeners of PBDE in the Mediterranean Sea and in the Atlantic Ocean, concentrations between 1 and 27 µg/Kg have been detected in these two locations. These levels of concentration are also reported by other studies as PBDE concentrations found in some aquatic organisms and in sediments and soils[44–46]. On the other hand, in the Pacific and Indian Oceans, only some congeners have been detected and in a concentration range close to the detection limit although most of them have not been detected. Moreover, the values presented in Table IV-14 evidence the concentration effect of microplastics. The results obtained are 10^8 orders of magnitude greater, meaning from ng to µg, having into account that one litre of seawater has circulated through the filter. Values of the order of ng/L in seawater in different locations have been also reported in other studies[47,48].

Then, the quantification of PAHs adsorbed on the same microplastic particles have been performed. Figure IV-16 shows a chromatograms superposition example of a 0.1 µg/mL PAHs standard solution and the solvent extract with the PAHs released from the microparticles.

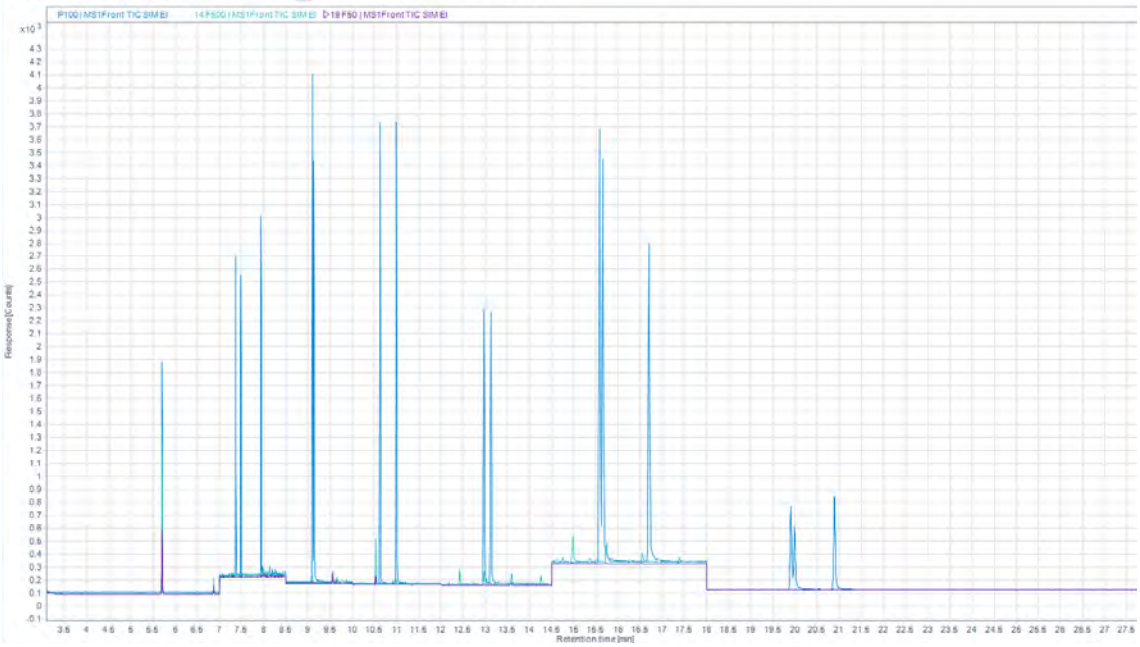

Figure IV-16. Chromatogram superposition of a 0.1 µg/mL PAHs standard solution in blue and the PAHs release from two filters related to the Mediterranean Sea and the Atlantic Ocean in green and purple respectively. The detection limit in this analysis is stablished at < 0.1 µg/L

As can be observed in the superpositions presented in Figure IV-16, the two chromatograms related to the PAHs release from microplastics collected in the Mediterranean Sea and the Atlantic Ocean any congener of PAHs has been quantified. So, no presence of PAHs has been detected in both cases.

In addition to the two filters from the Mediterranean and the Atlantic, as in the case of PBDEs, the PAHs adsorbed on the microplastics from the Pacific and Indian oceans have also been tried to quantify. From the four locations where the samples were taken only traces of PAHs have been found and with levels under the detection limit.

IV.4 Concluding remarks

In this chapter the concentration effect, i.e., the adsorption of persistent organic pollutants on different microplastic particles have been studied. It has been determined the dependence of the release process of POPs to the plastic composition and the extraction solvent used.

To reach this aim, four different types of polymeric microparticles and six extraction solvents have been tested using two families of pollutants: PBDEs and PAHs. The solubility parameter approach has been used to explain the process.

The concentration effect of microplastics of twelve congeners of PBDEs on PET, PP, LDPE and PS microplastic particles was investigated. The adsorption of PBDEs on different composition microplastics is complete. The results obtained from the extraction procedure demonstrates that it is function of the combination solvent-polymer used. This fact can be validated applying the "solubility parameter" approach which says that the more similar this parameter is between the solvent and the polymer, nearer to zero will be the enthalpy of the system causing the process to be spontaneous. Thus, will result in higher extraction. The solubility parameter ∂ for both the plastic and the solvent is the suitable parameter for helping in the solvent election.

The optimal solvent for PET is the ACN, in the case of PP and PS toluene results to be the most suitable solvent to use although is not recommendable due to its associated problematic. Thus, for PP and PS hexane should be used. Hexane is the optimal solvent in the case of having LDPE microparticles.

The differences in terms of adsorption/extraction using synthetic seawater instead of MilliQ® water to dissolve the PBDEs and study the effects of the presence of salts have been also analysed. The adsorption using synthetic seawater coincides with the ones obtained using MilliQ® water, almost a 100% of all PBDE congeners are adsorbed on the microplastics, confirming the conclusions extracted in the previous experiences.

The development of method and all the experiences have been done with GC-ECD, but it was validated with GC-HRMS, with a higher sensibility, to apply the method for the analysis of real samples.

The concentration effect of microplastics of sixteen congeners of PAHs on PET, PP, LDPE and PS microplastic particles was investigated. Three extraction solvents have been used in these experiences. The adsorption of PBDEs on different composition microplastics is complete. The results obtained from the extraction procedure demonstrates that it is function of the combination solvent-polymer used.

This fact can be validated again applying the "solubility parameter" approach. This approach is useful in the case of PP and LDPE where high recoveries have been obtained. In the case of

using PET or PS microparticles the recoveries obtained are pretty low so the approach cannot be applied. The hypothesis raised is the incompatibility between the structures of these two polymers (PET and PS) and the solvents used. A solvent with an aromatic ring in its structure, such as toluene, should be used. As has been stated before, the use of toluene is not recommendable due to its associated problematic.

The three solvents used for the extraction of PHAs from PET and PS show the same recoveries. In the case of PP and LDPE hexane should be used.

In summary, the approach described in this chapter, choosing in advance which is the best solvent for the extraction allows to ensure the maximum amount of pollutant extracted. This is very important when real and unique samples have to be analysed.

Microplastic samples collected from Mediterranean Sea, Atlantic, Pacific and Indian oceans during the Barcelona World Race 2014-2015 have been analysed to determine the concentration of PBDEs and PAHs adsorbed on them. From Mediterranean Sea and Atlantic Ocean, levels of PBDEs from 1 and 27 μg/Kg have been detected depending on the congener. From the analysis of Pacific and Indian Ocean, the amount of almost all the congeners found is pretty close to the detection limit. For the quantification of PAHs in microplastic samples collected in the same locations, any congener of PAH has been detected, only traces of this pollutant with values under the limit of detection of the instrument have been found.

After the concentration effect of microplastics have been demonstrate, in the next chapter the presence of organic pollutants suspended in seawater have been studied. To that end, an analytical methodology for the elution process of the pollutants from the HLB cartridges installed in the COA device have been developed.

Five different families of pollutants were selected for the study, phthalates, perfluorinated compounds (PFACs), pharmaceutical products, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). These pollutants were selected since their hydrophobic character makes them potential candidates because IQS has a wide experience in their chromatographic analysis.

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Chapter V.

Pollutants solved in seawater

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Pollutants solved in seawater

The present chapter is focussed in the analysis of those pollutants that do not have the ability to adsorb themselves on the plastic particles and therefore will be probably found suspended in seawater or those ones which are in concentrations at it will be found distributed between the plastic microparticles and the seawater. In order to do that, the chapter will explain the development of a methodology for the elucidation and analysis of five families of pollutants from a solid phase extraction cartridge used for their extraction and the development and optimization of different chromatographic methods for their analysis.

V.1 Introduction

As has already been discussed and demonstrated in the previous chapter, microplastics suspended in water have the ability of adsorb in their surface those hydrophobic pollutants that are around them in the ocean. In addition of that, there are also suspended in seawater other pollutants that do not have the ability to be adsorbed on microplastics but have also an interesting takeover due to their potential toxicity effects on the marine environment.

Pollutants such as plasticizers, pesticides, organochlorine biocides, polycyclic aromatic hydrocarbons have been detected in the oceans, including polar region, ocean sediments and biota, and open ocean surface waters, as well as in a few deep-sea water samples[1–3]. Furthermore, the growing use of pharmaceuticals, antibiotics, personal care products or illicit drugs, classified as the so-called emerging contaminants, has also become a new environmental problem, which has awakened great concern among scientists in the last few years. Even though they are found in very low concentrations, there is still a lack of knowledge about long-term risks that the presence of a large variety of pollutants families may pose for the environment, non-target organisms as well as for human health[4,5]. To have an approximate idea of which of all of them are going to remain in seawater, water solubility of organic pollutants is among the most important physical property to notice. The knowledge of this property will also allow to control the transport and fate of the chemicals in aquatic systems[6,7].

Consequently, there is a growing need to develop reliable analytical methods, which enable their rapid, sensitive and selective determination in environmental samples, at trace levels. Solid phase extraction (SPE), solid phase microextraction (SPME) and stir bar sorptive extraction (SBSE) have been reported as sensitive and reliable techniques for those families of analyte extraction[8–12].

Thus, it was decided that the designed device installed in the racing boat during the Barcelona World Race 2015, in addition to have three filters for trapping microplastic particles

also would have a Solid Phase Extraction cartridge capable of the retention of the pollutants solved in sea water[13,14].

The solid phase extraction is an analytical technique mainly used for the purification of a sample before their quantification and/or for the concentration of the analytes of interest present in the sample. The SPE aims to perform the separation of certain components of a sample through its distribution in two phases, a stationary and a mobile one. The stationary phase is mainly a solid retained on a support, while the mobile phase is liquid, in our scenario seawater. The extraction of an analyte of interests take place through solid-liquid systems using cartridges, discs or fibres. The use of SPE also allows to economize solvents since it makes the concentration of the analyte using a smaller amount of solvent in relation to the traditional processes, such as solid-liquid and liquid-liquid extraction[15,16].

For the type of sample that we pretended to collect, it was necessary that the SPE cartridge selected met some specific requirements. We need that the SPE cartridge was able to retain families of pollutants with very different physical and chemical properties. It was needed a cartridge with a universal stationary phase. That is why the SPE cartridge used was the Oasis HLB Cartridge.

Oasis HLB is a universal sorbent for acidic, neutral and basic compounds. It has a hydrophilic-lipophilic water-wettable reversed-phase sorbent and is made from a balanced ration of two monomers, the hydrophilic N-vinylpyrrolidone and the lipophilic divinylbenzene (see Figure V-1). A complete description of this SPE cartridge is available in Chapter II.

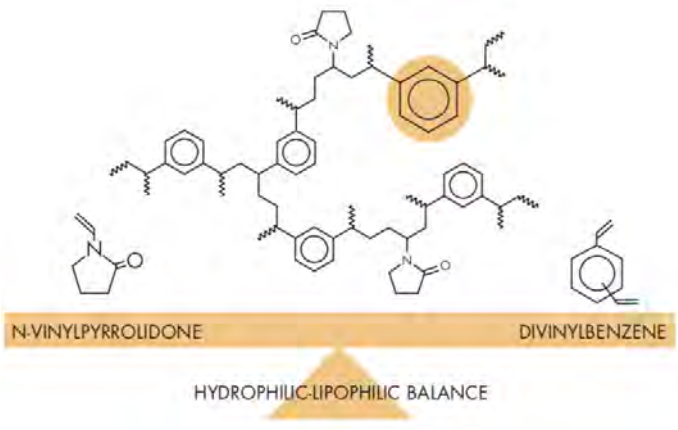

Figure V-1. Oasis HLB stationary phase polymer composition[17]

This balanced copolymer structure provides to the SPE cartridge a superior reversed-phase capacity with a special polar force for enhanced retention of polar analytes. The ability of this cartridge is adsorbing not only non-polar analytes as in most of the cases but also polar analytes.

This type of cartridge is widely used for agrochemical and environmental applications such as triazine or acid herbicides, metabolites, phenols or PAHs but is also used in food applications or pharmaceutical such as tetracyclines and basic drugs or tetracyclines and pesticides[18–22].

In addition to the elution and further analysis (known as target analysis) of specific families of pollutants as the ones mentioned above, new methodologies have been developed for the detection and analysis of a large number of compounds simultaneously, known as non-target analysis or analysis of unknowns.

Contamination of water resources is one of the major problems to be faced for environment preservation and sustainability. Although anti-pollution strategies taken in the last half-century have consistently reduced in surface water the amount and the presence of many recognised contaminants, other potentially hazardous chemicals are being released into the environment, together with new substances that are continuously synthesized and whose dangerous properties are not well known. Water-pollution monitoring typically makes use of methods for target analysis, which are normally focused on priority pollutants that are legally regulated or of public concern. The scope of such methods rarely exceeds several tens of analytes, and it is quite unusual to find analytical methods applied to more than 100 organic pollutants. Target-compound monitoring is often insufficient to assess the quality of environmental waters as only a limited number of analytes are recorded[23,24].

Because of the potentially adverse environmental and/or health outcomes associated with exposure to such chemicals, data concerning the presence and the concentration of these chemicals in biological matrices is needed. Analytical methods for a rapid and sensitive determination of a broad range of compounds in complex biological matrices are required. Multi-residue analytical methodologies are powerful tools, as they may provide greater knowledge about the overall contamination. Papers related to multi-residue analytical methodologies have increased over recent years though most are focused on targeted analysis methods and cover a relatively narrow range of chemicals class. These methods generally target only parent compounds whilst the metabolites and transformation products, which can be more toxic than their parent compounds. In recent years, the evolution of accurate mass high resolution mass spectrometry (HRMS) has initiated a new trend in analytical data processing towards non-target analytical methods. Non-target analytes might be therefore present in the samples and would not be detected in targeted analysis.

Global analysis, which can be used to search for a large number of compounds simultaneously, is one approach for addressing the increasingly diverse range of contaminants. Direct measurement of samples without any compound loss is ideal for complete, global detection of contaminants[25,26].

The analysis of the several samples collected during the BWR 2015 using the Oasis HLB cartridge allows to apply both techniques previously mentioned, the target and non-target analysis

to search the possible pollutants retained. The application of a non-target analysis to this samples will allow to have a global idea of which samples are those with a higher level of contamination and in function of which parameters them can be grouped.

Then, the main objective of this chapter is the elution and analysis of different families of pollutants retained in the SPE cartridge used in the BWR 2015 to be able to determine where the contamination focus is and how are the pollutants distributed in the oceans around the world.

In order to achieve this objective, the following tasks were proposed:

- Application and optimization of chromatographic methods developed in the Chromatography section for the analysis of five different pollutant families. Phthalates, perfluorinated acid and sulfonated compounds (PFACs), pharmaceutical products, polyaromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) are the pollutants selected.

- Development of an analytical methodology to achieve an efficient elution of all the families studied and all the possible pollutants retained in the SPE cartridge using a specific combination of organic solvents.

- Elution of some cartridges used in the BWR 2015 from different strategic sampling locations to try to determine the correlation between those zones and the diversity of pollutants found using the non-target analysis.

V.2 Material and Methods

As has been mentioned in the introduction section phthalates, PFACs, pharmaceutical products, PAHs and PCBs have been the pollutants selected for the development of the following experiences. The selection has been done according the IQS extensive experience in the analysis of these pollutants. Some of them has been analysed in the Chromatography section for many years[27–33].

As can be noticed, PAHs are a family of pollutants used in Chapter IV for the study of microplastics concentration effect. As has been concluded in the previous chapter, some of the PAH have not been adsorbed on the microplastics and with some of the polymers used the recovery values have not been high enough to equal the mass balance. Thus, PAHs have been used again in this chapter order to determine if part of them can be found in the aqueous solution (seawater), in other words, retained in the HLB cartridges used to collect pollutants solved in seawater during the BWR.

V.2.1 Reagents and reference substances

V.2.1.1 Phthalates

For the analysis of Phthalates, a standard mix containing 6 compounds was purchased from Dr. Ehrenstorfer™ (Reference Standards for Residue Analysis) with the reference XA08060100IO (Phthalate Esters – Analytes Mix 1 200 μg/mL in iso-octane, 1 mL of solution). In Table V-1 is shown the name of each compound present in the standard mixture.

Table V-1. Phthalates standard mixture compounds name and their abbreviations

Name	Abbreviation
Dimethyl phthalate	DMP
Diethyl phthalate	DEP
Dibutyl phthalate	DBP
Benzyl butyl phthalate	BBP
Bis(2-ethylhexyl)-phthalate	DEHP
Dioctyl phthalate	DOP

Because the standard mix is meant to be used in GC (solved in iso-octane) it is needed to do a change in the solvent in order to have the mix solved in methanol. When performing dilutions to change the solvent, solvent miscibility must be taken into consideration. Iso-octane and methanol are not miscible so an intermediate dilution in another solvent must be used. The selected solvent was acetone. Once the mixture is solved in methanol, a stock standard solution containing all the Phthalates at 10 μg/mL was prepared ***(SS-PHT)***. Working standard solutions

mixtures containing all the analytes at different concentrations were prepared by dilution of the stock solution with the appropriate volume of methanol. All the solutions were stored at -22ºC.

V.2.1.2 PFACs

In order to analyse PFACs, a standard mix containing 17 compounds including acids and sulfonated analytes was purchased from Wellington Laboratories (PFAC Mix-B 2 μg/mL in methanol, 1.2 mL of solution). A detailed table (see Table V-2) is presented below with the name of the compounds and their correspondent abbreviation.

Table V-2. PFACs standard mixture compounds and their abbreviations

Name	Abbreviation
Perfluoro-n-butanoic acid	PFBA
Perfluoro-n-pentanoic acid	PFPeA
Perfluoro-n-hexanoic acid	PFHxA
Perfluoro-n-heptanoic acid	PFHpA
Perfluoro-n-octanoic acid	PFOA
Perfluoro-n-nonanoic acid	PFNA
Perfluoro-n-decanoic acid	PFDA
Perfluoro-n-undecanoic acid	PFUdA
Perfluoro-n-dodecanoic acid	PFDoDA
Perfluoro-n-tridecanoic acid	PETrDA
Perfluoro-n-tetradecanoic acid	PETeDA
Perfluoro-n-hexadecanoic acid	PFHxDA
Perfluoro-n-octadecanoic acid	PFODA
Perfluorobutane sulfonate	L-PFBS
Perfluorohexane sulfonate	L-PFHxS
Perfluorooctane sulfonate	L-PFOS
Perfluordecane sulfonate	L-PFDS

A stock standard solution containing all the PFACs at 2 μg/mL in methanol was prepared ***(SS-PFACs)***. Working standard solutions mixtures containing all the analytes at different concentrations were prepared by dilution of the stock solution with the appropriate volume of methanol. All the solutions were stored at -22ºC.

V.2.1.3 Pharmaceutical products

For the analysis of pharmaceutical products five standards from different trading houses. A detailed table (see Table V-3) is presented below with the name of the standard, the producer and its reference.

Table V-3. Pharmaceutical products name, producers and references

Name	Trade house	Reference
Acetaminophen	Sigma-Aldrich	A500-100 g
Caffeine	Fluka	27600-25 g
Enrofloxacin	Fluka	33699-100 mg-R
Hydrochlorated Fluoxetine	Sigma-Aldrich	PHR1394-g
Norfloxacine	Sigma-Aldrich	N9890-5 g

A stock standard solution containing all the pharmaceutical products at 10 μg/mL in methanol was prepared ***(SS-PP)***. Working standard solutions mixtures containing all the analytes at different concentrations were prepared by dilution of the stock solution with the appropriate volume of methanol. All the solutions were stored at -22ºC.

V.2.1.4 PAHs

In order to analyse PAHs, a standard mix containing 16 compounds was purchased from Dr. Ehrenstofer™ (Reference Standards for Residue Analysis) with a reference XA20950009AL (PAHs Mix-9 100 μg/mL in acetonitrile, 1 mL of solution). A detailed table is presented in the experimental section of the Chapter IV with the name of the compounds present in the mixture, their abbreviations, their solubility in water at 25ºC and the coefficient octanol-water.

A stock standard solution containing all the PAHs at 10 μg/mL in methanol was prepared ***(SS-PAHs)***. Working standard solutions mixtures containing all the analytes at different concentrations were prepared by dilution of the stock solution with the appropriate volume of methanol. All the solutions were stored at -22ºC.

V.2.1.5 PCBs

The PCB-MIX 20 from Dr. Ehrenstofer™ (Reference Standards for Residue Analysis) with the reference LA20032000IO was selected which contains the 15 EPA-PCBs at 10 μg/mL in iso-octane. A detailed table (see Table V-4) is presented below with the name of the compounds present in the mixture.

Table V-4. PCBs standard mixture compounds name and their abbreviations

Name	Abbreviation
2,4,4'-Trichlorobiphenyl	PCB 28
2,4',5-Trichlorobiphenyl	PCB 31
2,2',5,5'-Tetrachlorobiphenyl	PCB 52
3,3',4,4'-Tetrachlorobiphenyl	PCB 77
2,2',4,5,5'-Pentachlorobiphenyl	PCB 101
2,3,3',4,4'-Pentachlorobiphenyl	PCB 105
2,3',4,4',5-Pentachlorobiphenyl	PCB 118
3,3',4,4',5-Pentachlorobiphenyl	PCB 126
2,2',3,3',4,4'-Hexachlorobiphenyl	PCB 128
2,2',3,4,4',5'-Hexachlorobiphenyl	PCB 138
2,2',4,4',5,5'-Hexachlorobiphenyl	PCB 153
2,3,3',4,4',5-Hexachlorobiphenyl	PCB 156
3,3',4,4',5,5'-Hexachlorobiphenyl	PCB 169
2,2',3,3',4,4',5-Heptachlorobiphenyl	PCB 170
2,2',3,4,4',5,5'-Heptachlorobiphenyl	PCB 180

Because the standard mix is meant to be used in GC (solved in iso-octane) it is needed to do a change in the solvent in order to have the mix solved in methanol. When performing dilutions to change the solvent, solvent miscibility must be taken into consideration. Iso-octane and methanol are not miscible so an intermediate dilution in another solvent must be used. The selected solvent was acetone. Once the mixture is solved in methanol, a stock standard solution containing all the PCBs at 10 μg/mL was prepared ***(SS-PCBs)***. Working standard solutions mixtures containing all the analytes at different concentrations were prepared by dilution of the stock solution with the appropriate volume of methanol. All the solutions were stored at -22ºC.

V.2.1.6 Mix working standard solution preparation

A standard solution with the five families of pollutants was prepared. In order to do that, a solution containing all the analytes was prepared in methanol by dilution of the different stock solutions. 1 mL of ***SS-PHT*** solution + 1 mL of ***SS-PFACs*** + 1 mL of ***SS-PP*** solution + 1 mL of ***SS-PAHs*** solution + 0.8 mL of ***SS-PCBs*** solution up to 10 mL with methanol.

The mixed working standard solution ***(SS-Mix)*** has all the analytes at the following concentrations: 1 μg/mL of phthalates, 0.2 μg/mL of PFACs, 1 μg/mL of pharmaceutical products, 1 μg/mL of PAHs and 0.8 μg/mL of PCBs.

A PAHs and PCBs working standard solution in n-hexane were prepared by dilution of stock solution in order to be able to inject them in the GC instrument.

V.2.1.7 Solvents

Acetonitrile (ACN), HPLC LC-MS grade, HiPerSolv Chromanorm, was from VWR Chemicals; Methanol (MeOH), LC-MS grade, was from Fisher Scientific; Acetone, for pesticide residue analysis, Pestinorm, was from VWR Chemicals; Ethyl acetate for pesticide residue analysis was from Panreac; Toluene, Chromasolv, for pesticide residue analysis was from Honeywell; n-Hexane for pesticide residue analysis, Pestinorm, was from VWR Chemicals.

V.2.1.8 Synthetic seawater

For the experimental procedure, 1 L of synthetic sea water was prepared using the compounds described in Table V-5 and adjusting the pH with hydrochloric acid (HCl, Panreac, 1 M) between 7.5 and 8.5.

Table V-5. Composition of synthetic sea water. Composition and product references[34]

Compounds	Concentration ($g \cdot L^{-1}$)	Product reference
NaCl	24	Panreac® 141659
$MgCl_2$	5.0	Sigma-Aldrich® M8266
Na_2SO_4	4.0	Panreac® 141716
$CaCl_2$	1.1	Sigma-Aldrich® 499609
KCl	0.70	Panreac® 141494
Na_2CO_3	0.20	Panreac® 141648
NaBr	0.096	Panreac® 141646
H_3BO_3	0.026	Panreac® 141015
$SrCl_2$	0.024	Sigma-Aldrich® 439665
NaF	0.0030	Sigma-Aldrich® 450022

V.2.2 Methods

Different chromatographic instruments have been used based on the pollutant analysed. Although several chromatographic techniques are suitable for the analysis of these families of pollutants, the election has been done according with the IQS extensive experience in the analysis of these pollutants. Phthalates, PFACs and pharmaceutical products has been analysed by UHPLC-MS/MS, the PAHs has been analysed by HRGC-MS and finally PCBs has been analysed by HRGC-ECD.

V.2.2.1 Phthalates analysis method by UHPLC-MS/MS

UHPLC-MS/MS analysis were performed using a Waters ACQUITY UHPLC H-Class coupled to a Xevo TQ-S micro triple quadrupole mass spectrometer. The suitable separation of the analytes was achieved connecting an ACQUITY UPLC BEH Phenyl 1.7 μm, 2.1 mm x 100 mm column. The column temperature was set at 45ºC. 0.1% of ammonia solution (A) and

acetonitrile (B) were the mobile phases used for the analysis and with a flow rate of 0.4 mL/min. The initial proportion was fix at 60% A:40% B, after 10 min was changed to 95% A: 5% B and held for 10 min (minute 20'). At the minute 21 the proportion was settled back to 60% A:40% B and held for 4 minutes. The total run time was 25 minutes. Ten microliters of a sample solution were injected. It was used a negative ionization mode (ESI-), a cone flow of 200 L/h (N_2), a desolvatation temperature of 550ºC, a desolvatation flow of 800 L/h and a capillary voltage of 2.5 kV. The acquisition was performed in MRM mode. Next, in Table V-6 is shown the collision energy and cone voltage parameters for all the analytes.

Table V-6. Collision energy and Cone voltage parameters for the phthalates analysed

Compound		Q1 (m/z)	Q3 (m/z)	Collision Energy (V)	Cone voltage (V)
Dimethyl phthalate	MRM 1	195	163	14	10
	MRM 2	195	135	28	20
Diethyl phthalate	MRM 1	223	177	6	30
	MRM 2	223	149	24	20
Dibutyl phthalate	MRM 1	279	149	12	40
	MRM 2	279	205	10	10
Benzyl butyl phthalate	MRM 1	313	149	8	40
	MRM 2	313	205	6	40
Bis(2-ethylhexyl) phthalate	MRM 1	391	149	22	50
	MRM 2	391	113	14	10
Dioctyl phthalate	MRM 1	391	261	8	50
	MRM 2	391	149	22	30

V.2.2.2 PFAC analysis method by UHPLC-MS/MS

UHPLC-MS/MS analysis were performed using a Waters ACQUITY UHPLC H-Class coupled to a Xevo TQ-S micro triple quadrupole mass spectrometer. The suitable separation of the analytes was achieved connecting an ACQUITY UPLC BEH C18 1.7 μm, 2.1 mm x 50 mm column. The column temperature was set at 45ºC. 0.1% of ammonia solution (A) and methanol (B) were the mobile phases used for the analysis and with a flow rate of 0.35 mL/min. The initial proportion was fix at 60% A:40% B, after 10 min was changed to 95% A: 5% B and held for 10 min (minute 20'). At the minute 21 the proportion was settled back to 60% A:40% B and held for 4 minutes. The total run time was 25 minutes. Thirty microliters of a sample solution were injected using methanol as an injection solvent. It was used a negative ionization mode (ESI-) and a cone flow of 200 L/h (N_2), a desolvatation temperature of 550ºC, a desolvatation flow of 800 L/h and a capillary voltage of 2.5 kV. The acquisition was performed in MRM mode. Next, in Table V-7 is shown the collision energy and cone voltage parameters for all the analytes.

Table V-7. Collision energy and cone voltage parameters for PFACs analysed

Compound	Q1 (m/z)	Q3 (m/z)	Collision Energy (V)	Cone voltage (V)
PFBA	213	169	6	20
PFPeA	263	219	8	20
PFHxA	313	269	10	10
PFHpA	363	319	10	10
PFOA	413	369	6	10
PFNA	463	419	10	10
PFDA	513	469	8	30
PFUdA	563	519	8	10
PFDoA	613	569	10	30
PFTrDA	663	619	10	20
PFTeDA	713	669	10	30
PFHxDA	813	769	12	20
PFODA	913	869	14	40
L-PFBS	299	-	-	50
L-PFHxS	399	-	-	10
L-PFOS	499	-	-	50
L-PFDS	599	-	-	50

V.2.2.3 Pharmaceutical products analysis method by UHPLC-MS/MS

UHPLC-MS/MS analysis were performed using a Waters ACQUITY UHPLC H-Class coupled to a Xevo TQ-S micro triple quadrupole mass spectrometer. The suitable separation of the analytes was achieved connecting an ACQUITY UPLC HSS T3 1.8 μm, 2.1 mm x 100 mm column. The column temperature was set at 40ºC. 0.3% of formic acid solution (A) and acetonitrile (B) were the mobile phases used for the analysis and with a flow rate of 0.30 mL/min. The initial proportion was fix at 90% A:10% B and held for 1 min, after 3 min (minute 4') was changed to 10% A: 90% B and held for 2 min (minute 6'). At the minute 8 the proportion was settled back to 90% A:10% B and held for 1 minutes. The total run time was 9 minutes. One microliter of a sample solution was injected. It was used a negative ionization mode (ESI+), a cone flow of 100 L/h (N_2), a desolvatation temperature of 550ºC, a desolvatation flow of 800 L/h and capillary voltage of 2.5 kV. The acquisition was performed in MRM mode. Next, in Table V-8 is shown the collision energy, the cone voltage parameters and the ionization mode used for all the analytes.

Table V-8. Collision energy, cone voltage parameters and ionization mode for pharmaceutical products analysed

Compound		ESI	Q1 (m/z)	Q3 (m/z)	Collision Energy (V)	Cone Voltage (V)
Acetaminophen	MRM 1	+	152	65	30	20
	MRM 2			110	15	
Caffeine	MRM 1	+	195	110	25	15
	MRM 2			138	20	
Enrofloxacin	MRM 1	+	360	316	20	25
	MRM 2			342	25	
Hydrochlorinated Fluoxetine	MRM 1	+	310	148	15	20
	MRM 2			259	20	
Norfloxacin	MRM 1	+	320	189	50	17
	MRM 2			302	20	

V.2.2.4 PAHs analysis method by HRGC-MS

HRGC-MS analysis was performed using an Agilent 6890GC Network Gas Chromatograph coupled to an Agilent 5975B inert XL mass spectrometry detector (MS). The suitable separation of the analytes was achieved connecting a 50% phenyl, 50% dimethylpolysiloxane bonded fused silica capillary column (VF-17ms, 20 m x 0.15 mm x 0.05 µm from Agilent). Helium was employed as a carrier gas at a flow of 1.5 mL/min. The column temperature was initially set at 60ºC and held for 3.1 min, then increased to 195ºC at a rate of 30ºC/min, then increased to 205ºC at a rate of 10ºC/min, after increased to 250ºC at a rate of 20ºC/min and finally increased to 310ºC at a rate of 5ºC/min and held for 5 min. The total run time was 27.85 min. one microliter of sample solutions were injected in split mode (ratio 5:1) at 300ºC and 40 psi. The acquisition was performed in SIM mode. Conditions of the MS were the following: capillary temperature at 300ºC, EI voltage in positive mode with seven time windows, from 3.00 min to 7.00 min analyzing the m/z of 128 for Na, from 7.00 min to 8.50 min analyzing the m/z of 152, 153 and 166 for Acy, Acp and Flu respectively, from 8.50 min to 10.00 min analyzing the m/z from of 178 for Phen and Ant, from 10.00 min to 12.00 min analyzing the m/z of 202 for Flt and Pyr, from 12.00 min to 14.50 min analyzing the m/z from of 228 for BaA and Cry, from 14.50 min to 18.00 min analyzing the m/z from of 252 for BbF, BkF and BaP and from 18.00 min to 27.00 min analyzing the m/z from of 276 for BghiP, DiBahA and IP.

V.2.2.5 PCBs analysis method by HRGC-ECD

GC-ECD analysis were performed using an Agilent Network Gas Chromatograph coupled to an electron capture detector (ECD). The suitable separation of the analytes was achieved connecting a 5% phenyl methyl siloxane column (HP5 30.0 m x 320 µm x 0.25 µm). Helium was employed as a carrier gas at a flow of 3.7 mL/min. The column temperature was initially set at 160ºC and held for 1 min, then increased to 260ºC at a rate of 2.5ºC/min and held for 1 min and

finally increased to 280ºC at a rate of 10ºC/min and held for 1 min. The total run time was 45 min. Two microliters of sample solutions were injected in splitless mode at 250ºC and 21.76 psi. Conditions of the ECD were the following: temperature at 350ºC and make up flow (N2) at 45 mL/min.

V.2.2.6 Oasis HLB cartridges analysis method by UHPLC-QTOF

UHPLC-QTOF analysis were performed using a SCIEX UHPLC Exion AD coupled to a SCIEX X500R QTOF (quadrupole-time of flight) with a SCIEX OS and Marker View software. The suitable separation of the analytes was achieved connecting a LUNA OMEGA POLAR C18 1.6 μm, 2.1 mm x 100 mm column. The column temperature was set at 40ºC. 0.1% of formic acid and 4 mM of acetic acid solution (A) and acetonitrile (B) were the mobile phases used for the analysis and with a flow rate of 0.40 mL/min. The initial proportion was fix at 95% A:5% B and held for 1 min, after 11.5 min (minute 12.5') was changed to 0% A: 100% B and held for 3.5 min (minute 16'). At the minute 16.1 the proportion was settled back to 95% A:5% B and held for 3.9 minutes. The total run time was 20 minutes. Three microliters of a sample solution were injected using methanol as an injection solvent. It was used a positive ionization mode (ESI+), a cone flow of 100 L/h (N_2), a desolvatation temperature of 550ºC, a desolvatation flow of 800 L/h and capillary voltage of 2.5 kV. The cone voltage was set up at 35 V with a collision energy of 15 V.

V.2.3 Experimental procedure

V.2.3.1 Elution of Oasis HLB cartridges

To study the elution process of the five families of pollutants chosen a laboratory scale procedure was carried out. 0.1 mL of **SS-Mix** in methanol was dissolved in 500 mL of synthetic seawater to mimic as much as possible the conditions to which the Oasis HLB cartridge was subjected during the BWR 2015. The use of synthetic seawater will allow us to detect the possible matrix effect due to the sea salts during the experiments. The 500 mL sample solution was stirred to solve the analytes.

Before loading the sample solution into the Oasis HLB cartridge, it has to be conditioned and equilibrated to activate the solid phase of inside. To do that, and with the help of a glass syringe, 5 mL of n-hexane followed by 5 mL of ACN and finally 5 mL of methanol was circulated through the cartridge. After that, the cartridge was dried circulating air through it. The sample solution was load into the HLB cartridge with a syringe and the solution that was not adsorbed into the cartridge stationary phase was collected in a glass container. To finish the loading step, 10 mL of MilliQ water were circulated through the cartridge to eliminate possible sea salt particles which could cause problems once the cartridge dries.

For the elution of the analytes from the cartridge, at first place, a bibliographic research has been done to determine which are the recommendable solvents for the elution of the pollutants chosen[16]. Then, the following procedure was applied: 2 mL of methanol + 2 mL of acetonitrile + 2 mL x 2 of n-hexane were circulated through the cartridge in this order. As can be seen, for the elution of the analytes, the descendent polarity of the solvents was followed. The three solvents were collected in different glass containers to then, be injected. In all cases, the amount of pollutant remained in glass container and in aqueous phase were determined by the addition of n-hexane to achieve the extraction.

Since the families of interest were analysed using techniques such as HRGC or UHPLC and the objective of the procedure was to determine the amount of pollutants in each solvent (MeOH, ACN and n-hexane) to optimize the elution, the extracts were manipulated in order to adapt them to the instrument. Thus, MeOH and ACN extracts, previously analysed by UHPLC, were evaporated and reconstituted in n-hexane. The same happens with the n-hexane extract, the n-hexane, after the injection in the HRGC, was evaporated by N_2 current and reconstituted in MeOH to be able to inject it in the UHPLC.

Since the analytes were extracted using 2 mL of each organic solvent, another standard solution with the analytes at the appropriated concentration were prepared (**SS-Mix-0**). The mixed standard solution used for the recovery calculation has all the analytes at the following concentrations: 0.05 µg/mL of phthalates, 0.01 µg/mL of PFACs, 0.05 µg/mL of pharmaceutical products, 0.05 µg/mL of PAHs and 0.04 µg/mL of PCBs.

V.2.3.2 Elution of Oasis HLB cartridges used during the BWR

Eighteen Oasis HLB cartridges used during the BWR were eluted in order to analyse the pollutants retained. The following figure (see Figure V-2) shows the location of the samples eluted. A table is also presented (see Table V-9) with the exact locations where the samples were collected.

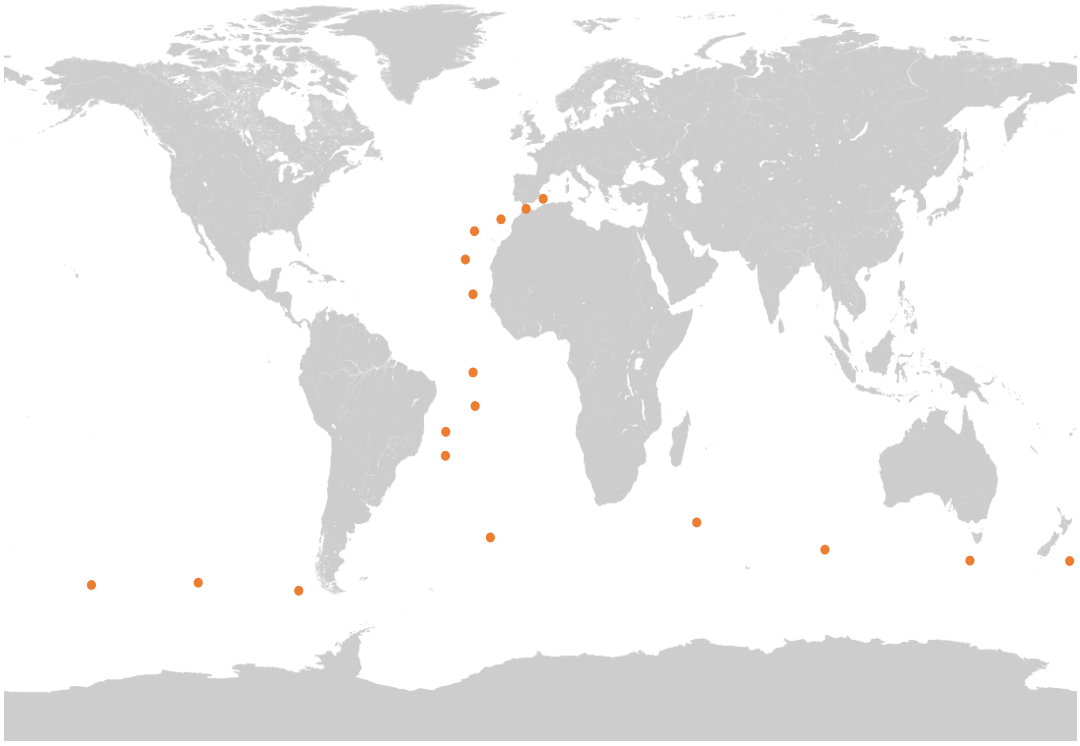

Figure V-2. Locations of the eighteen samples selected from the BWR 2015 to be analysed. Elution of pollutants retained in the HLB cartridge

Table V-9. BWR 2015 samples references, latitude-longitude and oceans where the HLB cartridge samples were collected

Reference	Latitude	Longitude	Ocean
5	34.7273	-10.8482	Atlantic
21	-26.2432	-34.5878	Atlantic
23	-32.2928	-31.6100	Atlantic
28	-43.5988	1.5920	Atlantic
36	-40.4578	52.5985	Indian
41	-42.4705	89.7912	Indian
44	-44.7021	113.1112	Indian
45	-45.6068	119.8872	Indian
48	-51.0833	146.6571	Pacific
52	-53.9912	-174.7275	Pacific
58	-52.0083	-121.9211	Pacific
63	-56.0454	-77.6918	Atlantic
72	-19.8272	-25.3792	Atlantic
81	10.9798	-31.2038	Atlantic
83	18.7358	-30.2214	Atlantic
88	33.3236	-16.572	Atlantic
91	35.7292	-6.2242	Mediterranean
93	37.6769	-0.2079	Mediterranean

As can be observed in the figure and table, two samples from the Mediterranean Sea, nine samples from the Atlantic Ocean, four samples from the Indic Ocean and three samples from the Pacific Ocean were chosen to obtain a representative distribution and a global information.

The elution of the Oasis HLB cartridges was performed using 2 mL of MeOH as organic solvent. With the elution of these cartridges a non-target analysis was pretended to perform (Analysis of Unknowns). Methanol is the organic solvent chosen since a liquid chromatographic instrument was used and it was deduced that would be the optimal to achieve a higher extraction. The extracts were directly injected in a UHPLC-QTOF.

V.3 Results and Discussion

The method development process has been divided in two main sections:

a) The detection of all the analytes shown in the pollutant's mixture (phthalates, perfluorinated compounds, pharmaceutical products, PAHs and PCBs). The **SS-Mix-0** solution containing all the pollutants has been injected in the respective chromatographic instruments for the analysis of each family of pollutants (see the experimental section). With that, the interferences study has been performed and the analytes detection has been optimized. For all the pollutants analysed, an experimental quantification limit of 1% has been established.

b) Elution method optimization. As has been mentioned in the experimental procedure section, MeOH, ACN and n-hexane have been the solvents chosen for the elution of the pollutants from the HLB cartridge. According to bibliographic research[15–17] these solvents are the most recommendable for the elution of all the pollutants families. After the elution, three fractions of eluate with the analytes of interest have been obtained. The objective is then, to determine which solvent or combination of solvents is the suitable for the elution of all the analytes.

Thus, each fraction has been analysed by chromatography and the recoveries have been calculated. The distribution of the pollutants among the different eluates has been analysed. For each pollutant, the percentage present in aqueous phase, in glass container and in the solvents has been quantified.

V.3.1 Elution of Phthalates

The analysis of this family of pollutants has been performed by UHPLC-MSMS. The method used was previously developed by the IQS Chromatography Section and a precolumn was added during the analysis since it was noticed that helps avoiding some contamination problems. The extended method development and optimization is found in bibliography[33].

Before starting the phthalates analysis, the interferences study has been performed. To do that, the solvent extracts used for the cartridge conditioning have been injected in the instrument. Some interferences have been detected with the MeOH extract injection in contrast with the ACN and hexane extracts where no interferences have been detected. A comparative figure is presented with two chromatograms, the MeOH extract and a 0.05 µg/mL phthalates standard solution in order to show the interferences (see Figure V-3). Moreover, a table with the phthalate's elution order and respective retention times is presented (see Table V-10).

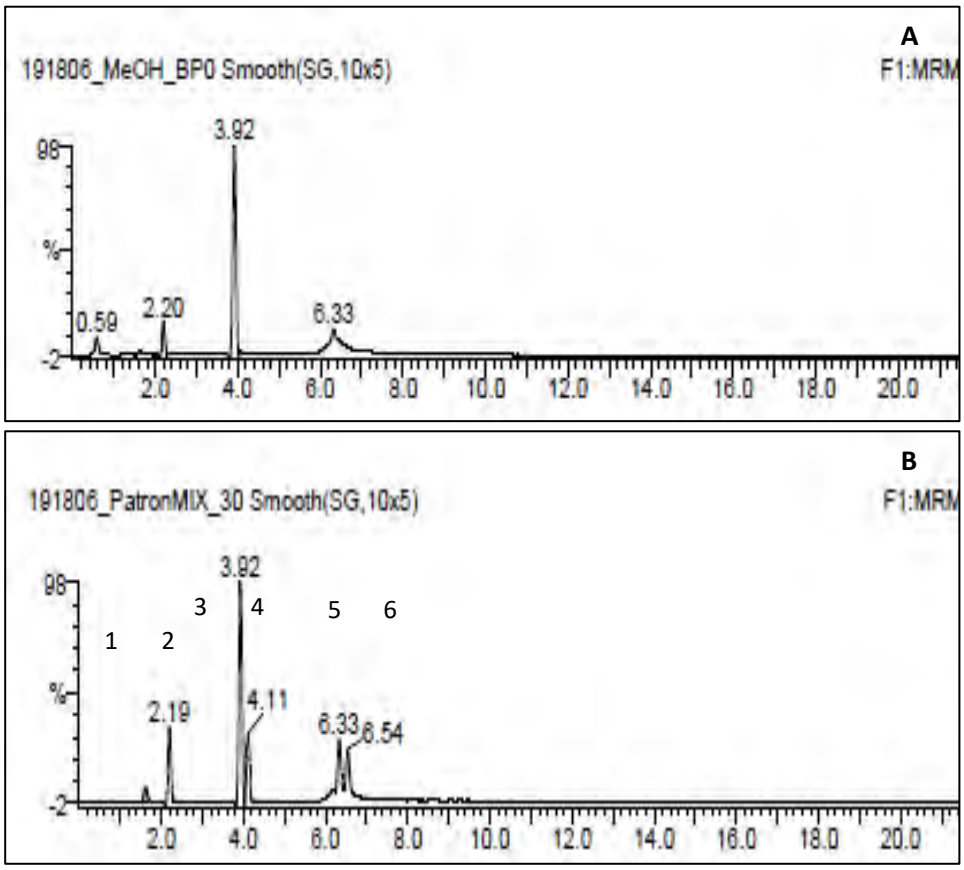

Figure V-3. Phthalates Interferences analysis. **A)** Chromatogram corresponding to MeOH extract after the cartridge conditioning. **B)** Chromatogram corresponding to a 0.05 µg/mL Phthalates standard solution

Table V-10. Elution order and retention time of the six phthalates

	Name	Retention time (min)
1	DMP	1.62
2	DEP	2.19
3	DBP	3.92
4	BBP	4.11
5	DEHP	6.33
6	DOP	6.54

In both figure and table presented above it can be observed the interferences detected during the blank's analysis. Two peaks appear in both chromatograms at the same retention time making impossible the recovery calculation. It is known that phthalates are commonly used as plasticizers, so although a precolumn was used to analyse this family of pollutants to avoid interferences it is not surprising found cross-contamination since during the laboratory procedure or in the proper UHPLC instrument plastic pieces are present.

Thus, only four of the six phthalates have been analysed during the following experiences. The two phthalates affected by the interferences are dibutyl phthalate with a retention time of 3.92 minutes and bis(2-ethylhexyl) phthalate with a retention time of 6.33 minutes. It is known that these two compounds, DBP and DEHP, are the most commonly used as plasticizers in PVC products like medical or analytical instruments tubing, toys or medical bags[35].

Once the interferences have been detected it can proceed with the phthalate's elution analysis. To corroborate the total retention of the phthalates on the cartridge stationary phase, the aqueous phase recovered after the cartridge loading and the glass container have been analysed. The following table (see Table V-11) shows the recovery values obtained for both fractions.

Table V-11. Recovery values of phthalates in the glass container and the aqueous phase. Three replicates of each experience have been done

Name	Glass container	Aqueous phase
DMP	-	-
DEP	-	18 ± 1
BBP	-	-
DOP	-	-

As can be observed in the Table V-11 only one of the analytes has been found in the aqueous phase (DEP), in a concentration of 20% approximately. This indicates that DEP has a lower affinity for the cartridge stationary phase than the rest of the analytes. It also has to take into account that there are two analytes which could not be detected.

It is determined then that almost all the pollutant load into the cartridge has been retained in the stationary phase. Thus, the phthalates elution has been performed and three solvents with different polarities have been used, MeOH, ACN and hexane. The figure presented below shows the recovery values obtained for the elution using MeOH and ACN as solvents. The use of hexane is not presented since no phthalates have been detected with this solvent (values under the experimental quantification limit). The differences in terms of polarity between the hexane and this pollutant can explain this fact.

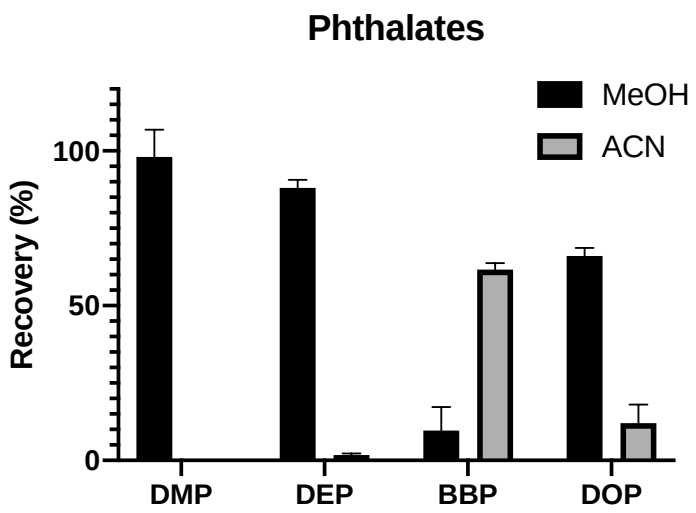

Figure V-4. Recovery values of phthalates in MeOH and ACN. Three replicates of each experience have been done

In Figure V-4 it can be observed that for all the analytes, the recovery values are above 70%. Three of the analytes, DMP, DEP and DOP have been eluted using MeOH as a solvent in contrast with BBP where higher recoveries have been obtained using ACN as a solvent. Analysing in more detail each congener, it can be said that for DMP the 100% of the analyte has been eluted using methanol. In the case of DEP, around 80% has been eluted with methanol but it is worth to remember that a 20% was remained solved in the aqueous phase so the mass balance is achieved. For the complete elution of BBP and DOP the use of first methanol and then ACN was needed. The sum of the recovery values obtained using both solvents results in a recovery of almost a 100% for the two last analytes.

V.3.2 Elution of PFACs

The analysis of PFACs has been done by UHPLC-MSMS. As in the case of phthalates analysis, the method used was previously developed by the IQS Chromatography Section and it was noticed that an installation of a precolumn was also needed for a proper analysis since it helps avoiding interferences and contamination problems during the blanks analysis[33].

With the initial PFACs standard solution injections, a problem with the UHPLC-MSM was detected. The instrument was not capable to detect those congeners with the highest molecular weights. The MSMS detector was shown a loss of sensibility when high molecular weight analytes were analysed. This is why, the last four PFAC congeners, i.e. the ones with the highest molecular weights (PFTrDA, PFTeDA, PFHxDA and PFODA) were not detected and so, not analysed during the following experiences (see Figure V-5). An instrument calibration should have been done to solve the problem.

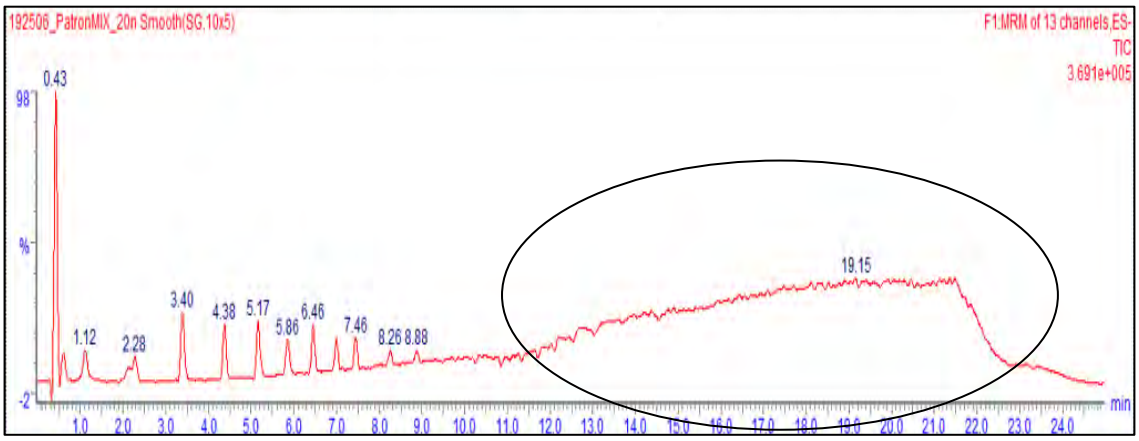

Figure V-5. Chromatogram corresponding to a 0.01 µg/mL PFACs standard solution. Example of the loss of sensibility of the MSM for the detection of high molecular weight PFACs. L-PFBS, L-PFHxS, L-PFOS and L-PFDS do not appear in the UHPLC-MSM chromatograms

At first place, and as in the previous experience, the interferences analysis has been performed. As an example, the comparation of two chromatograms corresponding to the methanol extract and a 0.01 µg/mL PFACs standard solution is shown in Figure V-6. The same chromatogram profile has been obtained when ACN or n-hexane are used. Furthermore, a table with the elution order of PFAC congeners and their retention time is presented (see Table V-12).

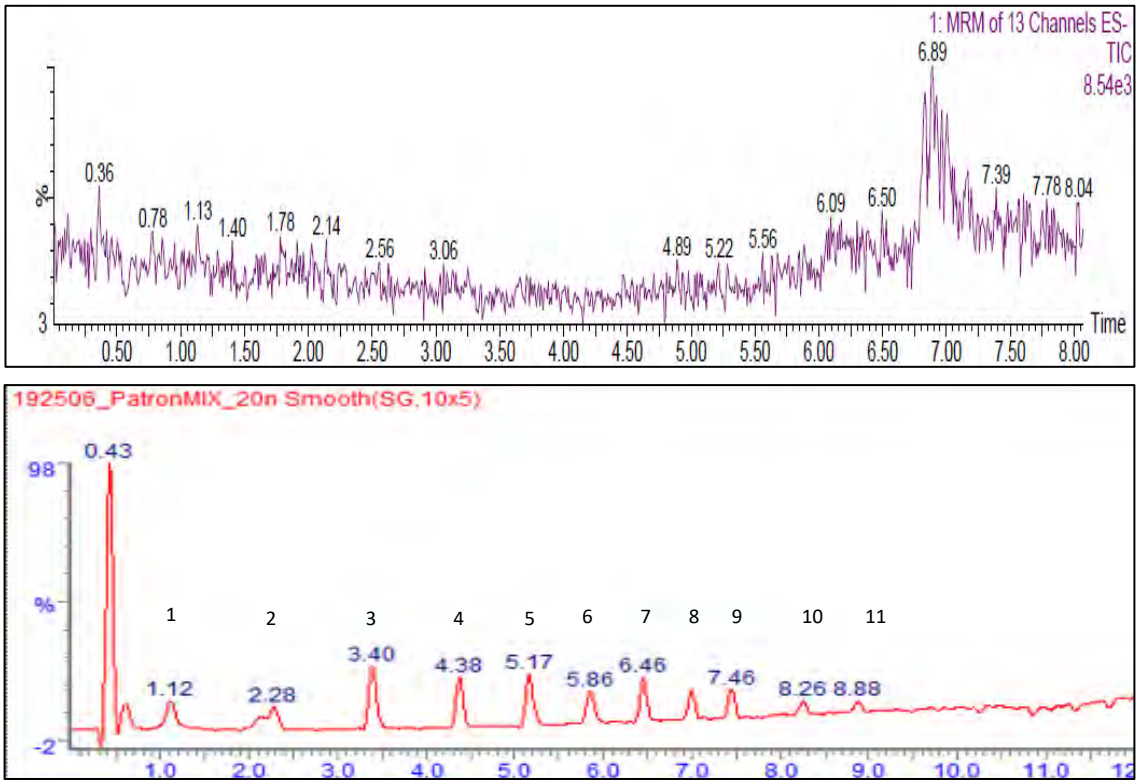

Figure V-6. PFACs Interferences analysis. **A)** Chromatogram corresponding to MeOH extract after the cartridge conditioning. **B)** Chromatogram corresponding to a 0.01 µg/mL PFACs standard solution

Table V-12. Elution order and retention time of PFACs

	Name	Retention time (min)
1	L-PFBS	1.12
2	PFHxA	2.28
3	PFHpA	3.40
4	L_PFHxS	4.38
5	PFOA	5.17
6	PFNA	5.86
7	L-PFOS	6.46
8	PFDA	6.98
9	PFUdA	7.46
10	L-PFDS	8.26
11	PFDoA	8.88

Several interferences have been detected during the analysis of the solvent extracts used for the cartridge conditioning. The two first PFAC congeners (PFBA and PFPeA) were also not analysed since part of them were retained in the precolumn used to avoid contamination problems and also appears in the chromatogram (see Figure V-6) at a pretty low retention times (0.43 and 0.44 minutes). So, during the following experiences, eleven PFAC congers have been studied.

After the interferences study, the PFACs elution analysis has been performed. As in the phthalates study, the aqueous phase and the glass container have been analysed in order to determine the analytes retention in the HLB cartridge. All the PFACs are found under the experimental quantification limit established in the glass container or in the aqueous phase. Thus, it is deduced that the total amount of PFACs load in the HLB cartridge has been retained.

Once the adsorption of the pollutant has been studied it proceed to their elution. MeOH, ACN and n-hexane extracts have been analysed in order to calculate the PFACs recovery values. Figure V-7 shows the recovery values obtained for the elution of PFACs using MeOH as extraction solvent.

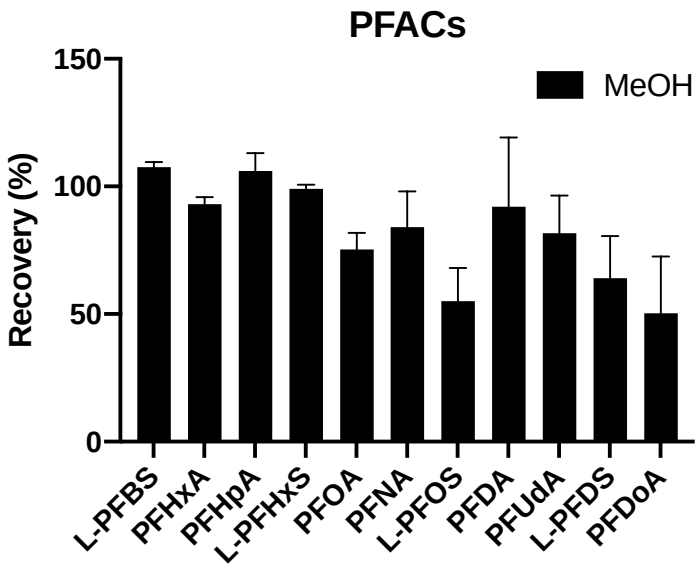

Figure V-7. Recovery values of PFACs in MeOH. Three replicates of each experience have been done

As can be seen in the previous figure, for the lightest perfluorinated acids and sulfonates which are those from four to seven fluor atoms (L-PFBS, PFHxA, PFHpA and L-PFHxS) recovery values around the 100% have been obtained, that is to say that the complete amount of analytes load in the HLB cartridge has been eluted using this solvent. For analytes such as PFOA, PFNA, L-PFOS and PFDA, recovery values between 90 and 70% have been obtained which could consider acceptable values. It also can be observed that for the heaviest analytes, the signal decreases with the increase of fluor atoms in their structure. This it can be explained using the same hypothesis about the detection problems of the instrument, the loss of signal in the UHPLC-MSMS used at high molecular weights.

ACN and n-hexane extracts do not show the presence of any PFAC congener. Thus, it is concluded that for the elution of PFACs from the Oasis HLB cartridge, methanol is most suitable solvent to use.

V.3.3 Elution of Pharmaceutical products

The analysis of pharmaceutical products has been done by UHPLC-MSMS[32]. As in the previous cases, the same procedure has been followed. First, the blanks have been checked in order to avoid contamination problems and detect interferences. After that, the adsorption of the pollutant on the HLB cartridge has been studied. Finally, the pollutant has been eluted to calculate his recovery and to determine which is the most suitable solvent to use.

First, the interferences analysis. For that, the three solvent extracts used for the cartridge conditioning have been analysed. As an example, Figure V-8 shows a chromatogram of a 0.05 µg/mL pharmaceutical products standard solution allowing us to observe the retention times

of the analytes. Moreover, a table where the pharmaceutical products elution order and their respective retention times is presented (see Table V-13).

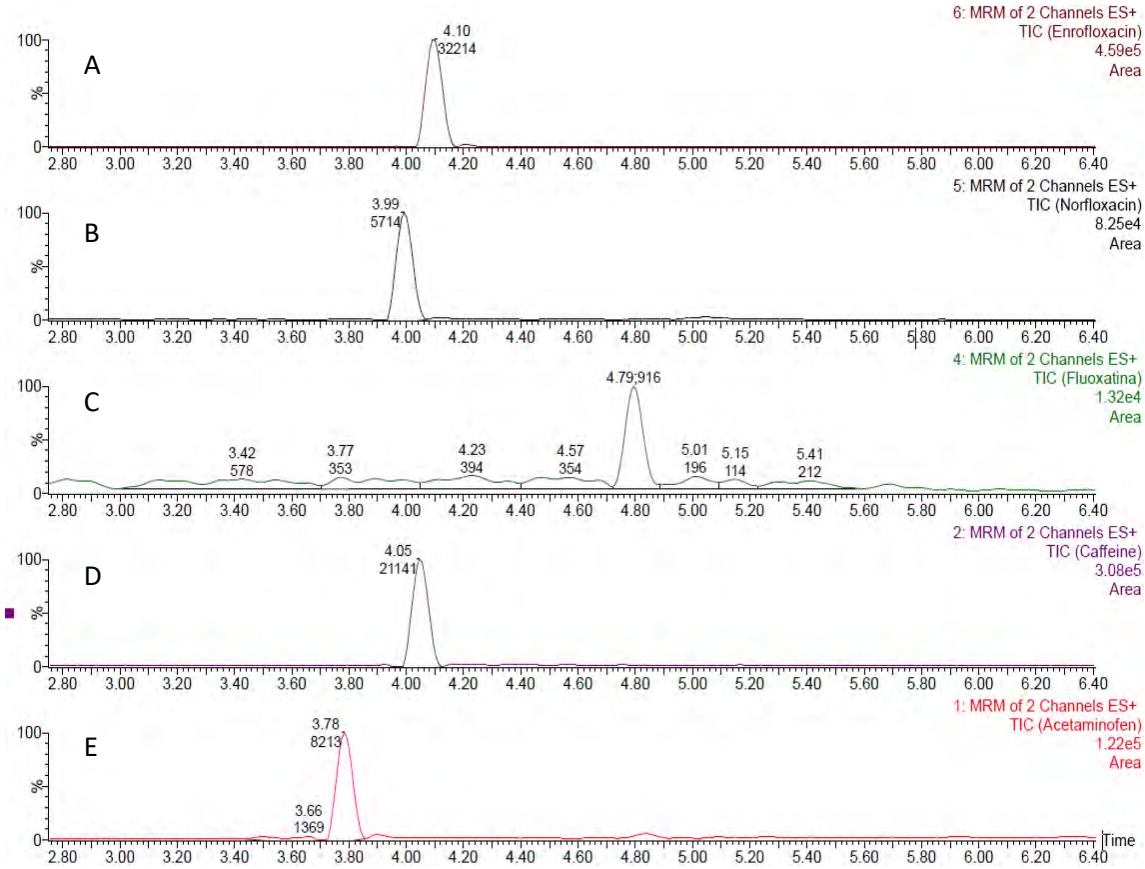

Figure V-8. Pharmaceutical products Interferences analysis. Chromatogram corresponding to a 0.05 µg/mL pharmaceutical products standard solution. **A)** Enrofloxacin MRM extraction. **B)** Norfloxacine MRM extraction. **C)** Fluoxetine MRM extraction. **D)** Caffeine MRM extraction. **E)** Acetaminophen MRM extraction

Table V-13. Elution order and retention time of pharmaceutical products

	Name	Retention time (min)
1	Acetaminophen	3.78
2	Norfloxacine	3.99
3	Caffeine	4.05
4	Enrofloxacin	4.10
5	Fluoxetine	4.79

As can be observed in both Figure V-8 and Table V-13, no interferences has been detected during the analysis so it can be proceeded with the pollutant's elution analysis.

In order to ensure the total adsorption of the pollutant on the cartridge stationary phase, the aqueous phase and the glass container have been analysed. As in the PFACs scenario, the

pharmaceutical products are found under the experimental quantification limit established in the glass container or in the aqueous phase, and that is to say that all the pollutant has been retained in the cartridge stationary phase. So, it is proceeded with the elution.

Figure V-9 shows a graphical representation of the recovery values obtained for the elution of the pharmaceutical products using methanol and ACN as solvents. With the elution with n-hexane no pharmaceutical products have been recovered.

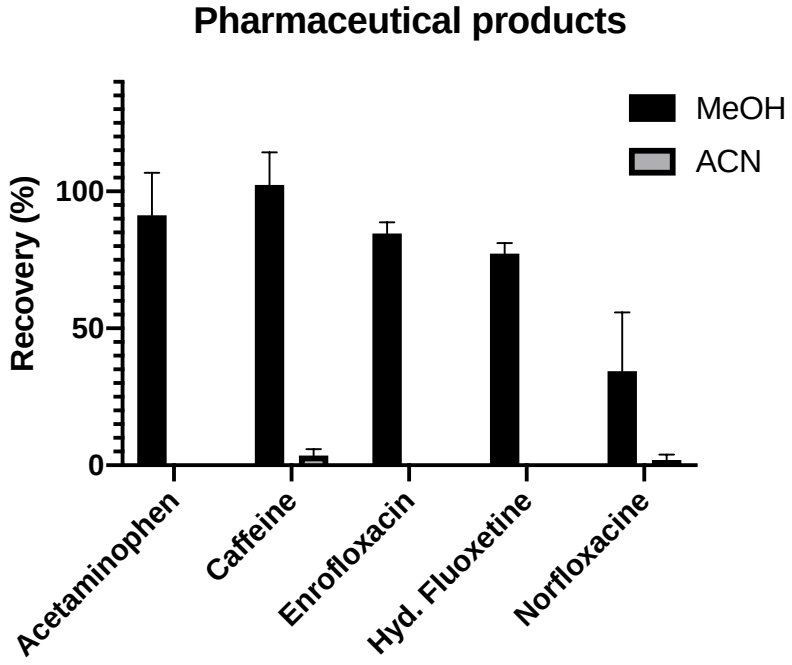

Figure V-9. Recovery values of pharmaceutical products in MeOH and ACN. Three replicates of each experience have been done

As can be seen in Figure V-9, the pharmaceutical products have been eluted using methanol and ACN. The use of ACN for the extraction gives rise very low recovery values since it is used after the elution with methanol with whom the majority of the analytes are eluted. Using ACN only caffeine and norfoxacine have given signal and in concentrations between 2 and 5%. For the acetaminophen, the caffeine, the enrofloxacin and the fluoxetine, concentrations between 90 and 100% have been obtained, this means that all the pollutant adsorbed on the cartridge stationary phase has been eluted using methanol. It also can be observed in the graphic presented that the norfloxacine recovery values decrease in comparison with the rest of the pharmaceutical products analysed until recovery values of 40%. This could be due to the fast degradation of this pharmaceutical product. The fact that the recovery of this product diminish corresponds to a signal loss during the analysis since its degradation starts in the analysis time.

n-Hexane extract has not shown the presence of any analyte which is logical having into account the differences in terms of polarity between the pharmaceutical products and n-hexane.

V.3.4 Elution of PAHs

All the experiences related with the analysis of PAHs have been performed by HRGC-MS. First, blanks have been checked to ensure that no interference problems will appear during the analysis. The chromatograms pertaining to the solvents analysed, to the solvent's extracts used for the cartridge conditioning and the glass containers used do not present any peaks that could cause interferences during the analysis, that is to say, recovery values under the 1%.

Next, a chromatogram corresponding to a PAHs standard solution where the elution order and the retention time of each congener is presented (see Figure V-10 and Table V-14).

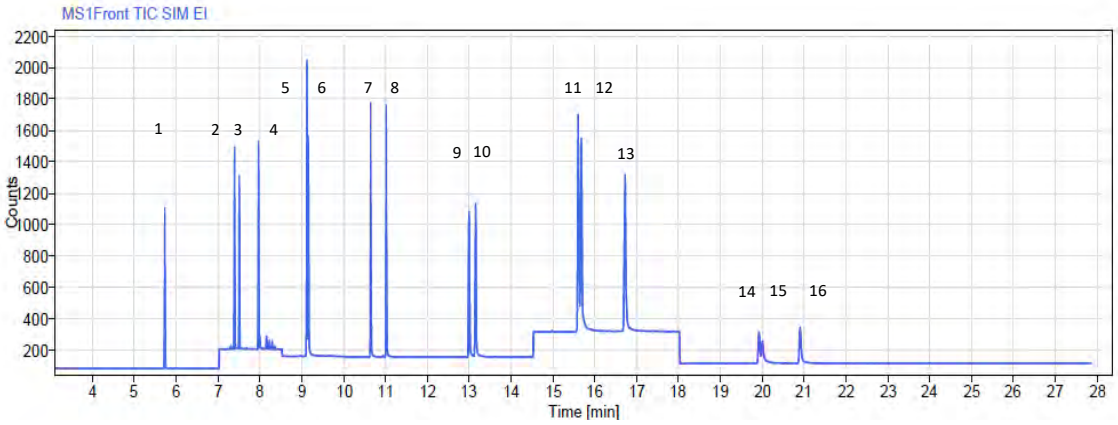

Figure V-10. PAHs 0.05 µg/mL standard solution GC-MS chromatogram

Table V-14. Elution order and retention time of the sixteen PAHs

	Name	Retention time (min)
1	Naphtalene	5.70
2	Acenaphthylene	7.36
3	Anaphtene	7.48
4	Fluorene	7.94
5	Phenanthrene	9.10
6	Anthracene	9.13
7	Fluoranthene	10.63
8	Pyrene	11.00
9	Benz[a]anthracene	12.97
10	Crysene	13.13
11	Benzo[b]fluoranthene	15.58
12	Benzo[k]fluoranthene	15.65
13	Benzo[a]pyrene	16.70
14	Benzo[ghi]perylene	19.90
15	Dibenzo[a,h]anthracene	19.98
16	Indene[1,2,3-cd]pyrene	20.88

To asses if the pollutant has been retained in the cartridge, the amount of PAHs in both glass container and aqueous phase have been analysed by a liquid-liquid extraction using n-hexane (see Table V-15).

Table V-15. Recovery values of PAHs in the glass container and the aqueous phase. Three replicates of each experience have been done

Name	Glass container	Aqueous phase
Naphtalene	2.6 ± 3	-
Acenaphthylene	-	-
Anaphtene	-	-
Fluorene	-	1 ± 2
Phenanthrene	-	-
Anthracene	-	-
Fluoranthene	-	-
Pyrene	-	1 ± 0
Benz[a]anthracene	-	1 ± 0
Crysene	-	1 ± 0
Benzo[b]fluoranthene	-	2 ± 0
Benzo[k]fluoranthene	-	2 ± 0
Benzo[a]pyrene	-	2 ± 0
Benzo[ghi]perylene	-	1 ± 0
Dibenzo[a,h]anthracene	-	-
Indene[1,2,3-cd]pyrene	-	2 ± 2

In the glass container the concentration of all the analytes detected is less than a 1% in almost all of the cases except the naphthalene where the concentration is 3%. From this recovery values, we can conclude that all the analytes, are in the aqueous phase or retained in the HLB cartridge. If we observe the amount of PAHs recovered from the aqueous phase after going through the cartridge, in all the cases the values are in a range from under the experimental detection limit to 2%.

Taking into account that for this type of pollutant, the amount of PAHs in the aqueous phase is under a 2% and the quantity found in the glass container is lower than a 1%, it can be concluded that the remaining amount of PAHs (almost all), has been adsorbed on the stationary phase of the cartridge, equalizing the mass balance.

Therefore, the extraction of this family of pollutants have been carried out. The final goal is to determine which solvent or combination of solvents is the optimal.

The recovery values obtained for the extraction of PAHs using ACN and n-hexane are shown in the Figure V-11.

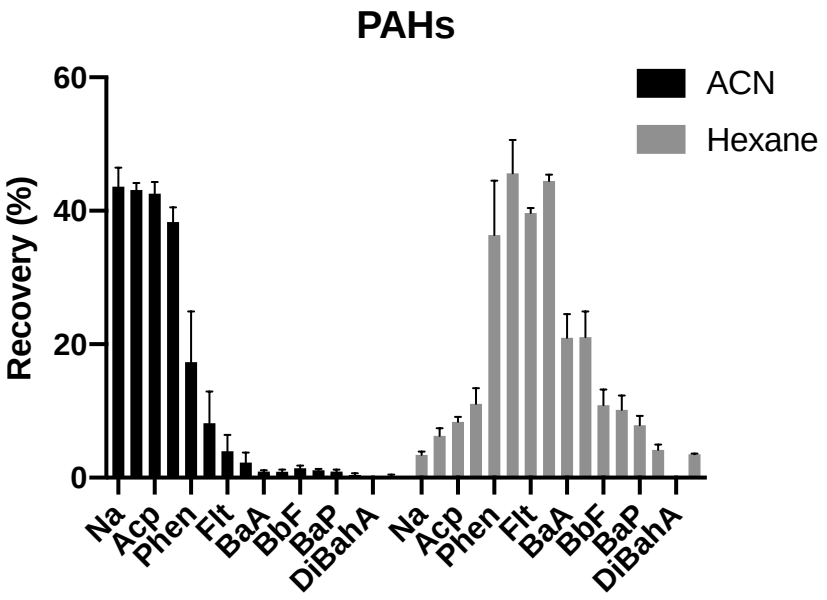

Figure V-11. Recovery values of PAHs in ACN and n-hexane. Three replicates of each experience have been done

In the previous figure, only the recoveries related with ACN and n-hexane fractions are shown since in the MeOH fraction the recovery values of PAHs are under a 0.5%.

As can be seen, using ACN and n-hexane two opposite profiles have been obtained. With ACN the lightest PAHs have been eluted, in contrast, using n-hexane heaviest compounds have been eluted. The differences in the elution using ACN or n-hexane can be explained by the polarity or the K_{ow} property of the analytes. Those more non-polar analytes are eluted with a non-polar solvent such as the n-hexane in contrast with those analytes eluted with ACN which have higher polarity or lower K_{ow} and do not need such a strong non-polar solvent.

Next, a figure is presented (see Figure V-12) where the total amount of PAHs recovered. To do that, the PAHs concentration in the ACN and n-hexane fractions have been taken into account.

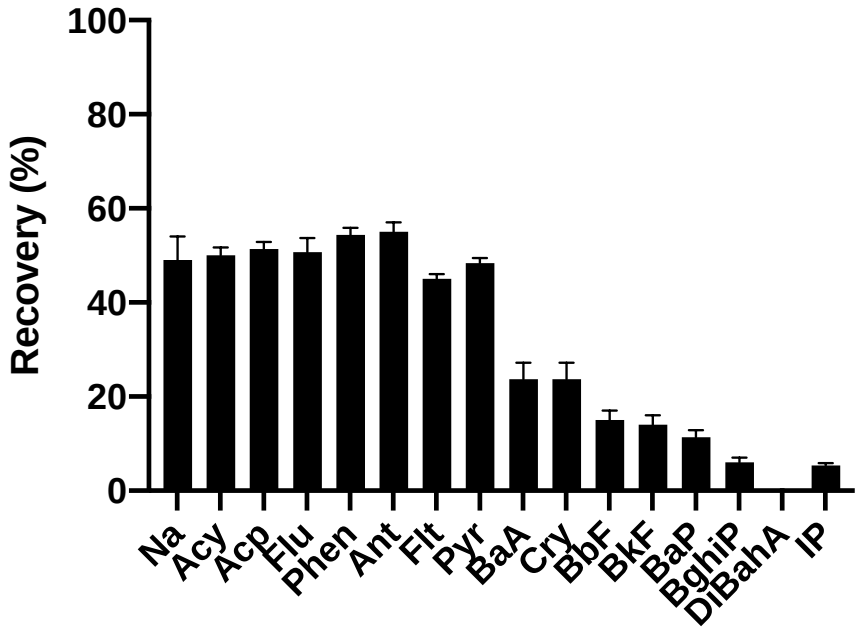

Figure V-12. Recovery values of PAHs in all the phases. Three replicates of each experience have been done

As can be seen in Figure V-12, a maximum of 40-60% of PAHs been recovered. Having into account the concentration obtained during the analysis of the aqueous phase or the glass container, almost the 40% of PAHs have still remained in the HLB cartridge. Moreover, as can be observed, the recovery values for the heaviest PAHs have diminished until values of 30-20%.

Thus, it can be concluded that for this family of pollutants, PAHs, the suitable solvents for their elution are the ACN and the n-hexane. Besides that, no presence of PAHs has been detected in the aqueous phase or the glass container meaning that we have not been able to elute the 100% of the pollutant from the cartridge.

V.3.5 Elution of PCBs

Finally, it is proceeded with the analysis of PCBs. All the experiences related with the analysis of PCBs have been performed by HRGC-ECD.

In this case, in the same way as with the rest of the pollutants the first step is the interferences analysis. Some interferences with the PCB congeners have been detected when the n-hexane conditioning extract has been analysed. The following figure shows two chromatograms corresponding to the n-hexane extract after the cartridge conditioning and a 0.05 ug/mL PCBs standard solution (see Figure V-13). Moreover, a table is presented where the elution order of PCB congeners and their respective retention time can be observed (see Table V-16).

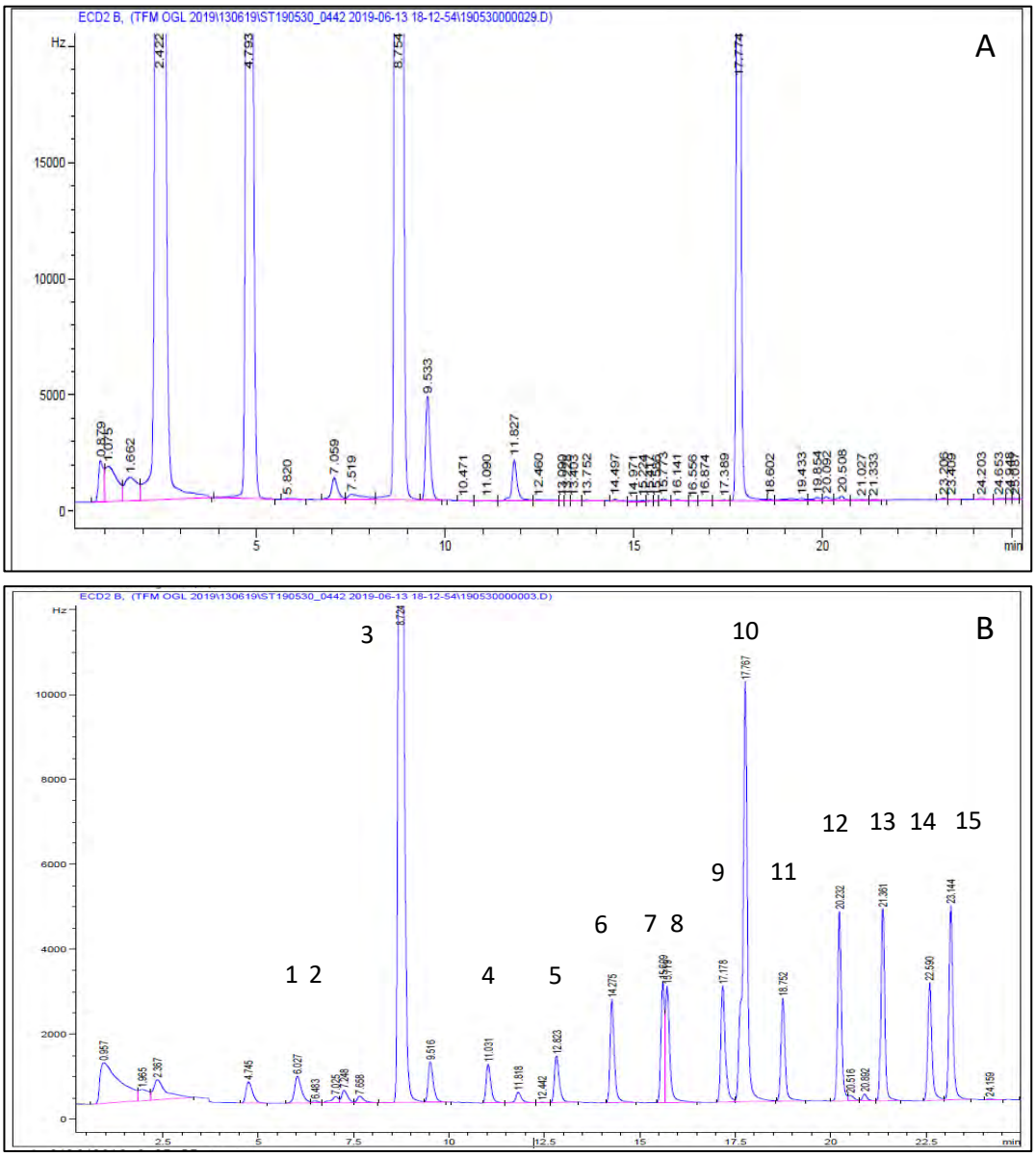

Figure V-13. PCBs Interferences analysis. **A)** Chromatogram corresponding to a n-hexane extract after the cartridge conditioning. **B)** Chromatogram corresponding to a 0.04 µg/mL PCBs standard solution

Table V-16. Elution order and retention time of the fifteen congeners of PCBs

	Name	Retention time (min)
1	PCB 28	6.03
2	PCB 31	6.03
3	PCB 52	8.72
4	PCB 101	11.04
5	PCB 77	12.82
6	PCB 118	14.28
7	PCB 153	15.61
8	PCB 105	15.72
9	PCB 138	17.18
10	PCB 126	17.78
11	PCB 128	18.75
12	PCB 156	20.24
13	PCB 180	21.36
14	PCB 169	22.59
15	PCB 170	23.15

As can be seen in Figure V-13, there are two peaks in the n-hexane chromatogram which have the same time retention of two PCB congeners and therefore will affect to the recovery calculation. Those two peaks affect to PCB 52 with a retention time of 8.72 minutes and PCB 126 with a retention time of 17.78 minutes. Thus, during the next experiences where PCBs have been analysed, PCB 52 and PCB 138 have not been quantified and thirteen of the fifteen PCBs have been taken into account. In addition to these two congeners, which cannot be quantified due to the interferences found, in Figure V-13 and Table V-16 can be observed that the congeners PCB 28 and PCB 31 appears in the chromatogram at the same retention time, 6.03. In the following experiences the peak corresponding to PCB 28 and PCB 31 has been symmetrically divided in order to calculate the area of both.

To continue with the experiences and, as in the rest of the pollutants, the glass container and the aqueous phase have been analysed to ensure that the total amount of PCBs has been retained in the HLB cartridge. Table V-17 shows the recovery values obtained for both glass container and aqueous phase.

Table V-17. Recovery values of PCBs in the glass container and the aqueous phase. Three replicates of each experience have been done

Name	Glass container	Aqueous phase
PCB 28	4 ± 7	-
PCB 31	4 ± 7	-
PCB 101	5 ± 3	7 ± 1
PCB 77	8 ± 3	8 ± 4
PCB 118	4 ± 2	6 ± 1
PCB 153	3 ± 1	7 ± 1
PCB 105	8 ± 3	13 ± 2
PCB 138	3 ± 1	6 ± 0
PCB 128	1 ± 0	6 ± 1
PCB 156	5 ± 1	9 ± 1
PCB 180	2 ± 1	8 ± 1
PCB 169	2 ± 1	9 ± 3
PCB 170	3 ± 0	10 ± 2

The concentration of all congeners detected is under a 10% both in the glass container and in the aqueous phase as can be seen in Table V-17. So, taking this into account, for this type of pollutant, it can be concluded that the remaining amount of PCBs, almost the 90% has been adsorbed on the stationary phase of the cartridge, equalizing the mass balance.

Then, it can proceed to the elution of this family of pollutants from the HLB cartridge. The following figure (see Figure V-14) shows the recovery values obtained for PCBs in ACN and n-hexane extracts.

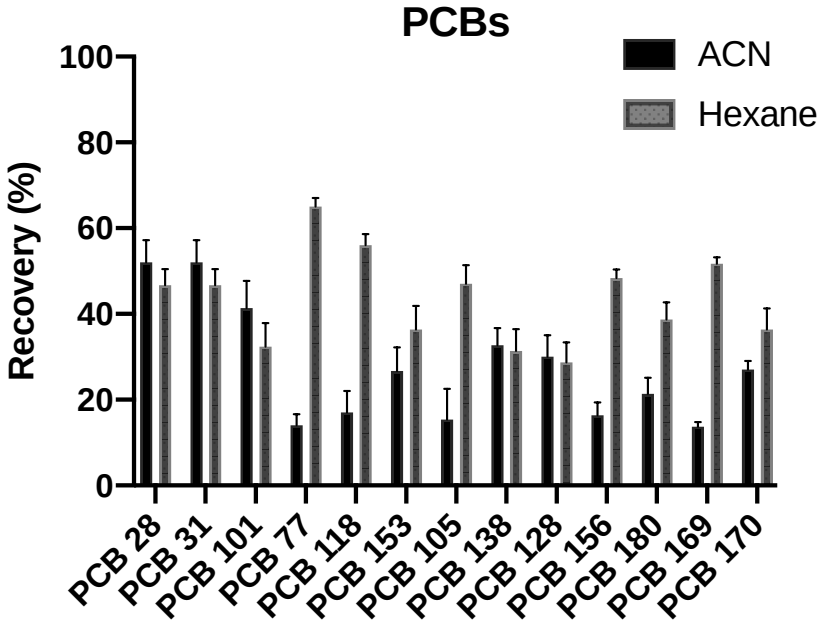

Figure V-14. Recovery values of PAHs in ACN and n-hexane. Three replicates of each experience have been done

As in the PAHs scenario, only ACN and n-hexane extracts recovery values are presented in the figure since during the analysis, in the methanol extract any PCB congener has been detected. The values obtained using ACN as a solvent do not show a clear tendency since recoveries from 20 to 60% have been obtained depending on the congener. Conversely, using n-hexane as extraction solvent recovery values around 50-70% have been obtained which indicates a more controlled elution. The objective of the experiences is to achieve the total elution of the pollutants using the optimal solvent or sequential order of solvents. Thus, the Figure V-15 shows the global recovery values obtained for this family of pollutants.

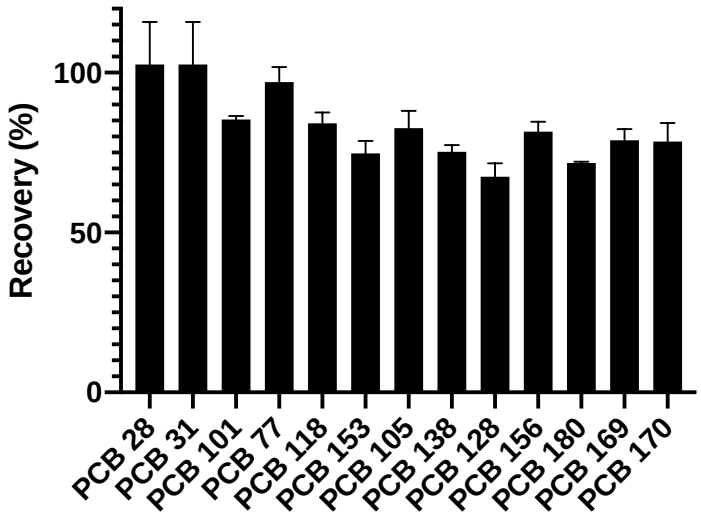

Figure V-15. Global PCBs recovery values of in all the extracts. Three replicates of each experience have been done

Having into account the values obtained, the total amount of PCBs adsorbed on the HLB cartridge stationary phase has been eluted using ACN and n-hexane as solvents. As can be seen the recovery values range from 80% for the heaviest congeners to 100% for the lightest congeners.

As previously described, a solution containing five families of pollutants with diverse properties have been eluted from an HLB cartridge to set an analytical methodology to be applied in the cartridges used during the BWR 2015. From the experiences can be extracted that the total amount of PFACs and PBCs load in the cartridge has been eluted. The recovery values obtained for the phthalates and the pharmaceutical products are higher than a 70% except the case of the norfloxacine. The elution of PAHs has shown recoveries around the 50-60% for the lightest congeners and around the 20-30% for the heaviest congeners, more experiences have to be done in order to achieve the total elution of this family of pollutants. In summary, it can be concluded that the phthalates, PFACs and pharmaceutical products have been eluted using mainly methanol and in some specific analytes using ACN. On the contrary, the PAHs and PCBs have been mainly eluted using n-hexane as organic solvent.

V.3.6 Principal Component Analysis of Oasis HLB cartridges from the BWR 2015

As has been extensively explained in the experimental procedure section, eighteen HLB cartridges used during the sample collection in the BWR 2015 were eluted in order to extract information regard to the pollutants retained in those cartridges (see Table V-9). The elution was performed with 2 mL of methanol since the analysis was performed by UHPLC-QTOF and is a solvent that allows the extraction of a higher number of pollutants families. The chromatographic conditions have been selected since a general method is wanted. The column used is valid for polar and non-polar analytes, a suitable mobile phase for weak and neutral acids is used and with a gradient that allows the elution of compounds with a wide range of lipophilia, which, by working in ESI+, will allow the ionizable compounds analysis (which are the most common). The eighteen samples were injected by triplicate so fifty-four chromatograms were obtained. With this analysis we have pretend to obtain global information about the seawater pollution using a representative number of samples. The UHPLC-QTOF is a technique through with a huge amount of data can be extracted from the injections.

As expected, a huge amount of information was generated during the HLB cartridges analysis. In Figure V-16 a 3D chromatogram example is presented in order to show the number of peaks generated in the injection of only two samples.

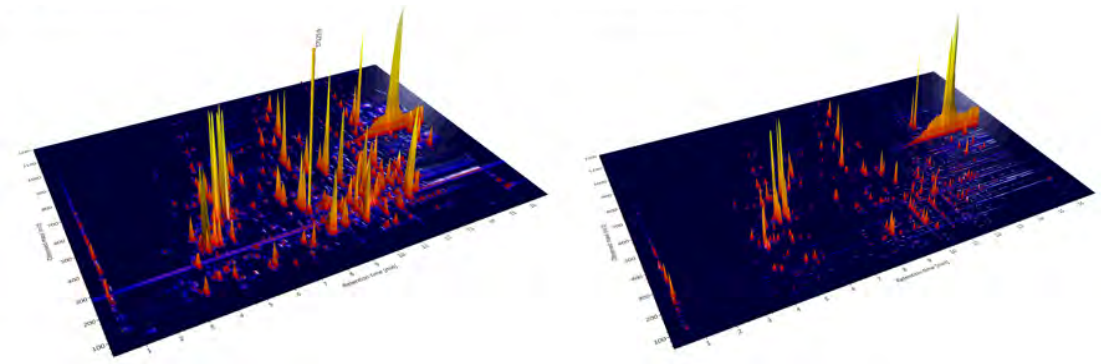

Figure V-16. 3D chromatogram example of HLB cartridge samples from the BWR injected in a UHPLC-QTOF

As can be seen in Figure V-16, the amount of information in each and every injection is huge. So, the assignation of all the peaks from those 3D chromatograms is a long-time work. Moreover, the 3D chromatograms presented corresponds to the injection of two sample and it has to be taken into account that we have injected eighteen samples with three replicates for each one. A more realistic methodology has to be applied to analyse this huge amount of information generated. Thus, the Principal Component Analysis (PCA) has been used.

PCA is a statistical procedure that uses an orthogonal transformation to convert a set of data of possibly correlated variables into a set of values of linearly uncorrelated called principal components. PCA is a statistical method that simplifies the complexity of sample spaces with

many dimensions while retaining its information. The PCA method therefore allows to condense the information provided by multiple variables into only a few components.

To start the generated data analysis contained in the injections, the 5000 most intense peaks in the ESI+ chromatograms of each sample were selected. These peaks were compared among the samples and were selected to follow with the analysis. The combination of all the information regarding the 5000 selected peaks in each of the eighteen samples results in a new data matrix. This new data matrix was used to perform the principal component analysis.

Figure V-17 shows the graphical representation resulting from the PCA analysis where the samples have been classified according to their locations. To obtain a more representative results, the first replicate of each injection (replicate a) has been eliminated since we have detected that provides a variation in the data due to the possible contamination with the previous sample injection. Thus, in the following figure are represented the replicates b and c of the eighteen samples.

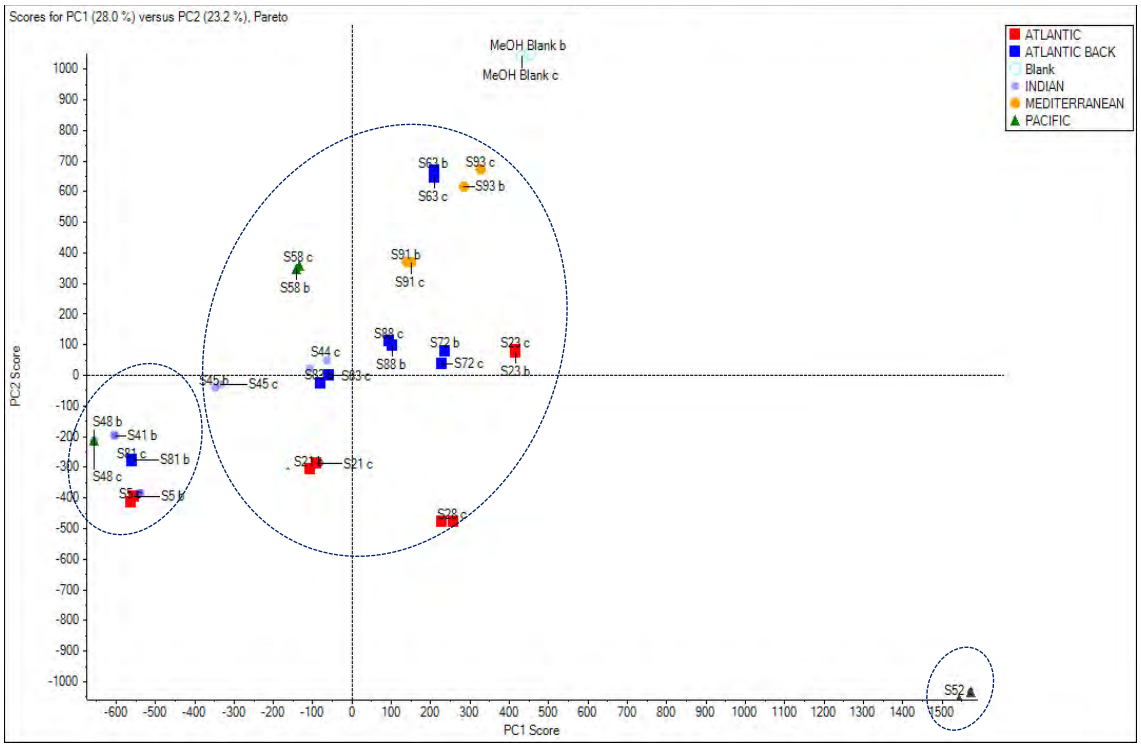

Figure V-17. Graphical representation of the PCA analysis, samples classified according to their location. The numbers in the samples are internal references, the colours used allow to differentiate between oceans

Figure V-17 shows the graphical distribution of two replicates of the eighteen samples in function of two variables, PC1 and PC2 which are the two components that presents the greatest data variability. The samples, as has been mentioned, are classified according their locations. In yellow the samples from the Mediterranean Sea, in red and blue the samples from the Atlantic

Ocean, in green the samples from the Pacific Ocean and finally, in purple the samples from the Indian Ocean.

As can be seen, the two replicates (b and c) for each sample appears in the graphic practically overlapped, which means that do not exist any variability between both of them. So, it can be deduced that the injections have been shown the same peaks validating the method repeatability.

It is also observed in Figure V-17 how the samples can be grouped in three different clusters. The first one, which contains the majority of the samples analysed and it is located in the origin of the graphic. A smaller cluster with four samples (S48, S41, S5 and S81) located in the negative PC1 and PC2 section and the last one with only one isolated sample, S52, appearing in high values of PC1.

It would be logical to think that the samples have to be grouped by oceans. The samples collected in near locations should present similar peaks in the result chromatogram. Therefore, do not have to present a high variability regarding the PC1 and PC2 components. This is what happens when the first cluster is analysed. The samples corresponding to the Mediterranean Sea are found and with pretty similar values of PC1 and PC2. The same happens with almost all the samples corresponding to the Atlantic Ocean (blue and red samples).

Regarding to the second cluster, samples collected in the Atlantic, Pacific and Indian oceans can be found. The position of this samples in the graphic shows that there are in their chromatograms specific peaks that cause a variability in their principal components which makes them pretty different than the rest. The next step to determine why this samples have a different behaviour is, first study the 5000 peaks used for the PCA and analyse which of them cause the variability in the data and then determine at which analyte corresponds that peaks.

Finally, an isolated sample can be observed in the graphic correspondent to a Pacific Ocean sample, S52. This sample shows a totally different behaviour than the rest of the samples. It can be deduced that the injection of this sample results in a chromatogram with a very different aspect. As in the case of the second cluster, to determine what are these differences due to the peaks used to perform the data matrix have to be studied in order to found which of them cause such a huge variability. Then, these peaks have to be analysed in order to deduce to what pollutants correspond.

In the previous figure, the samples are grouped, as has been mentioned, in function of their location, that is to say the ocean where they were collected. Besides that, the samples have been grouped having into account other properties in order to study if them can also explain their position in the PCA graphic. Thus, the samples have been also studied regarding having as a reference the equator.

Figure V-18 shows the PCA graphic obtained when the position of the samples regarding the equator have been taken account.

Figure V-18. Graphical representation of the PCA analysis, samples classified according to their location respect the equator. (-1) are samples located under the equator, (1) are samples located over the equator. The numbers in the samples are internal references

Figure V-18 shows as in the previous case, the graphical distribution of two replicates of the eighteen samples in function of the variables, PC1 and PC2. The samples are classified according their position regarding the equator. In red (-1) the samples which position is located under the equator line. In green (1) the samples which position is located above the equator line.

As can be seen, all the samples (except for one) collected in locations above the equator line show a positive tendency in both components PC1 and PC2. The peaks of these samples present pretty similar variability and besides that are found in the graphic near to the origin regarding PC1, which is the principal component for the variability explanation.

In contrast, the samples corresponding to locations under the equator line, as can be seen in the figure, any data aggrupation has been formed. Thus, in this analysis where the samples are classified in function of their position regarding the equator no global tendency can be used to explain all the samples behaviour.

All the samples were also classified in function of the existent superficial oceanic currents in the collecting locations. A new PCA graphic have been obtained and it is presented in Figure V-19.

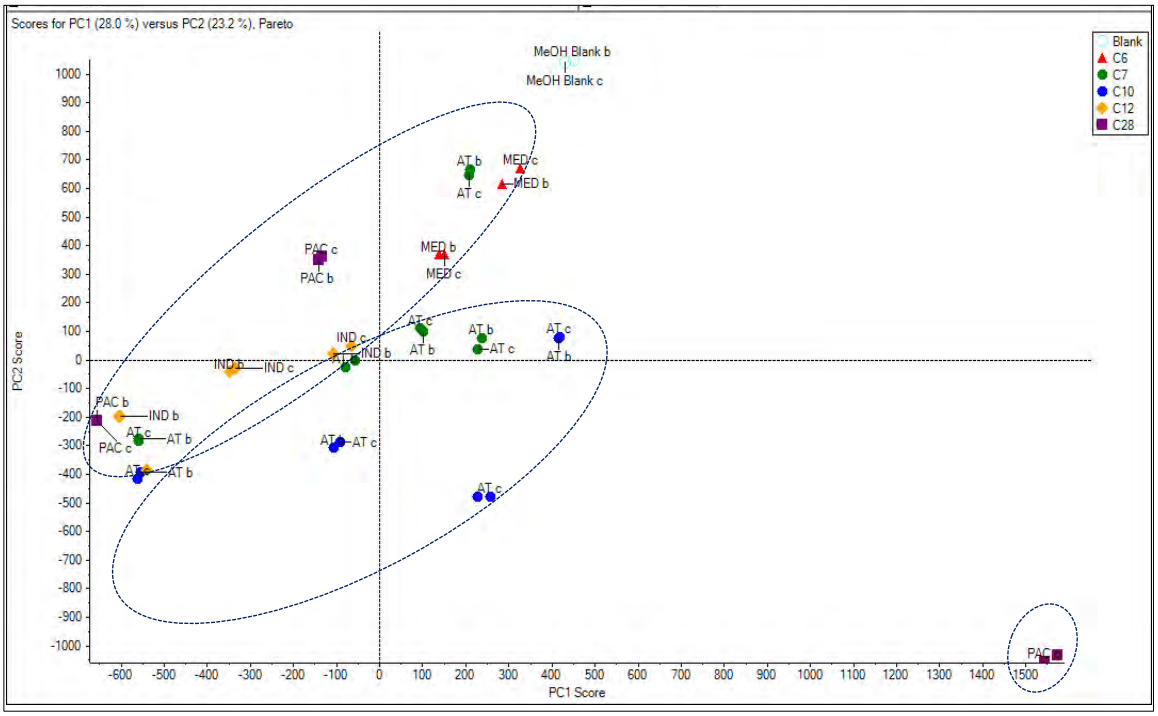

Figure V-19. Graphical representation of the PCA analysis, samples classified according to the superficial oceanic currents in the location where were collected. The colours used allow to differentiate between oceans

Figure V-19 shows the graphical distribution of two replicates of the eighteen samples in function of the variables, PC1 and PC2. The samples are classified according the superficial oceanic currents where the samples were collected. Each current in the graphic is identified with a number pertaining to its abbreviation. The name of each current is presented in more detail in Table V-18. Moreover, the samples in the graphic are classified by colours. In red the samples from the Mediterranean Sea, in green and blue the samples from the Atlantic Ocean, in purple the samples from the Pacific Ocean and finally, in yellow the samples from the Indian Ocean.

Table V-18. Oceanic current names, currents location and their correspondent abbreviation[36]

Current Abbreviation	Current Name	Current Ocean
C6	Canary current	Mediterranean Sea
C7	North-equatorial current	Atlantic Ocean
C10	Brazilian current	Atlantic Ocean
C12	Circumpolar current	Indian Ocean
C28	“Cabo de Hornos” current	Pacific Ocean

As is observed in the last PCA graphic, the samples can be grouped in two main clusters. In this analysis the samples have been grouped in function of if the currents where the samples were collected are warm or cold. C6, C12 and C28 corresponds to cold superficial currents and

are those present in the Mediterranean Sea, the Indian Ocean and the Pacific Ocean. C7 and C10 are those corresponding to warm superficial currents and are present in the Atlantic Ocean.

Therefore, those seawater samples affected by warm superficial currents present a common data variability. The analytes present in the sample has similar behaviour. The same happens with the samples affected by the cold superficial currents. The next step, as in the other analysis, is to determine which are the peaks in the corresponding chromatograms that causes the differences in terms of variability in the PC1 and PC2 components between those samples collected in cold or worm seawater and then, assign to which analyte or family of pollutants corresponds.

V.4 Concluding remarks

In this chapter the adsorption and elution process of different pollutants retained in the same solid phase extraction cartridges used during the BWR 2015 has been studied. Five families of pollutants with diverse behaviours have been used to do the experiences. In addition to that, several HLB cartridges from different locations used in the BWR 2015 has been eluted.

To reach this aim, a laboratory proof of concept in order to test the affinity of these pollutants to the HLB cartridge stationary phase has been developed. Moreover, the elution process optimization to achieve the highest recoveries using three organic solvents with different polarities has been studied. Six phthalates congeners, seventeen PFAC congeners, five pharmaceutical products, sixteen PAHs and fifteen PCB are the pollutants used during the procedure.

The HLB cartridge has been load with a standard solution containing a mix of all the pollutants. After that, three solvents, MeOH, ACN and n-hexane have been used to achieve their elution. The aim of this experiences is to determine what solvent or order of solvents is the most suitable for the elution of the total amount of each family.

During the phthalates analysis several interferences with the methanol used for the cartridge conditioning have been found. The methanol chromatogram shows two interferences with the DBP (3.92 minutes) and DEHP (6.33 minutes) congeners. These two congeners therefore have not been quantified. The total amount of the rest of phthalates retained in the cartridge stationary phase have been eluted using methanol and acetonitrile as solvents.

During the PFACs analysis no interferences with the solvents used during the cartridge conditioning have been found. Moreover, the total amount of pollutant load in the cartridge has been adsorbed in the stationary phase. A problem with the UHPLC-MSMS detector has been noticed during this analysis. The MSMS detector was shown a loss of sensibility when high molecular weight analytes were analysed. This is why, the last four PFAC congeners (PFTrA, PFTeA, PFHxA and PFDoA) were not detected and so, not analysed during the following experiences. The total amount of the rest of PFACs retained in the cartridge stationary phase have been eluted using methanol as solvent except for the PFUdDA, L-PFDS and PFDOA where the signal decrease due to the detector loss of sensibility.

No interferences during the pharmaceutical products analysis have been detected. In addition to that, any analyte has been found in the glass container or the aqueous phase, so the total amount load in the cartridge has been retained. The pharmaceutical products have been eluted using methanol and ACN. Using ACN only caffeine and norfoxacine have given signal and in concentrations between 2 and 5%. For the acetaminophen, the caffeine, the enrofloxacin and the fluoxetine, recoveries between 90 and 100% have been obtained. Norfloxacine recovery values decrease in comparison with the rest of the pharmaceutical products analysed until recovery values of 40%. This is due to the fast degradation of this pharmaceutical product.

For the elution of PAHs only the 50-60% of the total amount of PAHs load in the HLB cartridge has been eluted. Using ACN as a solvent mainly the lightest analytes has been eluted. The rest of analytes, the heaviest ones, have been eluted using n-hexane but in any of the cases recoveries of 60% have been exceeded.

Finally, the PCBs analysis have been performed. During the analysis several interferences with the n-hexane extract used during the cartridge conditioning have been detected. Peaks at 8.72 minutes and 17.78 minutes have been observed in the n-hexane corresponding with the elution times of PCB 52 and PCB 126, so these two congeners have not been quantified during the experience. For the rest of the congeners, the total amount load in the cartridge have been eluted using acetonitrile and n-hexane.

To end, eighteen Oasis HLB cartridges used to collect samples during the BWR 2015 have been eluted. 2 mL of methanol have been used for the elution and the extracts have been injected in a UHPLC-QTOF instrument. Two samples from the Mediterranean Sea, nine samples from the Atlantic Ocean, four samples from the Indic Ocean and three samples from the Pacific Ocean have been chosen to obtain a representative distribution and a global information.

The Principal Components Analysis have been the methodology used for the data analysis since a huge amount of information have been obtained for all the injections. The variability is the parameter used in the PCA to find an aggrupation between the samples. The location where the samples were collected, their position regarding the equator and the superficial oceanic currents have been used as the three common characteristics for all the samples to explain the aggrupation. It has been determined that the samples are grouped according the ocean where they were collected. Furthermore, the samples can be also grouped according the superficial oceanic currents of their locations.

The conclusions extracted from the PCA analysis generate a record of the potential of this thesis. The amount of information that can be extracted in future works from the samples collected during the BWR 2015 is limitless.

V.5 References

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Chapter VI.

Conclusions

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Conclusions

In this thesis, the role of microplastics suspended in seawater has been studied. This study has intended to understand the current environmental situation in a global way. The effect of microplastics besides the organic pollutants suspended in seawater has been described and the following conclusions have been extracted from the study:

1. An improving sampling methodology (COA device) has been developed and optimized with the collaboration of the Sailing Technologies Group. A filtration system (filters of 500 µm, 50 µm and 5 µm) has been selected to collect superficial microparticles. Furthermore, SPE (Oasis HLB Plus from Waters) has been selected for the retention of the pollutants dissolved in seawater.

2. Through the collaboration of our research group, GEMAT, and the FNOB the device has been installed in one of the racing boats of the BWR 2015 so more than hundred samples of different locations along all the oceans could be taken. The COA device has been capable to collect microparticles in suspension (microplastics and microplankton) and pollutants solved in seawater.

3. Microplastic particles such as polyethylene, polypropylene, polyacetal or cellulose have been found in the Mediterranean Sea. In the Atlantic Ocean, microplastics such as polyethylene terephthalate, polyethylene and polypropylene have been also detected. From the analysis of the filters from the Indian Ocean, microparticles corresponding to microplastics but also microplankton or proteins have been identified. The majority of the microparticles from the Pacific Ocean analysed corresponds to microplankton particles.

4. The location points where the samples were collected in the Mediterranean Sea were relatively near to the coast. So, the probability of found plastic particles coming from the industries or human activities is higher than in the other oceans. In contrast with the Pacific Ocean, the currents generated in the locations where the samples were collected probably have been caused the movement of plastic particles towards areas further away from the location points. Thus, has been concluded that the Pacific Ocean is rich in microplankton.

5. Plastic particles of sizes between 500 µm and 50 µm are the most abundant. So, the majority of microplastics retained in the filters have the same size of microplankton. This fact increases the risk that aquatic organisms introduce them to the trophic chain due to their confusion with microplankton.

6. The microplastics concentration effect has been demonstrated. The adsorption and release of twelve congeners of PBDEs and sixteen PAHs on PET, PP, LDPE and PS microplastic particles have been studied. The results obtained from the extraction procedure demonstrates that the release is function of the combination solvent-polymer used. This fact has been validated applying the "solubility parameter" approach. The solubility parameter ∂ for both the plastic and the solvent is the suitable parameter for helping in the solvent election. The approach described allows to ensure the maximum amount of pollutant extracted. This is very important when real and unique samples have to be analysed.

7. Microplastics collected from Mediterranean Sea, Atlantic, Pacific and Indian oceans during the Barcelona World Race 2015 have been analysed to determine the concentration of PBDEs and PAHs adsorbed. From Mediterranean Sea and Atlantic Ocean, levels of PBDEs from 1 and 28 μg/kg have been detected depending on the congener. From the analysis of Pacific and Indian Ocean, the amount of almost all the congeners found is pretty close to the detection limit. For the quantification of PAHs in microplastic samples collected in the same locations, any congener of PAH has been detected, only traces of this pollutant with values under the limit of detection of the instrument have been found.

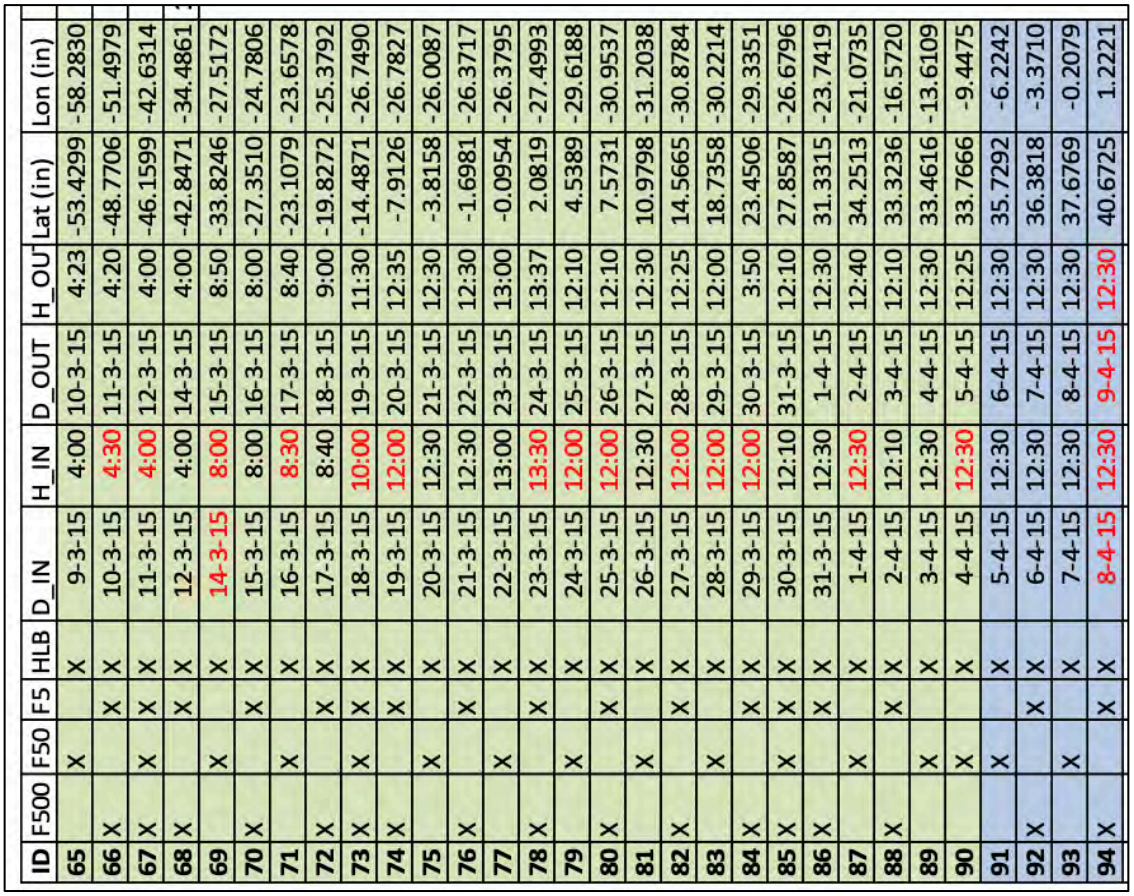
8. An analytical methodology for the SPE cartridges elution has been developed. The combination of three solvents, MeOH, ACN and n-hexane have been used to achieve the elution of a mixture of PAHs, PCBs, PFACs, pharmaceutical products and phthalates. Several chromatographic techniques for the analysis and quantification of all these pollutants have been used.

9. To end, eighteen Oasis HLB cartridges used during the BWR 2015 have been eluted and injected in a UHPLC-QTOF instrument. Two samples from the Mediterranean Sea, nine samples from the Atlantic Ocean, four samples from the Indic Ocean and three samples from the Pacific Ocean have been chosen to obtain a representative distribution and a global information.

10. The Principal Components Analysis have been the methodology used for the analysis of the data obtained. The variability is the parameter used in the PCA to find an aggrupation between the samples. The location where the samples were collected, their position regarding the equator and the superficial oceanic currents have been the parameters used as the three common characteristics for all the samples to explain the aggrupation. It has been determined that the samples are grouped according to the ocean where they were collected, that is to say, the location and furthermore, the samples have been also grouped according the superficial oceanic currents from where there were collected.

Annexes

ID	F500	F50	F5	HLB	D_IN	H_IN	D_OUT	H_OUT	Lat (in)	Lon (in)	ID	F500	F50	F5	HLB	D_IN	H_IN	D_OUT	H_OUT	Lat (in)	Lon (in)
1	X	X	X	X	31-12-14	15:26	1-1-15	14:46	41.1720	2.1482	**33**		X		X	3-2-15	23:35	4-2-15	22:35	-42.8513	33.4745
2	X		X	X	1-1-15	15:26	2-1-15	14:26	37.1097	0.1629	**34**	X			X	4-2-15	23:35	5-2-15	22:35	-42.6000	39.8545
3		X		X	2-1-15	15:26	3-1-15	14:26	35.5770	-2.1611	**35**		X		X	5-2-15	23:35	6-2-15	22:35	-42.3990	47.1230
4	X		X	X	3-1-15	15:26	4-1-15	14:26	35.5409	-6.2016	**36**	X		X	X	7-2-15	0:09	8-2-15	23:09	-40.4578	52.5985
5		X		X	4-1-15	15:26	5-1-15	14:26	34.7273	-10.8482	**37**	X	X	X	X	8-2-15	0:57	9-2-15	0:07	-42.5977	57.1142
6	X		X	X	5-1-15	15:26	6-1-15	14:26	33.0542	-12.4968	**38**	X			X	9-2-15	0:57	10-2-15	0:07	-43.4773	62.5067
7		X		X	6-1-15	15:37	7-1-15	14:37	29.9130	-14.6138	**39**		X		X	10-2-15	0:57	11-2-15	0:07	-43.6772	70.6037
8	X		X	X	7-1-15	15:57	8-1-15	14:37	26.0628	-16.8955	**40**	X		X	X	11-2-15	0:57	12-2-15	21:34	-42.5517	78.7592
9		X		X	8-1-15	15:57	9-1-15	15:37	24.1227	-22.3008	**41**		X		X	12-2-15	1:52	13-2-15	12:15	-42.4705	89.7912
10	X		X	X	9-1-15	16:19	10-1-15	19:47	19.3002	-25.4982	**42**	X		X	X	13-2-15	12:15	14-2-15	13:15	-44.3617	97.0842
11		X		X	10-1-15	20:48	11-1-15	19:48	13.0470	-27.0662	**43**		X		X	14-2-15	12:16	15-2-15	13:16	-44.4594	105.2176
12	X		X	X	11-1-15	20:48	12-1-15	19:48	7.8472	-27.2897	**44**	X		X	X	15-2-15	12:22	16-2-15	13:22	-44.7021	113.1112
13	X	X	X	X	12-1-15	21:31	13-1-15	20:31	4.1802	-27.0257	**45**		X		X	16-2-15	12:20	17-2-15	13:20	-45.6068	119.8872
14	X		X	X	13-1-15	21:31	14-1-15	20:31	2.1005	-27.3702	**46**	X		X	X	17-2-15	12:35	18-2-15	13:25	-47.5470	127.2617
15		X		X	14-1-15	21:31	15-1-15	20:31	-1.0557	-29.7325	**47**		X		X	18-2-15	13:39	19-2-15	14:39	-49.4135	136.7532
16	X		X	X	15-1-15	21:31	16-1-15	20:31	-4.8917	-31.0583	**48**	X		X	X	19-2-15	12:19	20-2-15	13:19	-51.0833	146.6571
17		X		X	16-1-15	21:31	17-1-15	20:31	-9.4815	-31.7910	**49**	X	X	X	X	20-2-15	13:45	21-2-15	13:49	-53.2721	164.4644
18	X		X	X	17-1-15	23:44	18-1-15	22:45	-14.2463	-32.4993	**50**	X		X	X	21-2-15	13:49	22-2-15	13:20	-53.2721	164.4644
19		X		X	18-1-15	23:44	19-1-15	22:45	-17.9212	-33.7240	**51**		X		X	22-2-15	13:15	23-2-15	13:15	-54.0331	174.1315
20	X		X	X	19-1-15	23:44	20-1-15	22:44	-22.4970	-35.2742	**52**	X		X	X	23-2-15	13:30	24-2-15	13:30	-53.9912	-174.7275
21		X		X	21-1-15	1:02	22-1-15	0:02	-26.2432	-34.5878	**53**		X		X	24-2-15	13:30	25-2-15	13:30	-52.9931	-158.0803
22	X		X	X	22-1-15	1:02	23-1-15	0:02	-29.3632	-32.5643	**54**	X		X	X	25-2-15	15:00	26-2-15	15:05	-52.8786	-157.6566
23		X		X	23-1-15	2:37	24-1-15	1:37	-32.2928	-31.6100	**55**		X		X	26-2-15	15:10	27-2-15	15:20	-52.4606	-152.6948
24	X		X	X	24-1-15	2:37	24-1-15	14:00	-33.8870	-27.8480	**56**	X		X	X	27-2-15	15:40	28-2-15	15:00	-52.6243	-145.6208
25	X	X	X	X	24-1-15	14:18	25-1-15	20:25	-34.5595	-25.6643	**57**		X		X	1-2-15	15:00	2-3-15	1:40	-52.3336	-126.2048
26					25-1-15	21:21	26-1-15	20:21	-36.2800	-21.9015	**58**	X		X	X	2-3-15	1:30	3-3-15	1:30	-52.0083	-121.9211
27		X		X	26-1-15	21:35	29-1-15	20:57	-38.3373	-18.3045	**59**		X		X	3-3-15	1:30	4-3-15	1:30	-51.8885	-113.4399
28	X		X	X	29-1-15	21:57	30-1-15	20:57	-43.5988	1.5920	**60**	X		X	X	4-3-15	1:45	5-3-15	1:55	-53.0961	-105.0273
29		X		X	30-1-15	21:57	31-1-15	20:57	-43.6782	9.3625	**61**	X	X	X	X	5-3-15	1:30	6-3-15	1:35	-52.7821	-96.7240
30	X		X	X	31-1-15	21:57	1-2-15	20:57	-44.3158	15.9515	**62**	X		X	X	6-3-15	2:00	7-3-15	2:20	-53.3245	-87.7077
31		X		X	1-2-15	23:18	2-2-15	22:18	-43.9880	23.4555	**63**		X		X	7-3-15	4:00	8-3-15	4:10	-56.0454	-77.6918
32	X		X	X	2-2-15	23:18	3-2-15	22:18	-43.5422	28.4488	**64**	X		X	X	8-3-15	4:00	9-3-15	4:05	-56.0355	-66.9008



ID	F500	F50	F5	HLB	D_IN	H_IN	D_OUT	H_OUT	Lat (in)	Lon (in)
65		X		X	9-3-15	4:00	10-3-15	4:23	-53.4299	-58.2830
66	X		X	X	10-3-15	4:30	11-3-15	4:20	-48.7706	-51.4979
67	X	X	X	X	11-3-15	4:00	12-3-15	4:00	-46.1599	-42.6314
68	X		X	X	12-3-15	4:00	14-3-15	4:00	-42.8471	-34.4861
69		X		X	14-3-15	8:00	15-3-15	8:50	-33.8246	-27.5172
70	X		X	X	15-3-15	8:00	16-3-15	8:00	-27.3510	-24.7806
71		X		X	16-3-15	8:30	17-3-15	8:40	-23.1079	-23.6578
72	X		X	X	17-3-15	8:40	18-3-15	9:00	-19.8272	-25.3792
73	X	X	X	X	18-3-15	10:00	19-3-15	11:30	-14.4871	-26.7490
74	X		X	X	19-3-15	12:00	20-3-15	12:35	-7.9126	-26.7827
75		X		X	20-3-15	12:30	21-3-15	12:30	-3.8158	-26.0087
76	X		X	X	21-3-15	12:30	22-3-15	12:30	-1.6981	-26.3717
77		X		X	22-3-15	13:00	23-3-15	13:00	-0.0954	-26.3795
78	X		X	X	23-3-15	13:30	24-3-15	13:37	2.0819	-27.4993
79		X		X	24-3-15	12:00	25-3-15	12:10	4.5389	-29.6188
80	X		X	X	25-3-15	12:00	26-3-15	12:10	7.5731	-30.9537
81		X		X	26-3-15	12:30	27-3-15	12:30	10.9798	-31.2038
82	X		X	X	27-3-15	12:00	28-3-15	12:25	14.5665	-30.8784
83		X		X	28-3-15	12:00	29-3-15	12:00	18.7358	-30.2214
84	X		X	X	29-3-15	12:00	30-3-15	3:50	23.4506	-29.3351
85	X	X	X	X	30-3-15	12:10	31-3-15	12:10	27.8587	-26.6796
86	X		X	X	31-3-15	12:30	1-4-15	12:30	31.3315	-23.7419
87		X		X	1-4-15	12:30	2-4-15	12:40	34.2513	-21.0735
88	X		X	X	2-4-15	12:10	3-4-15	12:10	33.3236	-16.5720
89		X		X	3-4-15	12:30	4-4-15	12:30	33.4616	-13.6109
90		X		X	4-4-15	12:30	5-4-15	12:25	33.7666	-9.4475
91		X		X	5-4-15	12:30	6-4-15	12:30	35.7292	-6.2242
92	X		X	X	6-4-15	12:30	7-4-15	12:30	36.3818	-3.3710
93		X		X	7-4-15	12:30	8-4-15	12:30	37.6769	-0.2079
94	X		X	X	8-4-15	12:30	9-4-15	12:30	40.6725	1.2221

Figure 0-1. Latitudes and longitudes where each sample was collected. Information about the day and the exact timing of collection