

Linear alkylbenzene sulfonates in the aquatic environment

Study of the analysis, sorption processes and sediment toxicity

Ángeles Rico Rico

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Linear alkylbenzene sulfonates in the aquatic environment

Study of the analysis, sorption processes and sediment toxicity

Linear alkylbenzene sulfonaten in het aquatisch milieu
Een studie van de analyse, sorptie en sediment toxiciteit

(met een samenvatting in het Nederlands)

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A mis padres

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Chapter 1

General introduction

1. Surfactants

Surfactants are an extensive group of surface-active agents that can be biologically or synthetically produced. Biosurfactants are usually produced by bacteria or yeast [1], and they play an important role in vital functions such as nutrient transport, cell motility, etc. Soap was the first recognized surface-active agent used for medical and cleaning purposes, and it was originally synthesized by mixing animal fat with wood ashes. Synthetic surfactants began to be produced in the 19th century, and they are increasingly used for a wide variety of industrial and domestic purposes (cleaning and dispersive agents, emulsifiers, polymerisation processes, etc).

All surfactants molecules have a hydrophilic (“head”) and a hydrophobic (“tail”) group. The hydrophobic part usually consists of an alkyl chain (branched or not) that is attached to the functional hydrophilic group. According to the charge of their hydrophilic moieties, surfactants are classified in four categories: anionic, cationic, non-ionic and amphoteric (see Figure 1). At low aqueous concentrations, surfactants are present as single molecules (monomers) and they like to be at the surfaces or interfaces (Figure 2). At higher concentrations, the solubility of the monomer form is exceeded, and surfactants molecules will associate and form micelles, with their hydrophilic groups oriented to the exterior. The surfactant concentration at which this occurs is known as the critical micelle concentration (CMC). The amphiphilic character, the ability to lower surface tension, and the detergency power, is what make surfactants especially attractive for their use in the formulation of industrial and household detergents [2,3]. Surfactant behavior and effectiveness will depend on the molecular structure and the media characteristics [4,5].

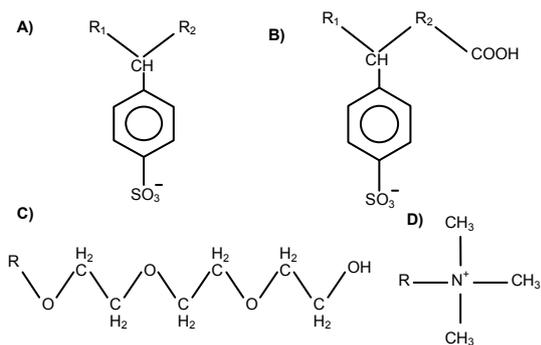


Figure 1. Examples of synthetic surfactant structures. (A) The anionic linear alkylbenzene sulfonate (LAS); (B) the LAS intermediate product sulfophenyl carboxylic acid (SPC); (C) the non-ionic alkyl ethoxyate (AE3); (D) the cationic trimethyl ammonium chloride (TMAC). “R” groups correspond to alkyl chains with variable length.

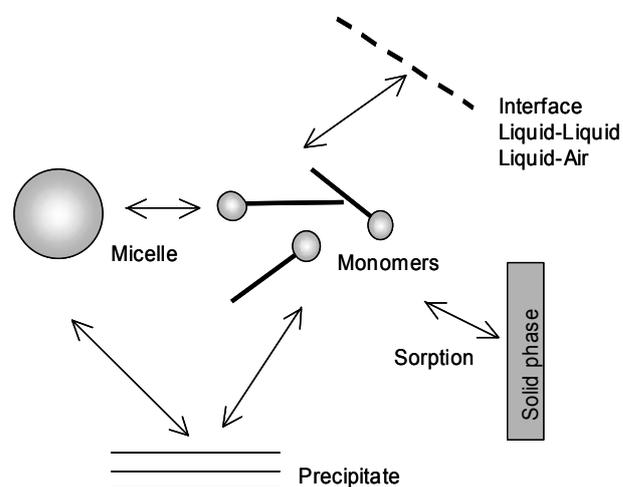


Figure 2. Forms and behaviour of surfactants molecules in water.

1.1 Linear alkylbenzene sulfonates (LAS)

Linear alkylbenzene sulfonate (LAS) is one of the most used synthetic anionic surfactants worldwide. They were introduced in the sixties as the principal component of household detergents to replace the highly branched alkylbenzene sulfonates (ABS). LAS use was favoured because of the high biodegradability, excellent detergent properties and lower production costs [6,7]. The European consumption of LAS in 2005 was 430 kt, from which more than the 80 % was used for the formulation of household cleaning products [8].

Commercial LAS consist of a mixture of more than 20 individual constituents, with different alkyl chain length and different position of the benzene-sulfonate group in the alkyl chain. The average length of the alkyl chain is of 11.6 carbon atoms [8-11]. The raw materials used for the production of LAS compounds are typically petrochemically derived materials, such as ethylene, linear paraffin and benzene. The LAS precursor, the linear alkylbenzene (LAB) is generally manufactured by the alkylation of benzene with mono-olefins [12], which result in a group of homologues from 10 to 13 methyl groups, with all possible isomers except for the 1-phenyl. LAS is subsequently obtained by the sulfonation in the para position of the LAB. This sulfonation is generally performed by using SO₃ gas [3], followed by the neutralization with NaOH to form the LAS sodium salt.

Biodegradation of LAS compounds in the environment is primarily initiated by the ω -oxidation of the terminal carbon atom of the alkyl chain, and continues with successive β -oxidations that shorten the alkyl chain by 2 methyl units [13-15]. The main intermediates of LAS degradation are the sulfophenyl carboxylic acids (SPC), which do not present surfactant properties any longer. Ultimate biodegradation of the intermediates seems to be slower than of LAS. However, the toxicity of SPC's is several orders of magnitude lower than that of LAS [16,17].

2. Fate and behaviour of LAS in the aquatic environment

The fate of a consumer product in the environment depends on its specific use, the way of discharge, and the characteristics and environmental behaviour of the product. Degradation, volatilisation, sorption and dilution are the processes generally affecting the environmental fate and distribution of a substance. The understanding of these processes is primordial for the prediction of contaminant concentration and its possible adverse effects.

A relatively large industrial and domestic use of LAS have led to high concentrations of these compounds in the wastewater effluents [18]. In developed countries, the majority of the waste discharges are treated via wastewater treatment plants (WWTPs), which have been reported to efficiently reduce the LAS concentration present in the receiving waters [19-21]. LAS can also be introduced in the terrestrial environment by leakage of sewer lines, septic tanks or application of sludge for agriculture use [6,20] (see Figure 3). When untreated urban wastewater is discharged, LAS dissolved concentrations of up to 1.68 mg/L have been found in surface waters [18]. Furthermore, sediment concentrations up to 238 mg/kg were detected in estuarine areas [22]. However, these concentrations are measured in areas close to the

wastewater effluents, since LAS concentration considerably decreases at relatively short distances from the discharged point [22,23]. Monitoring studies have reported the importance of the sorption processes in freshwater and marine media for the removal of LAS from the water column [24-26]. Besides, LAS present in sediment and water has shown to be rapidly biodegradable under aerobic conditions [14,27-29], and there is evidence of its biodegradation under anoxic conditions [30,31].

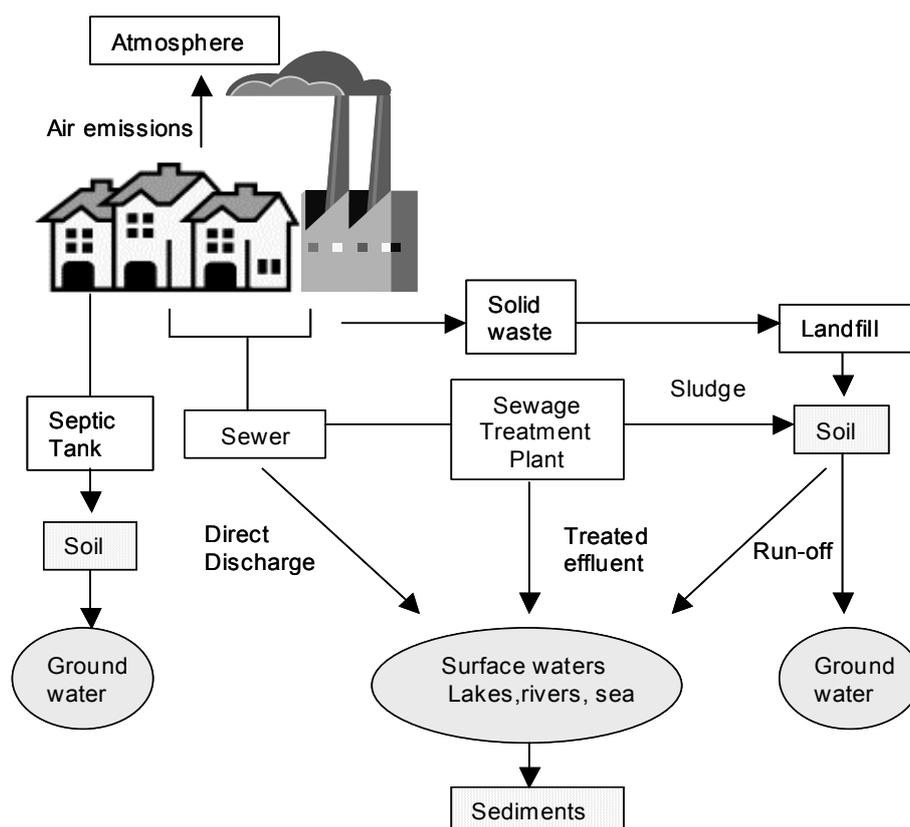


Figure 3. Transport and distribution of consumer product chemicals in the environment.

An overview of the studies from the last decade reporting LAS (total) concentrations in different aquatic systems is presented in Table 1. Many of them have been conducted in areas receiving untreated wastewater. High LAS concentrations have been found in sediments from low energetic areas (e.g. lagoon, lakes) close to non treated effluents, but concentrations are close to the limit of detection in locations > 10-15 Km from the discharged points [23,32].

The European directive 91/271/EEC [33] demands that all wastewater in the European Union countries should be properly collected and treated, at least with secondary treatment, prior to being discharged into surface waters. Therefore, high concentrations of LAS or other xenobiotics in recent European marine sediments should reflect the inappropriate implementation of the directive by the European Union states. In the case of LAS, influent concentrations are decreased up to 99 % in WWTP with a secondary treatment

[16]. Maximum reported concentration in the areas very close to discharge points from treated effluents are 80 µg/L in the water column, and 12.8 mg/kg in the sediment [23].

Table 1. LAS concentrations in marine and freshwater sediments. Overview of the published monitoring studies during the last decade.

Description of Location	Location	LAS sediment (mg/kg)	LAS pore water (mg/L)	Type of sediment	OC (%)	Year-Ref
Estuary area close to urban effluent discharge	Cádiz, Spain	26.8	0.048	NS	NA	2000 [34]
Estuary area with high bioturbation	Cádiz, Spain	0.8 ± 0.2	0.023 ± 0.009	Sand/Clay	1.61	2001 [35]
Estuary area close to urban effluent	Cádiz, Spain	138 ± 14	0.045 ± 0.013	Clay	2.96	2001 [35]
Lagoon close to urban effluent	Cádiz, Spain	238		Clay/Sand	NA	2002 [22]
Marine nautic club	Cádiz, Spain	1.23		Clay	NA	2002 [22]
Tidal channel	Cádiz, Spain	< 10		NS	NS	2005 [36]
Reservoir receiving primary treated wastewater	Cádiz, Spain	<1.71		NS	NS	2008 [23]
Discharge outlet of secondary treatment WWTP	Cádiz, Spain	12.8		NS	NS	2008 [23]
Marine nautic club	Marbella, Spain	3.72		Clay	1.31	2002 [22]
Outflow municipal wastewater	Almeria, Spain	65		Clay/Sand	2.77	2002 [22]
Coastal area, 1 Km North from outflow of sludge	Barcelona, Spain	78		Clay	NA	2002 [22]
Outflow of industrial plant	Tarragona, Spain	0.49		Clay	NA	2002 [22]
Bight, different urban areas	Germany	< 0.106		NS	NS	2001 [37]
River, 2 m below outfall	Lowell, Indiana		0.001	Sand	0.54	2006 [32]
River, 1 Km below outfall	Lowell, Indiana		0.005	Sand	0.53	2006 [32]
River, 2 m below outfall	Bryan, Ohio		0.011	Sand	0.55	2006 [32]
River, 1 Km below outfall	Bryan, Ohio		0.006	Sand/Silt	0.95	2006 [32]
River, 2 m below outfall	Wilmington, Ohio		0.005	Sand	0.51	2006 [32]
River, 1 Km below outfall	Wilmington, Ohio		0.005	Sand	0.57	2006 [32]

NS= Not specified. NA= Not analyzed.

2.1 Factors influencing the distribution of LAS in the aquatic environment

Sorption plays an important role in the environmental fate and effect of LAS in the aquatic environment. Apart from the structural characteristics of the LAS molecules, the sorption of LAS to natural sorbents is dependent of the properties of the sorbent and the physico-chemical characteristics of the media. The most relevant parameters are discussed below.

2.1.1 Sorption of LAS

Sorption of organic compounds to sediments and other particulate materials affects their mobility and potential to reach the surface and interstitial water compartments [3,38,39]. Besides, the affinity of a compound to be sorbed has direct consequences for its bioavailability, and therefore on its toxicity and possible biodegradation.

The sorption of a compound to a specific sorbent is described by the sorption coefficient (K_d) that is defined as the relation of the concentration of the compound in the sorbent (C_s , mg/kg) and in the aqueous phase (C_w , mg/L) (Equation 1):

$$K_d = \frac{C_s}{C_w} \quad (1)$$

Sorption of hydrophobic chemicals to sediment is well correlated with the organic carbon fraction (f_{oc}) of the sediment, because non-polar organic compounds generally only bind to this fraction. For those compounds, the sorption coefficient is normalized to the organic fraction, resulting in Equation 2:

$$K_{oc} = \frac{K_d}{f_{oc}} \quad (2)$$

where K_{oc} is the normalized organic carbon sorption coefficient. However, when dealing with ionizable organic compounds such as LAS, other properties of the sediment have to be considered, because the overall sorption process is most probably the result of more than one binding mechanism. In the case of LAS, the functional sulfonate group might interact with other sediment sites by either specific or non-specific interactions. These interactions are often based on adsorption instead of absorption. Indeed, apart from the correlation between sorption and the organic carbon content in most of the sorption studies with LAS [39-42], other sediment parameters such as sesquioxide and clay content affect the sorption affinity [39,43-45]. Non linear isotherms are usually obtained in most of the studies. For these reasons, the most common sorption isotherm used to fit LAS sorption data is the Freundlich equation, which considers multiple types of sorption sites:

$$C_s = K_F \cdot C_w^n \quad (3)$$

K_F is the Freundlich constant, and n is the Freundlich exponent, and both parameters are deduced from a fit of experimental data with equation 3. As it is graphically shown in Figure 4, a linear isotherm is obtained for $n=1$, while for $n \neq 1$, sorption is enhanced ($n>1$) or

reduced ($n < 1$) at higher concentrations of the sorbate. Generally, experimental data is fitted using the logarithmic form of the Freundlich equation:

$$\text{Log}C_s = \text{Log}K_F + n \cdot \text{Log}C_w \quad (4)$$

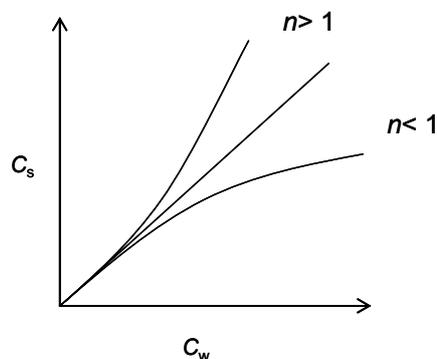


Figure 4. Freundlich isotherms.

2.1.2 Sediment properties

Sediments can be classified based upon their composition or grain size distribution. Sediments from high-energetic environments are mainly composed of larger particles, while sediments from low-energetic environments contain small particles (silts and clays). The particle size is an important factor to characterize sediments, because it affects the transport of particles, and therefore the contaminants associated to them. Besides, other properties such as the organic or clay content are directly related to the grain size distribution of the sediment.

The sand (63-2000 μm diameter) and most of the silt particles (2-63 μm) are resistant materials with very low surface/volume ratio and no surface charge. These two sediment constituents are generally poorly related to LAS sorption to sediments [39,44,46]. The clay fraction of the sediments ($< 2 \mu\text{m}$) commonly consists of very fine particles that cover a wide range of mineral and non-mineral constituents. Layer silicate clays, non-crystalline aluminosilicates and amorphous and crystalline oxides, are commonly part of this fraction [47]. Sorption studies of anionic surfactants to specific clay minerals have shown the importance of these sediment (and soil) components on the adsorption processes [48-50]. Although the surface of clay minerals is usually negatively charged, the composition of the aquatic phase might change the initial charge of the mineral or particle surface, making it easier to interact with the anionic surfactant molecule [39,48,51]. Metal oxides are also affecting the sorption of LAS to sediments and soils [39,44,45,52]. Sorption affinity is increasing in the presence of amorphous oxides [45], likely because of the higher surface area of the amorphous minerals.

Finally, the organic carbon content has a very important influence on the distribution of organic compounds in sediments. The relative contribution of the organic carbon and the clay content in the sorption of chemicals to sediments is nonetheless difficult to characterize, because these two parameters are generally well correlated to each other [51,53]. Natural

organic matter has many different origins. Organic matter in marine areas far from the main land is mainly originated from the plankton. While in river, estuary or coastal areas, organic matter is from terrestrial and aquatic sources, as well as from human activities. The structure of sediment and soil organic matter includes large hydrophobic parts but also many different types of hydrophilic domains [54]. The polar parts of the organic matter may interact with other ions (e.g. Na^+ , K^+ , Ca^{2+} , Mg^{2+} , Al^{3+}) and be sensitive to changes of the pH and ionic strength.

2.1.3 Dissolved organic matter

Dissolved organic matter (DOM) includes an ample range of constituents with many different characteristics that differ depending on the origin (aquatic or terrestrial), age, and environmental parameters (pH, salinity, etc) [54,55]. Quantification of DOM is commonly made by organic carbon content analysis and therefore the term “dissolved organic carbon” (DOC) is generally used. The presence of DOC in the aquatic systems plays an important role in the transport and fate of contaminants in the environment [56]. Besides, it has been demonstrated that DOC may decrease the bioavailability of chemicals in solution [38,57] and thus, their toxicity. The determination of the sorption of chemicals to DOC is difficult because the phase separation by traditional techniques (e.g. centrifugation) can not be easily applied to dissolved sorbents. Careful evaluation of the experimental method to be used for the DOC-sorption determination is therefore required. Differences in the DOC nature and the environmental characteristics will lead to different molecular interactions with the sorbate, and thus, different sorption affinity. Sorption of hydrophobic compounds to DOC is dependent of the characteristics of the aquatic media [54,58-60], and effects of aqueous composition on both the sorbate and the sorbent have been observed for ionizable compounds in humic acids solutions [61].

Surfactants have been considered to strongly affect the fate of humic acids in the environment, because their presence influences the solubility of humic acid substances [38,56]. However, research have been more focused on the sorption of non polar organic compounds to DOC, and little is known about the sorption processes of ionic and non ionic surfactants to DOC.

2.1.4 Salinity, water hardness and pH: Marine versus freshwater media

The solution chemistry is strongly influencing the extent of sorption of ionic compounds to natural sorbents [39,62]. The presence of ions of an opposite charge on the surface of the sorbent will promote sorption of the ionic compounds. For anionic surfactants, the sorption is correlated to the ionic strength of the media [3]. Besides, the effect of the ion concentration in solution is also affecting the solubility of ionic surfactants, because high concentration of cations will lead to the precipitation of the molecules. Both sorption and precipitation will lead to a decrease of the bioavailable surfactant fraction in the water column. Literature has shown that the toxicity of LAS compounds to *Daphnia magna* decreases with increasing water hardness [63]. Studies focused on the effect of the aqueous composition on the performance of LAS as detergent have also shown the relevance of the divalent (mainly Ca^{2+}

and Mg^{2+}) versus the monovalent cations (e.g. K^+ , Na^+) in the precipitation and solubility of LAS [64-67]. The influence of Na^+ and Ca^{2+} on the sorption of LAS and other anionic compounds have been studied using single electrolyte solutions [39,62,68-70], and in general, field sorption coefficients in marine areas are higher than in the freshwater media [3,22].

With regard to the effect of pH, studies have shown that low pH favours the sorption of anionic compounds because of the formation of a positively charged sorbent surface [39,71,72]. The potential effect of the pH on the sorption of LAS will be relevant in particular in freshwater systems or in coastal and estuarine areas close to wastewater discharges, while the marine environment will be characterized by a high and relatively constant salt content and buffered pH.

3. Toxicity of LAS in sediments

LAS is considered to be a polar narcotic compound, with higher affinity for the more polar lipids such as phospholipids [73,74]. LAS toxicity is caused by the disruption of the biomembranes and denaturation of proteins [6]. The narcotic toxicity is the common mode of action for hydrophobic organic compounds. It relies on the assumption that once the chemical has partitioned into the membrane, the toxic effect is compound independent. For non-polar organic compounds, it has been shown that the dissolved fraction of the toxicant in the pore water is responsible for the toxicity in sediment (Equilibrium Partitioning approach – EqP-) [75-79] (Figure 5). According to this theory, the hydrophobic organic compounds are distributed between the pore water, the sediment and the lipids of the organisms, and the distribution of the contaminant among these phases is at equilibrium. Therefore, if the concentration of the chemical in the pore water can be estimated or measured, and since this fraction is the most relevant route of exposure, sediment toxicity can be predicted by toxicity data from water-only experiments.

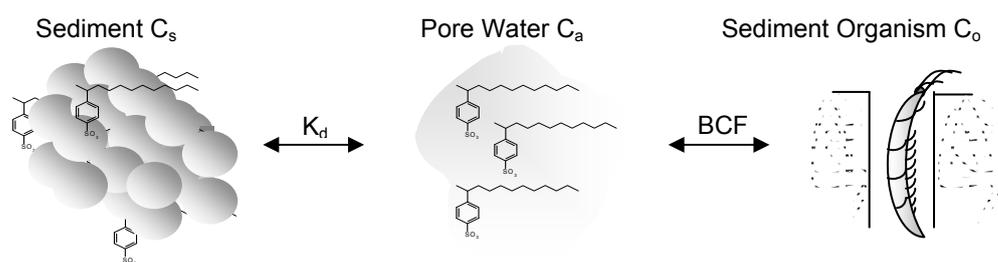


Figure 5. The equilibrium partitioning approach (EqP).

Although the database on the effects of LAS to fresh and seawater column organisms is extensive, data on the toxicity of LAS to benthic organisms is more limited, and most of them are focused on fresh water media [80]. The toxicity of the highly branched alkylbenzene sulfonate (ABS) to the freshwater amphipod *Hyalella azteca* in a sediment system was well predicted from the data generated via water exposure [81]. However, in a recent study with

the deposit feeder *Hydrobia ulvae*, the LAS toxicity was clearly affected by feeding habit [82]. Body residues have been proposed as a dose-metric for LAS exposure [83]. Nevertheless, other mechanisms like the biotransformation (involving kinetics of the process) can affect this measurement. For example, Tolls et al. [84] showed that biotransformation reduce the internal LAS concentration in fish with up to a 40 %.

4. Freely dissolved concentrations of LAS compounds

The free concentration of a chemical is believed to be the bioavailable fraction, and therefore the responsible for the toxicity. In sediment, this fraction corresponds to the freely dissolved concentration in the pore water, and the toxic effect will take place when the freely dissolved concentration in the pore water exceeds the toxic concentrations in water-only exposure. However, measuring freely dissolved concentrations in pore water can be difficult due to the fact that traditional techniques are not sufficient to separate dissolved sorbents (such as dissolved organic matter) from the system [76].

There are several experimental techniques for the determination of the freely dissolved concentrations of organic compounds in complex matrices, such as fluorescence quenching [38,85], headspace partitioning, dialysis [86], etc. Solid phase micro extraction (SPME) is an increasingly applied technique for the analysis of freely dissolved concentrations of organic compounds (see, for instance refs [87-91]). Although the SPME method is in some cases less sensitive than other techniques that work with larger extraction volumes, its simplicity, fast equilibrium and avoidance of clean up steps, however, make this tool suitable for a wide variety of matrices. Application of the SPME method to ionic compounds is more challenging, because careful evaluation of the experimental conditions is needed [85,92].

SPME methods use different polymer coatings on glass fibers (generally optical fibers used for data transmission) to extract compounds from the aqueous solutions (see Figure 6). For the extraction of non-polar hydrophobic compounds, polydimethylsiloxane (PDMS) is the most widely used polymer coating [93-96], while polyacrylate (PA) is generally more efficient extracting compounds with polar groups [92,97]. For hydrophobic compounds, partitioning is the process that governs the extraction of the chemical from the aqueous phase to the fiber polymer. Only molecules that are freely dissolved (and not those already bound to other sorbent phases) will absorb into the polymer. When the system is in equilibrium, the ratio of the concentration in the aqueous and polymer phase will be constant, and this is defined by the sorption coefficient K_{fw} . The porous polymer Carbowax/Templated Resin and PDMS have already been tested as passive samplers for the analysis of LAS compounds from wastewater samples [98,99]. However, both studies considerably change the initial conditions of the water samples by changing the solution pH or adding ion-pairing reagents for counterion association. In summary, while the SPME method has been successfully calibrated and applied for a large number of non-polar hydrophobic compounds, research and improvement of the application of the method for anionic compounds like LAS is still

needed, and its development is useful for the study of the presence and behavior of these compounds in the environment.

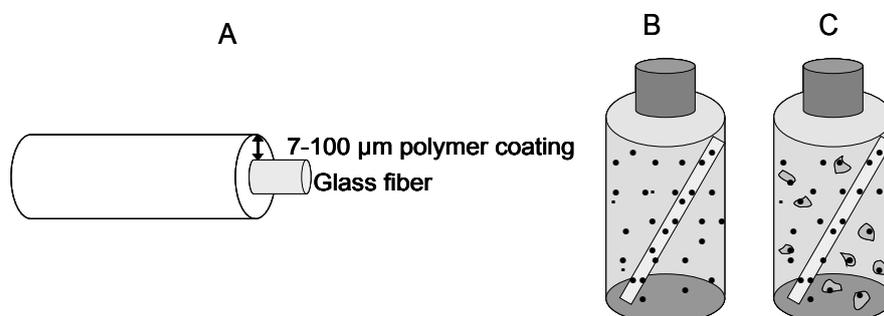


Figure 6. SPME fiber (A). SPME fiber in equilibrium in a clear solution (B), and in a solution with a particulate sorbent (C). Adapted from Ter Laak [100] and Droge [101].

5. Risk assessment of surfactants in coastal ecosystems: RISICO project

The research in this thesis is performed within the framework of the RISICO project (MEST-CT-2004-505182) and this project is supported by the European Commission through the FP6 program (Early Stage Training Marie Curie Fellowship). The project involved four PhD students working in different research centers in Europe: P&G Eurocor in Belgium, the Institute of Marine Sciences in Andalusia (Spain), the Institute for Risk Assessment Sciences of Utrecht University (Netherlands), The Plant Bioengineering Department of the Free University of Brussels (Belgium) and the International Atomic Energy Agency-Marine Environment Laboratory (Monaco). Four main working areas are defined for the project:

1. Bioavailability of surfactants in marine sediments.
2. Bioaccumulation of surfactants in marine organisms.
3. Biodegradation and ecotoxicity of surfactants in marine environments.
4. Aerosolization of surfactants in coastal environments.

The main objectives of the RISICO project are to refine the ecological risk assessment of various classes of surfactants in the coastal environment and to develop analytical methods to predict the exposure of surfactants in sea-sprays and marine sediments. These aspects are thought to contribute in the development of marine risk assessment guidance (EU TGD, Commission directive 93/67/EEC) for the safe use of surfactants in Europe as requested by the EU White Paper (Com (2001) 88 Final). The specific objectives and contribution from IRAS includes the development of analytical methods for the analysis of surfactants in the pore water, and gains insights into the bioavailability and sorption of surfactants in marine sediments.

6. Scope and outline of the thesis

The main objective of the present PhD thesis is to gain insights into the sorption processes of the anionic surfactant linear alkylbenzene sulfonate in relation to sorbent, aqueous media, and compound characteristics, and to study the implication of the sorption processes on the bioavailability of these compounds to benthic organisms. The outcome will serve to reduce the uncertainty in the risk assessment of surfactants in the aquatic environment.

The Equilibrium partitioning approach is considered as a key element during the research. If this approach is valid for LAS, risk assessment of contaminated sediments can be simplified to water-only exposure testing and the prediction (by sorption coefficients) or direct measurement of the compound concentration in the pore water. With these objectives, the major activities of this thesis are focused on the following areas:

- to develop an analytical tool to measure freely dissolved concentrations of LAS in complex matrices during sorption studies and other laboratory or *in situ* conditions.
- to understand the sorption of the anionic surfactant LAS in terms of sediment, surfactant and aqueous characteristics by sorption studies at laboratory conditions.
- to test the validity of the EqP approach for LAS by comparing the toxic effect from the aqueous phase with the effect from sediments in benthic organisms.

Chapter 2 describes the calibration and application of the solid phase micro extraction method (SPME) for the analysis of freely dissolved concentration of LAS congeners in seawater. In this work, the effect of the pre-treatment of the SPME fiber is tested, and the optimized method is applied to measure the solubility of LAS compounds in seawater. **Chapters 3** and **4** are focusing on the study of the sorption of LAS compounds to sediments. **Chapter 3** focuses on the sediment properties that are mainly affecting the sorption of LAS to marine sediments, using single sediment components and field natural sediments. **Chapter 4** explores the effect of the molecular structure and the aqueous composition on the sorption of LAS to sediments, using different LAS constituents and sea and freshwater dilutions. Besides, the application of the fiber-water sorption coefficients to predict LAS sorption to sediments and other hydrophobicity driven processes is discussed. In **Chapter 5**, the sorption of LAS to different types of dissolved humic acids is studied for different molecular structures, and the investigation is also extended to test the effect of the aqueous composition on the sorption affinity of LAS to dissolved humic acids. In **Chapter 6**, the sediment toxicity of LAS to a marine benthic organism is investigated in two different sediments and in water-only exposure, and the application of the equilibrium partitioning approach for LAS is tested. Special attention is given to the spiking procedures and the stability of the compound during the bioassays. **Chapter 7** summarizes the main results of the present thesis and discusses them in relation to the potential applications of the SPME method, and environmental relevance of the main sorption and toxicity outcomes. Finally, concluding remarks and future perspectives from the author's point of view are given.

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Chapter 2

Freely dissolved concentrations of anionic surfactants in seawater solutions: Optimization of the nd-SPME method and application to linear alkylbenzene sulfonates

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ABSTRACT

The solid-phase microextraction method (SPME) has been optimized for the analysis of freely dissolved anionic surfactants, linear alkylbenzene sulfonates (LAS), in seawater. An effect of different conditioning treatments of the polyacrylate fiber coating was demonstrated for both uptake kinetics and sorption isotherm linearity. The most likely cause is a modification of the polymer structure from a glassy to a rubbery state. Thermal conditioning at 120 °C yielded linear sorption isotherms and reproducible SPME measurements for several individual LAS compounds, with detection limits at the low microgram per liter range. Sorption of LAS to the conditioned SPME fiber was independent of LAS co-solutes in mixtures. The method has been applied to study the precipitation of LAS in seawater, and solubility data for a wide range of individual LAS constituents is presented for the first time. Hence, the developed SPME method for the anionic LAS has shown to be a useful tool in complex matrices. The advantage of the SPME analyses in complex matrices is, besides its simplicity, that it also leads to clean extracts for chromatographic analyses. The method is still limited by ubiquitous background LAS contamination.

INTRODUCTION

The freely dissolved concentration of a chemical controls many environmental and industrial processes, such as evaporation, sorption, precipitation, biodegradation, bioconcentration and toxicity [1-3]. It is therefore a key entity for process based studies. Several techniques have been developed that distinguish the freely dissolved concentration from the fraction bound to particles or macromolecules (see e.g. [4-8]). The solid-phase microextraction (SPME) method has been introduced ten years ago as a relatively simple technique to measure freely dissolved concentrations in all kinds of matrices [6,9-11]. The freely dissolved concentration can simply be calculated from the measured concentration in the fiber polymer and the specific fiber-water partitioning coefficient (K_{fw}). Most applications of SPME, however, focus on hydrophobic neutral organic chemicals, while fewer studies included more polar or ionized compounds [12-16].

This study was focused on the development of a method for measuring freely dissolved concentrations of linear alkylbenzene sulfonates (LAS). LAS is globally the most abundantly used type of anionic surfactant, and many monitoring studies have reported its presence in marine areas in the vicinity of wastewater discharge points [17,18]. Laboratory studies on the fate and behavior of LAS in seawater could benefit from a simple method to determine the freely dissolved concentration. The influence of changes in solution chemistry on sorption of anionic compounds to SPME will likely be minimal in seawater due to the buffered pH, and the already high salt content. Application of SPME fibers to study anionic surfactants in marine samples might therefore also be easier compared to application of the SPME method in weakly buffered solutions.

Commercial LAS is composed of a mixture of homologues that generally vary in the alkyl chain length from 10 to 13 carbon atoms, and isomers with different positions of the benzene-sulfonate moiety. Different molecular structures imply differences in the physico-chemical characteristics (e.g. solubility, critical micelle concentration –CMC-, etc.) [19], that clearly affect the environmental behaviour of these compounds. For example, the hydrophobicity of organic compounds is closely related to the accumulation in organisms or partitioning into sediments and soils [20]. Sorption and bioconcentration of LAS also increase with the alkyl chain length. Within the LAS isomer series, those with the most external phenyl positions showed the highest sorption coefficients in sediment [22]. For ionic organics, such as anionic surfactants, the calibration of this technique is markedly challenging, because the partitioning of ionisable compounds will also be strongly influenced by the solution chemistry (pH, salinity, type of counterions, etc.) [13,14,19,23]. Nevertheless, SPME has been proven to be a useful extraction technique for ionisable compounds when experimental conditions are optimized [14,15,24,25]. For both acids and bases, the affinity of ionized species for the polyacrylate SPME coating is orders of magnitude lower compared to the neutral species [13-15]. At most environmental conditions, the LAS structures exist only as ionized species. Previous testing with the SPME technique for LAS has therefore included a pre-treatment of the sample to enhance sorption of the ionized species to the fiber. Ceglarek *et al.* [24] has used a porous polymer for the analysis of LAS in wastewater samples at pH 3. Alzaga *et al.*

[25] extracted LAS homologues and isomers from aqueous samples using poly(dimethylsiloxane) (PDMS) by adding the ion-pairing reagent tetrabutylammonium. Both analytical procedures considerably improved recoveries and detection of total LAS concentrations. However, both methods modify sample conditions, and this could strongly alter the actual freely dissolved concentration of the compounds in the sample.

Whether surfactants truly partition into the SPME coating, or mainly adsorb to the polymer surface, is an important question for the application of SPME. The partitioning of non-ionic surfactants (alcohol ethoxylates, AE) from seawater to disposable polyacrylate (PA) coated SPME fibers is linear, and the K_{fw} of individual AE is not influenced by cosolutes [12]. Based on the successful results for non-ionic surfactants with PA coated fibers, the same phase was considered for the extraction of anionic surfactants.

While most commercial SPME fibers are normally thermally conditioned prior to their (first) use, the disposable polyacrylate SPME fibers gave excellent results for the non-ionic surfactants without any prior treatment. The conditioned state of the PA phase, however, is crucial for the successful extraction of LAS compounds, as will be shown in this study. Several lines of evidence presented here suggest that the pre-treatment's of the fiber affects the state of the polymer coating (e.g. glassy versus amorphous rubbery) and, thereby, the sorption behaviour of the LAS surfactants. The main issue that hinders us to clearly substantiate this hypothesis is that the composition of the PA polymer is not well defined [26]. Sodium polyacrylate is a strongly water absorbing polymer phase [27]. The resulting volume expansion for this phase is unwanted for the original purpose of the polymer coating (protective coating of optical fibers), and swelling is moreover not observed for PA SPME fibers, implying that the coating is not pure polyacrylate. Poly(alkyl)acrylates are all rubbery at room temperature [28], with a glass transition temperature (T_g) below 5 °C. In contrast, many poly(alkylmethacrylates) are glassy polymers at room temperature, with T_g varying from 120 to 20 °C [29]. The state of the polymer in the disposable fibers, therefore, strongly depends on the exact composition of the polymer. Moreover, the T_g can strongly decrease when a polymer phase is fully hydrated, or by the increasing concentrations of plasticizers [30,31].

The aim of the present work was to test the applicability of the SPME method for the analysis of individual LAS compounds in seawater. Pilot studies showed a strong influence of the type of pre-treatment of the fibers on the sorption characteristics, and therefore specific attention was paid to different conditioning treatments of the fiber coating. For this purpose, uptake kinetics and sorption isotherms were obtained for different fiber conditioning methods with one single LAS compound. Based on these experiments, an optimal conditioning method was selected, and sorption isotherms for each compound to the polyacrylate SPME were determined. The sorption isotherms for single LAS compounds were then compared to those determined in LAS mixtures. If adsorption to the fiber surface significantly contributes to overall extraction by SPME, co-solutes will compete for sorption sites at elevated concentrations. Electrostatic repulsion might play a role in the sorption of anionic surfactants [32], and this repulsion is likely increased by the presence of other anionic surfactants in the polymer phase, or on its surface.

As a first application of the method to determine the freely dissolved monomer concentration of an anionic surfactant, precipitation of LAS in seawater was studied. Precipitation of LAS in seawater limits the surfactant solubility for concentrations 200 times lower than the critical micelle concentration in freshwater [33], but this process is scarcely studied. Whereas some precipitation data are available for complex LAS mixtures and single counter ions [33-35], data on pure compounds are limited to industrial in-house reports. The outcomes of this study are discussed addressing the applicability to other LAS compounds present in commercial mixtures, and the sensitivity of the method for quantification of LAS concentrations in *in situ* studies.

MATERIALS AND METHODS

Chemicals and solutions

LAS compounds are abbreviated as C_n-m-LAS, where n is the length of the alkyl chain and m indicates the position of the phenyl group in the alkyl chain. C₉-1-LAS, C₁₀-1-LAS, C₁₀-2-LAS, C₁₁-2-LAS, C₁₁-5-LAS, C₁₂-1-LAS, C₁₂-2-LAS, C₁₂-3-LAS, C₁₂-4-LAS, C₁₂-5-LAS, C₁₂-6-LAS, C₁₃-2-LAS and C₁₃-5-LAS (purity > 97%) were synthesized as sodium salts by Dr. J.Tolls [19]. Surfactant stocks were made in analytical methanol (Lab-Scan, Dublin, Ireland). GP2 medium (Table S-1 in Appendix) was used as artificial seawater, and prepared according to standard procedures [36]. Except for KBr (Sigma-Aldrich, Zwijndrecht, The Netherlands), the salts used to prepare GP2 were obtained from Merck (Darmstadt, Germany). NaN₃ (Merck, Darmstadt, Germany) was added to avoid biodegradation in all tests.

Conditioning procedures for polyacrylate SPME fibers

SPME fiber with a glass core of 108-109 μm diameter and coated with polyacrylate (PA, 7.5-7.6 μm thickness, PA volume 2.72 μL/m) was obtained from Polymicro Technologies (Phoenix, AZ), and disposed after single use. All polyacrylate fibers were cut to 40±0.1 mm length. C₁₂-2-LAS was used to study the uptake kinetics and to compare the effect of the different conditioning treatments. A summary of the applied conditioning treatments is given in Table 1.

For non-conditioned “7-μm” PA coated fibers (used just as received from the supplier, as in ref [12]), SPME uptake profiles were studied during three months of exposure for four different aqueous concentrations. In these experiments, C₁₂-2-LAS was spiked using stock solutions in pure water with 25 mg/L NaN₃, always using less than 1 mL stock per vial (see below). A separate vial was prepared for each time point, in which both the aqueous phase and the SPME fiber were sampled and analyzed. Sorption isotherms, for the concentration range between the detection limit of the SPME extracts and the concentration at which precipitation occurs in seawater (~2 mg/L), were obtained for non-conditioned fibers after four days of exposure (short term apparent equilibrium), and after three months of exposure (long term equilibrium).

For the three other conditioning treatments, uptake kinetics of C₁₂-2-LAS were studied for a period up to 54 d (seawater-conditioned fibers) and 21 d (autoclave-conditioned and temperature-conditioned fibers), and sorption isotherms were determined after 4 d for autoclave-conditioned and temperature-conditioned fibers. The seawater-conditioning consisted of storage of fibers for 1 month in clean artificial seawater (with 25 mg/L NaN₃). Autoclave-conditioned fibers were autoclaved for 8 h in pure water (120 °C at 15 psi), after which the samples were left to cool down slowly inside the autoclave. Temperature-conditioned fibers were kept in the oven of a gas chromatograph under helium flow at 120 °C overnight. After this thermal treatment, the fibers were kept for at least a day in pure water before use, since this significantly lowered the background noise during LC-MS/MS analysis.

Table 1. Conditioning treatments of the disposable 7- μ m polyacrylate SPME fibers for which uptake kinetics and sorption isotherms were determined for C₁₂-2-LAS.

Treatment Name	Procedure
Non-conditioned	SPME fibers were used as received from the supplier.
Seawater-conditioned	SPME fibers were stored for 1 month in seawater, containing 25 mg/L NaN ₃ .
Autoclave-conditioned	SPME fibers were autoclaved (120 °C; 15 psi) in pure water during 8 h.
Temperature-conditioned	SPME fibers were kept in GC oven at 120 °C for 16 h under Helium flow, then stored for at least 1 day in pure water.

SPME measurements with temperature-conditioned fibers

The uptake kinetics were also studied with the temperature-conditioned fibers for the other C_n-2-LAS compounds for periods up to 20 d, at 100 μ g/L with duplicates at each time point. All experiments were performed at room temperature (19 \pm 1 °C) in 20 mL scintillation vials with polyethylene-lined screw caps (Perkin Elmer, Boston, MA). To spike the aqueous phase, a weighed aliquot (<1% of the total volume) of the surfactant stock in methanol was added to 20 mL of artificial seawater. Vials were then completely filled with seawater to reduce the systems headspace in order to prevent differences in the homogeneity of the solution as was observed with alkylethoxylates [12], and SPME fibers were then introduced. The test vials were gently shaken on a Roller mixer SRT9 (Stuart, Omnilabo, Breda, The Netherlands). After exposure, the fibers were wiped with a wet tissue (Milli-Q water), which also reduced inter-fiber variability, and cut in small pieces. LAS compounds were extracted from SPME fibers in HPLC vials (Chromacol, Welwyn Garden City, UK) using 0.5 mL of a solution with the same composition as the LC-MS eluents, consisting of 10 mM ammonium acetate in 80:20 (v:v) methanol-water. Aluminium foil was used to cover the mouth of the vials in order to avoid the relatively high and variable LAS background concentration in the rubber/PTFE material of the screw caps, as has been observed during pilot experiments. Vials were stored at -20 °C until analysis.

The sorption isotherms of all individual LAS compounds were determined after at least 2 d exposure with duplicate temperature-conditioned fibers and two vials for each tested concentration. Again, the range in test concentration depended on both the detection

limit and the solubility limit of each specific compound. LAS in seawater solutions was extracted with C18 SPE columns (Supelclean™ ENVI™-18, 0.5 g, Supelco, Bellefonte, PA), as described in [12]. Deionized water with ammonium acetate was added to the methanol SPE extracts, in order to make the solution comparable to the LC-MS eluent.

Influence of LAS co-solutes on sorption isotherms of individual LAS compounds in simple mixtures

The sorption isotherms of C₁₀-2-LAS, C₁₁-2-LAS or C₁₂-2-LAS were also studied in binary mixtures with C₁₃-2-LAS. If competition would occur, the strongest competition is expected with C₁₃-2-LAS, since this compound has the highest affinity for the SPME. Only iso-molar aqueous concentrations were tested in order to prevent precipitation problems at the highest test concentrations. The experimental set-up was similar as described above for testing individual isomers. In addition, the sorption isotherms of the C_n-2-LAS compounds were also determined in a mixture with all four compounds at equivalent aqueous concentrations. All tests were carried out using at least two separate vials for each concentration.

Analysis of the freely dissolved LAS at concentrations below and above their aqueous solubilities

Solutions of single LAS compounds in seawater were prepared as described above for all the LAS test compounds. Five to seven concentrations for each compound were tested. LAS-seawater concentration tested ranged from up to 3.1 mg/L for the C₁₃-m-LAS isomers to up to 95 mg/L for C₉-1-LAS. In addition, the precipitation of C₁₂-2-LAS and C₁₃-2-LAS was tested in single solutions containing CaCl₂ or MgCl₂ at the molarities of these salts in seawater (0.009M and 0.047M, respectively). Surfactant solutions were kept at 0 °C during 24 h to accelerate the precipitation process, since equilibrium between the precipitate and the dissolved form can take a relatively long time [33,37]. Next, vials were placed at room temperature (19 ± 1 °C) in a shaking device during 24 h after which two fibers were introduced as duplicate samplers. The test systems were then shaken for 72 h, and LAS from SPME fibers was extracted and analyzed.

Chemical analysis

Samples were analyzed on a LC-MS/MS system that consisted of a Perkin Elmer (Norwalk, CT) liquid chromatography system with a triple quadrupole/linear ion trap mass spectrometer (Q-TRAP®, MDS Sciex Applied Biosystems, Foster City, CA), and with a RP-C18 column (3 µm Econosphere, 50 x 3 mm, Alltech, Deerfield, IL). The interface was a Turbo Ion Spray source used in the negative mode, the source temperature was 450 °C and ion-spray voltage was set to -1200 V. Injection volume was 20 µL and the isocratic flow rate was 0.4 mL/min. Eluent solution consisted of 10 mM ammonium acetate in 80:20 (v:v) methanol-water. LAS compounds were measured by screening of the molecular ion [M-H]⁻ 339, 325, 311, 297 and 283 *m/z* and the daughter ion 183 *m/z*, except for the C_n-1-LAS compounds, for which the daughter ion was 170 *m/z*.

RESULTS AND DISCUSSION

Optimizing the SPME method for anionic surfactants

Laboratory equipment often contain background concentrations of LAS [38,39]. Studies in which very low concentrations are reported have normally used ^{14}C labeled LAS (see e.g. [22,40-42]). We detected LAS background concentrations in an ample variety of lab material and in the analytical system. The presence of LAS in eluents was clearly demonstrated when trapping it on the HPLC column (data not shown), as used during gradient elution in e.g. ref [43]. The cleanest glassware was selected and lab material was washed with analytical methanol, although some background LAS always remained. The quantification limit for most of the LAS compounds was restricted by this background concentration to 1 $\mu\text{g/L}$. For the calibration of the SPME method and the calculation of the K_{fw} , the equilibration time has to be known. In a previous study with alcohol ethoxylates, using 35- μm polyacrylate SPME fibers [12], the equilibration time was between 2 and 4 days. However, in a pilot experiment with C_{12} -2-LAS using the same 35- μm PA fiber during 20 days, equilibrium was not observed (data not shown). Due to the larger surface/volume ratio, faster equilibration kinetics were expected to occur for the 7- μm PA SPME fiber. Figure 1A shows the uptake kinetics of C_{12} -2-LAS for the non-conditioned fibers at several aqueous concentrations. The measured aqueous concentrations remained constant throughout time, indicating that both the depletion of the aqueous phase by uptake in the SPME, and losses to the vial surfaces were negligible (<5%). An apparent equilibrium for the fiber-water distribution coefficient (D_{fw}) was observed for the non-conditioned fibers after 4 days. It becomes clear from the uptake curves for the different concentrations that the D_{fw} at this apparent equilibrium is strongly concentration dependent. At prolonged exposure of the non-conditioned fibers, however, the higher apparent equilibrium plateau of the D_{fw} for the lower LAS concentrations steadily decrease after seven days. Somewhere between days 60 and 110, a new equilibrium occurs and all D_{fw} values are comparable to that for the highest concentration. This “excessive uptake” in the first days of exposure for lower LAS concentrations, is clearly reduced in seawater-conditioned, autoclaved, and temperature-conditioned fibers (Figure 1B). Whereas the seawater-conditioned fibers still display some excessive uptake of LAS, the D_{fw} of the autoclave-conditioned fibers and temperature-conditioned fibers obtained from the first few days of exposure are comparable to the long term equilibrium D_{fw} for the non-conditioned fibers, even though the latter 2 fibers were tested at lower LAS concentrations. The sorption isotherms for C_{12} -2-LAS with the differently conditioned SPME fibers (Figure 2), also demonstrate the effect of conditioning on the concentration dependency of the D_{fw} . At the apparent 4-day equilibrium, the isotherm for the non-conditioned fiber is nonlinear (slope of linear regression in Figure 2 is 0.57), whereas the sorption isotherm for non-conditioned fibers exposed for 110 days is linear (slope of 1.01). The sorption isotherms obtained with the autoclave-conditioned fibers and the temperature-conditioned fibers after a 4-day exposure period are also linear, and overlap the data obtained with the non-conditioned fiber exposed for 110 days.

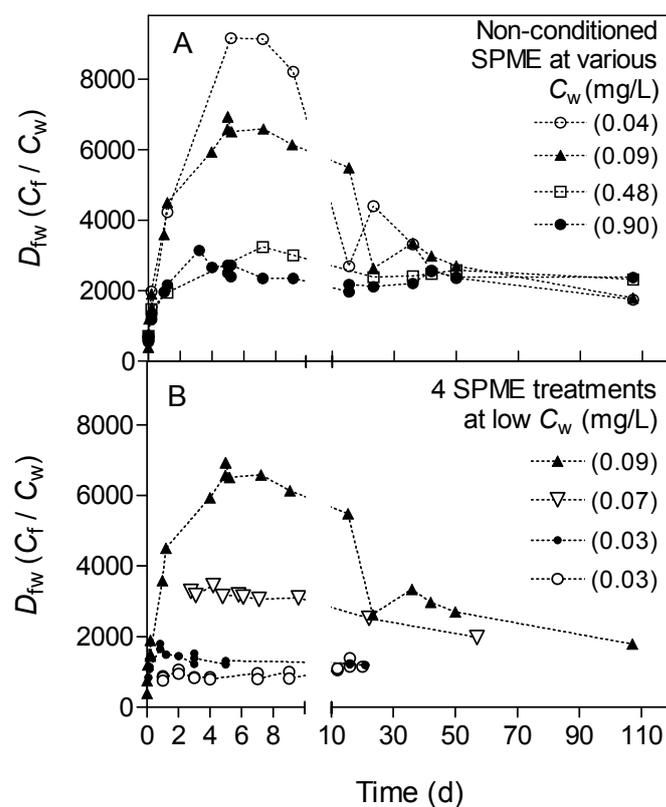


Figure 1. SPME uptake kinetics of C_{12} -2-LAS from seawater during 110 days, using (A) nontreated PA fibers at varying aqueous concentrations and (B) different conditioning procedures at relatively low concentrations: non-conditioned (\blacktriangle), seawater-conditioned (∇), autoclave-conditioned (\bullet), and temperature-conditioned (\circ). Dotted lines connect the sampling points of each experiment in time.

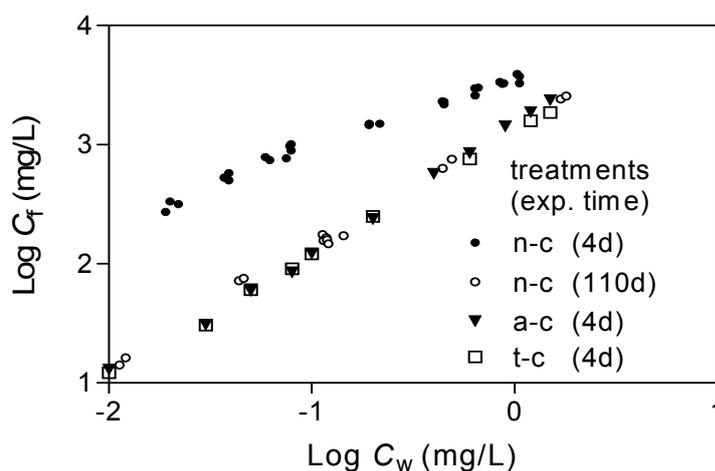


Figure 2. Concentration of C_{12} -2-LAS in SPME fibers plotted against aqueous concentrations after a certain time of exposure. Non-conditioned fibers were either sampled after 4 days (\bullet , n-c) or 110 days (\circ , n-c). Autoclave-conditioned fibers (\blacktriangledown , a-c) and temperature-conditioned fibers (\square , t-c) were exposed for 4 days.

Tentative interpretation of the conditioning effect

The excessive uptake of LAS in SPME is reduced by both hydration (seawater conditioned fibers and long term exposure of non-conditioned fibers) and heating of the fibers (autoclaved and fibers conditioned under helium at 120 °C), as shown in Figure 1. This suggests that the mechanism underlying the decreasing D_{fw} after the apparent equilibrium is related to a transition of (part of) the polymer phase from a glassy to a rubbery state. Although the exact sorption sites remain unidentified, we hypothesize that LAS sorbs relatively strong to sorption sites in the initially glassy polymer structure of the non-conditioned fiber, or at the fiber surface. As the polymer of the non-conditioned fiber slowly becomes fully rubbery, because of a lowering of the glass transition temperature (T_g) due to hydration, LAS absorbs into the polymer phase with a constant sorption coefficient. By heating, and slowly cooling down, the polymer phase of autoclave- and temperature-conditioned fibers may also be brought to the rubbery state, and maintains this state after the conditioning. For the 4-days exposed non-conditioned fibers, the D_{fw} for the highest test concentrations is in the same range as the concentration independent D_{fw} after 110 days exposure (Figure 2). This could be interpreted as a plasticizing effect of LAS itself, lowering the polymer's T_g to below room temperature, so that D_{fw} is only related to the absorption affinity for the rubbery polymer state.

The linear isotherm for the model compound C₁₂-2-LAS indicates a simple partitioning process of the LAS compound into the polymer, with a single sorption coefficient (K_{fw}). On the other hand, there are indications that untreated fibers in methanolic solutions enhance the actual LAS signal in the LC-MS/MS via a matrix effect that remains to be identified (unpublished data). Therefore, an alternative or additional explanation for the reduction of 'excessive uptake' at prolonged exposure to relatively low concentrations, is via a slowly decreased matrix effect after prolonged exposure to water, or total removal of the matrix effect by the applied conditioning treatments. Although we realize that these explanations are speculative, the data clearly show the potential effect of the pre-treatment on the sorption characteristics of the polyacrylate coating.

Temperature-conditioned fibers were selected for further experiments with other LAS compounds and mixtures, being the most reproducible and convenient method (a large batch of more than 50 fibers of e.g. 40 mm can be conditioned simultaneously under helium in most GC ovens).

K_{fw} of single LAS compounds for conditioned polyacrylate SPME and the influence of co-solutes

Uptake experiments for other LAS homologues (C₁₀-2-, C₁₁-2 and C₁₃-2-LAS) were performed to determine which equilibration period is sufficient to reach equilibrium for all LAS compounds in temperature-conditioned fibers. The longest exposure period required to obtain 95% steady state, was 33.3 h for C₁₃-2-LAS (Figure S-1 in Appendix). For the subsequent experiments with all LAS compounds, the systems were always equilibrated within 48 h. Linear sorption isotherms were fitted to the sorption data for all individual LAS compounds (Figure 3, and S-3 in Appendix), resulting in concentration independent K_{fw} in all

cases (Table 2). In the applied set-up, the extraction from the aqueous phase by the SPME fibers is non-depletive for all tested LAS compounds, with the maximum depletion of 1.8 % for C₁₃-2-LAS. As discussed further on, K_{fw} values vary with the alkyl chain length (Figure 3A) as well as with the position of the benzene-sulfonate group (Figure 3B). The linear isotherms indicate that sorption of LAS to the temperature-conditioned fibers is driven by passive diffusion into the fiber polymer, without a significant effect of adsorption to the fiber surface. The assumption of an absorption process is also supported by the results of the experiments with LAS mixtures, for which the fitted K_{fw} values are presented in Table 2 (the sorption isotherms are presented in Figure S-2 of the Appendix). The K_{fw} values fitted to the data with the binary mixtures (C_n-2-LAS and C₁₃-2-LAS) as well as to the data of the mixture of four C_n-2-LAS compounds were not significantly different from those obtained from the experiments with single compounds. Although aqueous concentrations were isomolar, C₁₃-LAS concentrations in the fiber were up to a factor 30 higher than the other compounds due to its higher affinity. The effect of LAS co-solutes on the K_{fw} of single LAS compounds is thus negligible. The presented K_{fw} values should therefore be applicable to all LAS mixtures in seawater samples at a comparable temperature.

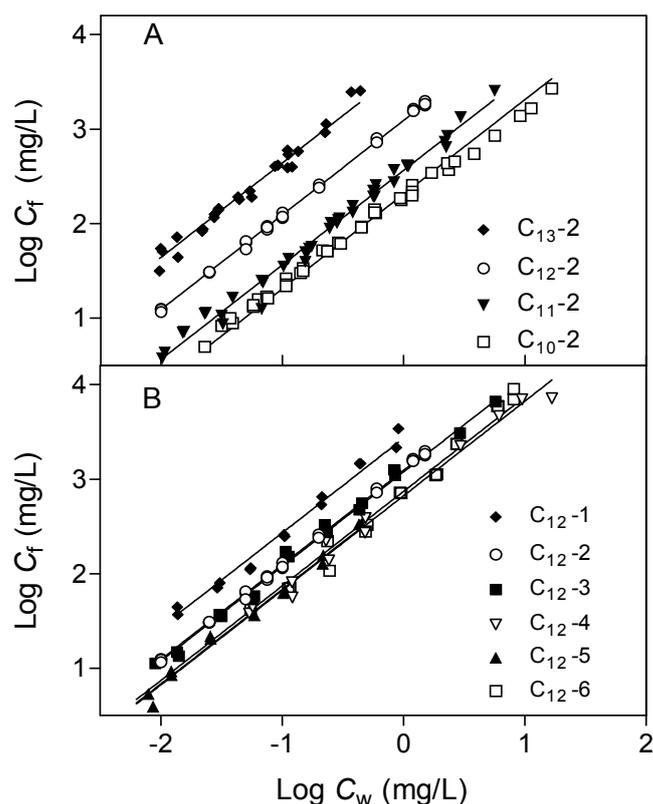


Figure 3. Fiber-seawater isotherms with thermally conditioned 7- μ m PA fibers for (A) C_n-2-LAS homologues, and (B) C₁₂-m-LAS isomers.

Table 1. Sorption coefficients for linear alkylbenzene sulfonates between polyacrylate SPME fibers and seawater (K_{fw}), aqueous solubility and solubility Products (K_{sp}) for Ca^{2+} and Mg^{2+} .

Compound	Log $K_{fw} \pm SE$	N^e	Aqueous Solubility (mg/L)	K_{sp}	
C ₉ -1-LAS	2.1 ± 0.02	16	17.6		
C ₁₀ -1-LAS	2.6 ± 0.03	9	8.6		
C ₁₀ -2-LAS	2.3 ± 0.01	34	17.4		
	2.4 ± 0.03 ^a	11			
	2.3 ± 0.02 ^b	12			
C ₁₁ -2-LAS	2.6 ± 0.01	39	8.1		
	2.5 ± 0.02 ^a	12			
	2.4 ± 0.02 ^b	14			
C ₁₁ -5-LAS	2.4 ± 0.02	17	19.0		
C ₁₂ -1-LAS	3.4 ± 0.02	16	1.2		
C ₁₂ -2-LAS	3.1 ± 0.01	25	2.2		
	3.1 ± 0.02 ^a	11			
	3.1 ± 0.02 ^b	20			
	3.0 ± 0.06 ^c	4		3.1 ^c	8.0·10 ^{-13 c}
	2.7 ± 0.04 ^d	4		4.0 ^d	7.1·10 ^{-12 d}
C ₁₂ -3-LAS	3.1 ± 0.02	17	5.3		
C ₁₂ -4-LAS	2.8 ± 0.02	12	13.0		
C ₁₂ -5-LAS	2.8 ± 0.02	16	3.8		
C ₁₂ -6-LAS	2.9 ± 0.03	17	7.7		
C ₁₃ -2-LAS	3.6 ± 0.02	26	0.3		
	3.7 ± 0.02 ^a	12			
	3.5 ± 0.06 ^c	3		0.5 ^c	2.3·10 ^{-14 c}
	3.2 ± 0.01 ^d	3		1.5 ^d	8.9·10 ^{-13 d}
C ₁₃ -5-LAS	3.3 ± 0.02	16	1.8		

^a Obtained from solutions with C_n-2-LAS mixtures simple-mixture experiment (Data are represented in Figure S-3)

^b Obtained from solutions with C₁₃-2-LAS (Data are represented in Figure S-3).

^c Obtained from the experiment with only CaCl₂

^d Obtained from the experiment with only MgCl₂

^e N = number of samples

Interpretation of K_{fw} values

The concentration independent K_{fw} values for all individual LAS compounds and mixture experiments are presented in Table 2, while Figure S-2 and S-3 provide additional plots of sorption data. The effect of the number of methyl groups on the sorption to the SPME fiber is shown in Figure 3A. The difference of 0.2 log units between the K_{fw} for C₁₀-2-LAS and C₁₁-2-LAS was markedly smaller than the difference between the other LAS compounds. The increment of 0.5 log units between C₁₁-2-LAS, C₁₂-2-LAS and C₁₃-2-LAS, however, corresponds to sorption data of LAS compounds to sediment, which is suggested to be mainly the result of a higher hydrophobicity [22,42]. Similar variation is also found among the measured and estimated parameters such as K_{ow} and aqueous solubilities for unsulfonated linear alkylbenzenes [44]. A considerable difference of 0.3 log units in the K_{fw} is observed between LAS molecules with the phenyl groups in the first and the second position of C₁₀-m-LAS and C₁₂-m-LAS (Table 2, Figure 3B). Long alkyl chains and/or long un-substituted alkyl fragments in the molecule will enable more Van der Waals interactions [22]. This is again in agreement with the differences in the measured K_{ow} values for the alkylbenzene isomer groups [44]. While the K_{ow} for the second and the most internal phenyl positions differed less than 0.2 log units, up to 0.5 log unit difference was observed between the 1-phenyl and the 2-phenyl alkylbenzene isomers. Figure 3B shows that, besides the C₁₂-1-LAS compound, the other inner isomers of C₁₂-m-LAS form two groups: the K_{fw} values for external phenyl positions (C₁₂-2-LAS and C₁₂-3-LAS) are indistinguishable, but differ from the K_{fw} values of the cluster of the LAS molecules with the phenyl group in a central position (C₁₂-4-LAS, C₁₂-5-LAS and C₁₂-6-LAS). The same effect was also observed in the separation of the isomers in the LC/MS/MS analysis. A complete separation of the external and internal isomers of C₁₂-m-LAS was possible with the described method, but separation of the specific isomer could not be achieved. Log K_{fw} difference between the second and the fifth phenyl position isomers for C₁₁-m-LAS is 0.2, but 0.3 and 0.4 for C₁₂-m-LAS and C₁₃-m-LAS isomers, respectively. Although the number of studies focused on specific LAS isomers is limited, compounds with the most external phenyl position also showed a higher sorption affinity to fresh water sediments [22]. and higher tendency to accumulate in fish, compared to the inner isomers [45]. Because the incremental factor for Log K_{fw} is not perfectly constant for both the alkyl chain length and the position of the benzene-sulfonate group along the alkyl chain (Table 2), extrapolation of K_{fw} values to other LAS congeners not used in this study should be done with care.

Application of K_{fw} : Precipitation studies

Precipitation of LAS with seawater salts limited the concentration range that could be tested in this study. Still, this phenomenon forms an interesting test case for demonstration of the applicability of SPME fibers with anionic surfactants. When the solubility limit is reached, the freely dissolved concentration will remain constant at a certain maximum, corresponding to the aqueous solubility. As demonstrated in Figure 4 for C₁₂-m-LAS and C₁₃-m-LAS isomers, the concentration in the equilibrated SPME fiber also reaches a maximum. Flocks of precipitated salt were visually detected in the solutions corresponding to this plateau region.

SPME fibers have been used in a similar way in a study to demonstrate that aqueous solubility limits the concentrations in pore water of polycyclic aromatic compounds in spiked soils [46] and in another study that reported the critical micelle concentration of nonionic surfactants [12]. To estimate the maximum soluble concentration of each compound in the artificial seawater (Aqueous Solubility-Table 2), the average surfactant concentration in the fiber at the plateau region was divided by the K_{fw} value of each compound in seawater.

The shorter alkyl chain homologues precipitated at the highest concentrations, and internal phenyl isomers were more soluble than the external ones. Within the C_{12} -m-LAS isomers, C_{12} -4-LAS was the most soluble compound. A similar effect has been previously detected for single isomer LAS solutions [47], for which the solubility in a 10 mM $NaSO_4$ solution was explained by the alkyl chain length and symmetry of the specific isomers. Interactions are favored for the longer alkyl chain LAS molecules and for the isomers with the most symmetrical distribution [47]. Consequently, solubility will be higher for the most asymmetrical isomers such as C_{12} -4-LAS.

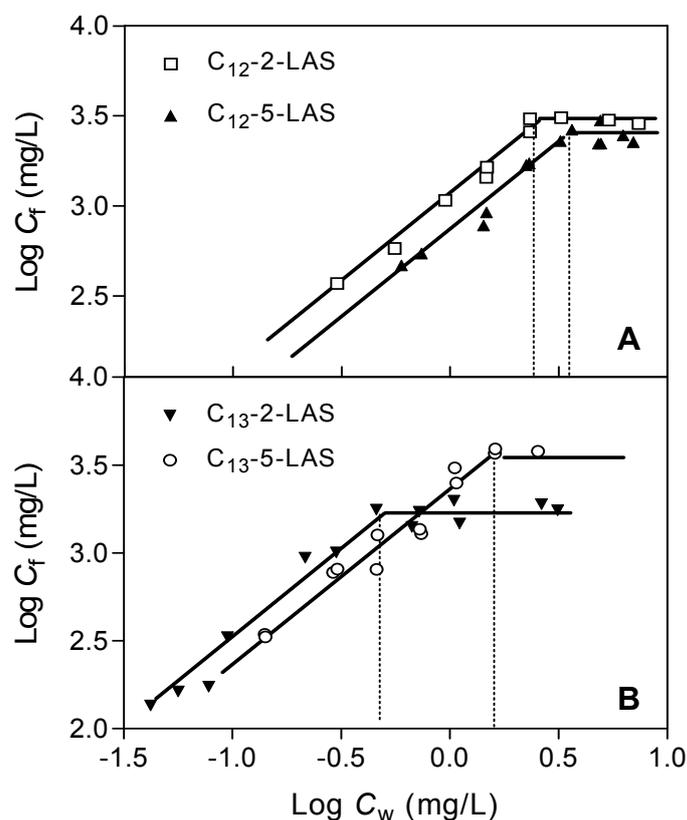


Figure 4. LAS concentration in SPME fibers (C_f) at different total surfactant concentrations in seawater for two isomers of C_{12} -m-LAS (A) and two isomers of C_{13} -m-LAS (B). Broken lines separate the region below and above the precipitation of each compound with the seawater salts. The aqueous solubilities were calculated by dividing the C_f average (calculated from the level where C_f is constant) by the K_{fw} from Table 2.

Although sodium is the most abundant cation in seawater, it is well known that the divalent cations calcium and magnesium are mainly responsible for LAS precipitation [34,48]. Ca^{2+} and Mg^{2+} concentrations in the artificial seawater solution are 0.009 and 0.047 M, respectively. In order to know if LAS precipitation in seawater can be explained by the presence of these cations, separate experiments were performed with solutions containing the salts CaCl_2 or MgCl_2 at the same molarity as in artificial seawater, and using the compounds C_{12} -2- and C_{13} -2-LAS. Data obtained from these experiments are shown in Figure 5. As it was observed for the artificial seawater, C_f increases linearly when increasing the concentration of these two compounds in CaCl_2 and MgCl_2 solutions. Obtained K_{fw} values are higher for the compound with longer alkyl chain. Due to the fewer number of data points for these experiments, sorption data were not fitted to an isotherm, and K_{fw} values were estimated by the average of the K_{fw} values from the data points before the plateau region. Standard errors of the K_{fw} values are all considerably small ($\text{SE} \leq 0.06$). K_{fw} values obtained from the experiments with CaCl_2 are similar or slightly inferior to the K_{fw} values obtained for the complex seawater solutions. However, K_{fw} values from the MgCl_2 experiment are approximately 0.4 log units lower than that obtained in seawater. This result may be explained by the mechanism at which LAS molecules are absorbed into the SPME fiber. Ion pairing is believed to be the process that governs partitioning of ionisable organic compounds into hydrophobic phases such as lipids or octanol [14,23]. If such assumption is also valid for LAS sorption into the SPME polymer, the sorption data obtained for the two salt solutions might indicate that LAS molecules are more readily to form a neutral complex (in the form $\text{CaLAS}^+ + \text{LAS}^-$) with Ca^{2+} than with Mg^{2+} .

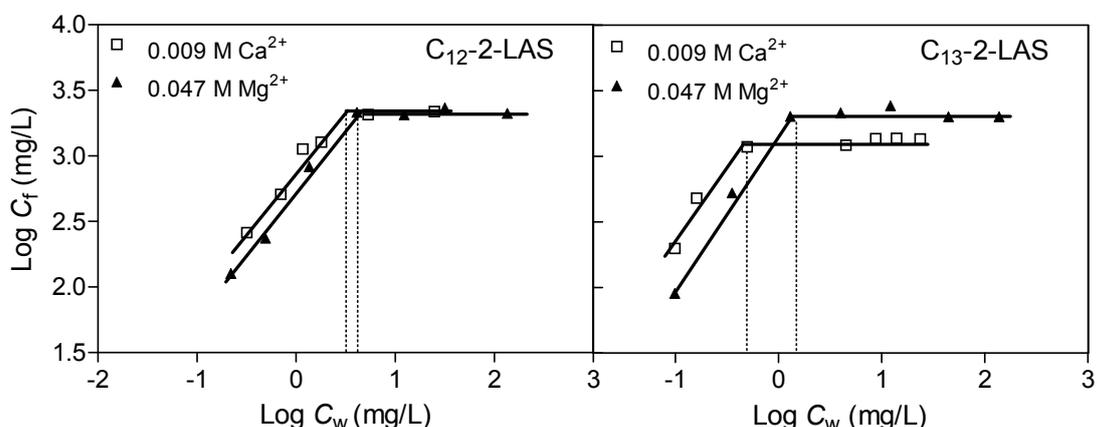


Figure 5. LAS concentration in SPME fibers (C_f) at different total surfactant concentrations in CaCl_2 or MgCl_2 solutions for C_{12} -2-LAS and C_{13} -2-LAS. Broken lines separate the region below and above the precipitation of each compound with the seawater salts. The aqueous solubilities were calculated by dividing the C_f average (calculated from the level where C_f is constant) by the K_{fw} from Table 1.

Maximum soluble concentrations for the experiments with single CaCl_2 or MgCl_2 solutions were calculated as for the seawater experiments. Maximum soluble concentrations were used to calculate the solubility product (K_{sp}) of LAS with Ca^{2+} and Mg^{2+} by using the expression:

$$K_{\text{sp}} = [\text{LAS}]^2 \cdot [\text{M}^{2+}]$$

Obtained K_{sp} values for the $\text{Ca}(\text{LAS})_2$ and $\text{Mg}(\text{LAS})_2$ salts and LAS aqueous solubility in CaCl_2 and MgCl_2 solutions are listed in Table 1. According to the precipitation diagram for a commercial C_{11.5}-m-LAS mixture with Mg^{2+} [34], the K_{sp} for Mg^{2+} with such a LAS mixture at seawater conditions (0.047 M Mg^{2+}) is $1.3 \cdot 10^{-10}$. The K_{sp} for Ca^{2+} at seawater conditions (0.009 M Ca^{2+}) with a C_{11.9}-m-LAS mixture is $1.0 \cdot 10^{-12}$ [33]. Comparison of the obtained results with the literature data is nonetheless complicated, because previous studies have always used a mixture of homologues or isomers. However, the obtained K_{sp} values for the $\text{Mg}(\text{LAS})_2$ complex are also higher than for the $\text{Ca}(\text{LAS})_2$, and this is in agreement with literature observed trends [33,34,49]. Based on the available data and lower K_{sp} for $\text{Ca}(\text{LAS})_2$ than for $\text{Mg}(\text{LAS})_2$, LAS dissolved in seawater is thus expected to precipitate with Ca^{2+} first, but the effect of Mg^{2+} and other ions in solution can not be ruled out, since the solubility of LAS in seawater is not only explained by the Ca^{2+} concentration.

The reported results with concentrations above the solubility of LAS are again a clear indication that only freely dissolved LAS molecules are sorbed into the PA SPME, showing the strength of the SPME analysis to be performed in matrices difficult to separate by traditional techniques.

Potential application of nd-SPME for LAS compounds

In the present study, the extraction method with polyacrylate SPME fibers has been calibrated for its use in seawater for a wide range of LAS compounds. Although not all LAS compounds present in commercial mixtures were tested, the simplicity and sensitivity of the method makes it a promising tool for systems where extraction of the dissolved fraction is complex. Reported concentrations of LAS from monitoring studies in effluent receiving waters in the Catalanian region in Spain [18], and in the pore water of the sediments from the South of Spain [50], are in the range of the quantification limits of the developed SPME method. However, studies generally report total homologue concentration, since isomer differentiation and quantification is difficult in the absence of specific standards [51]. The applicability of the current non-depletive SPME method for environmental sampling is limited though, due to the relatively small proportion of each LAS structure in commercial mixtures, in combination with both relatively high background contamination from various sources and low solubility products in seawater. However, application of the nd-SPME method for LAS can be readily extended to other laboratory based studies, such as aqueous solubility, sorption of LAS to complex matrices as sediments and dissolved organic material, etc. Such studies are highly needed to improve both the qualitative and quantitative understanding of a wide variety of environmental processes of LAS.

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APPENDIX

A. Artificial seawater composition

Table S-1. Salt composition of the artificial seawater used. According to EPA protocol [36]. Salts were added in the listed order to MilliQ water. NaN_3 was added when needed. The final seawater was of 31‰ salinity and pH of 8.14.

Salt	Concentration (g/L)
NaCl	21.03
Na_2SO_4	3.52
KCl	0.61
KBr	0.088
$\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$	0.034
$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$	9.5
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	1.32
$\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$	0.02
NaHCO_3	0.17

B. Data analyses

Uptake curves were generated using the first-order one-compartment model. This model has been widely applied to describe the kinetics using SPME for organic compounds [7,52] and it is expressed by the equation:

$$D_t = D_{ss} \cdot (1 - e^{-k \cdot t}) \quad (1)$$

where D is the ratio C_f/C_w for each time point (D_t) and at the steady state (D_{ss}), t is the time. The rate constant k is used to estimated the time needed to reach the 95% equilibrium in the system by the expression:

$$t_{95\%} = \frac{\ln(0.05)}{-k} \quad (2)$$

at the considered steady state, depletion from the aqueous phase was calculated as:

Depletion:

$$\text{Depletion}(\%) = \frac{100 \cdot V_f \cdot K_{fw}}{V_w} \quad (3)$$

where V_f and V_w are the volumes of the fiber and the aqueous phase respectively.

Each data range obtained to calibrate the SPME fiber for the different LAS compounds was fitted to the log-log regression line:

$$\text{Log}C_f = \text{Log}K_{fw} + \text{Log}C_w \quad (4)$$

All data analyses were performed using Graphpad Prism version 4.00 for Windows, (GraphPad Software, San Diego, CA, USA).

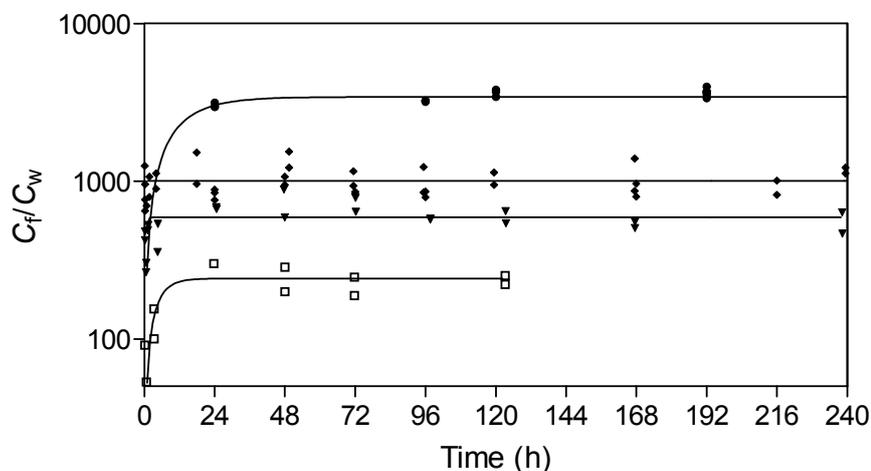


Figure S-1. Uptake profiles in thermally conditioned SPME fibers, expressed as the fiber-seawater distribution coefficient, for four LAS compounds varying in alkyl chain: C₁₀-2-LAS (■), C₁₁-2-LAS (▼), C₁₂-2-LAS (◆) and C₁₃-2-LAS (●).

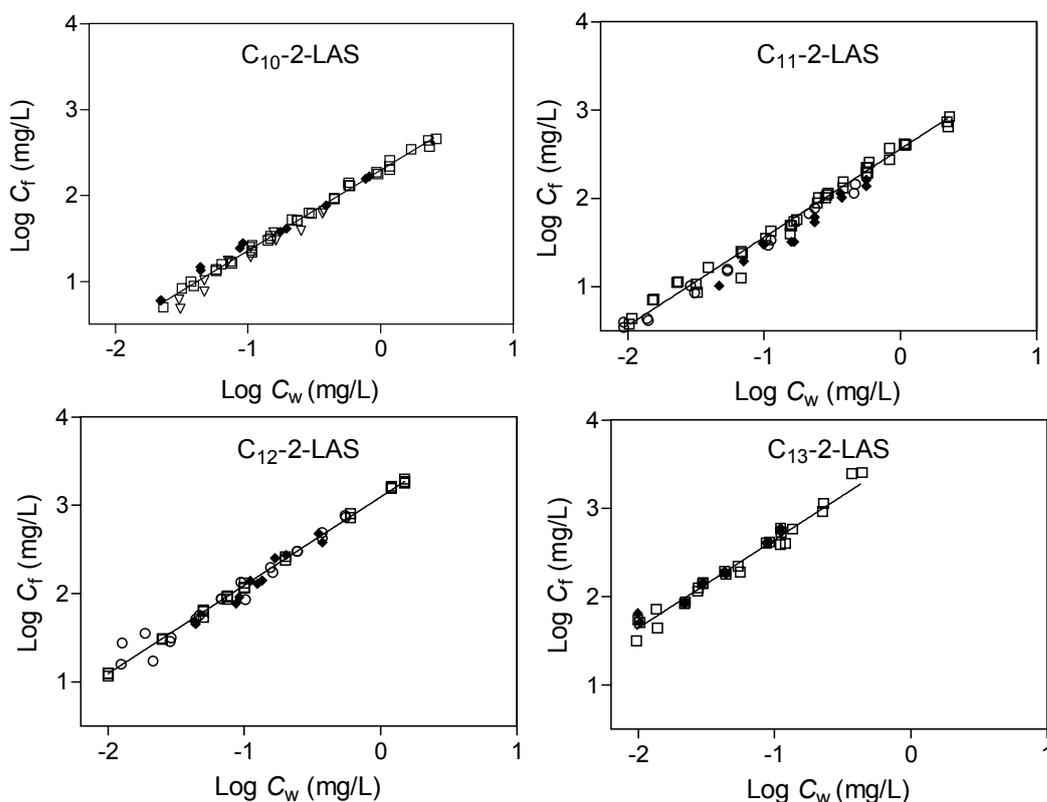


Figure S-2. Fiber-seawater isotherms with thermally conditioned 7- μ m PA fibers of C_n-2-LAS compounds: single exposure (□), binary mixtures (○) and mixture with all 4 compounds (◆).

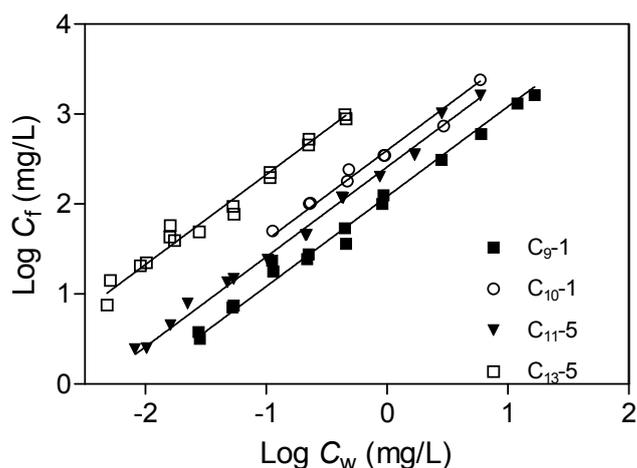


Figure S-3. Fiber-seawater isotherms with thermally conditioned 7- μ m PA fibers of C₉-1-LAS, C₁₀-1-LAS, C₁₁-5-LAS and C₁₃-5-LAS.

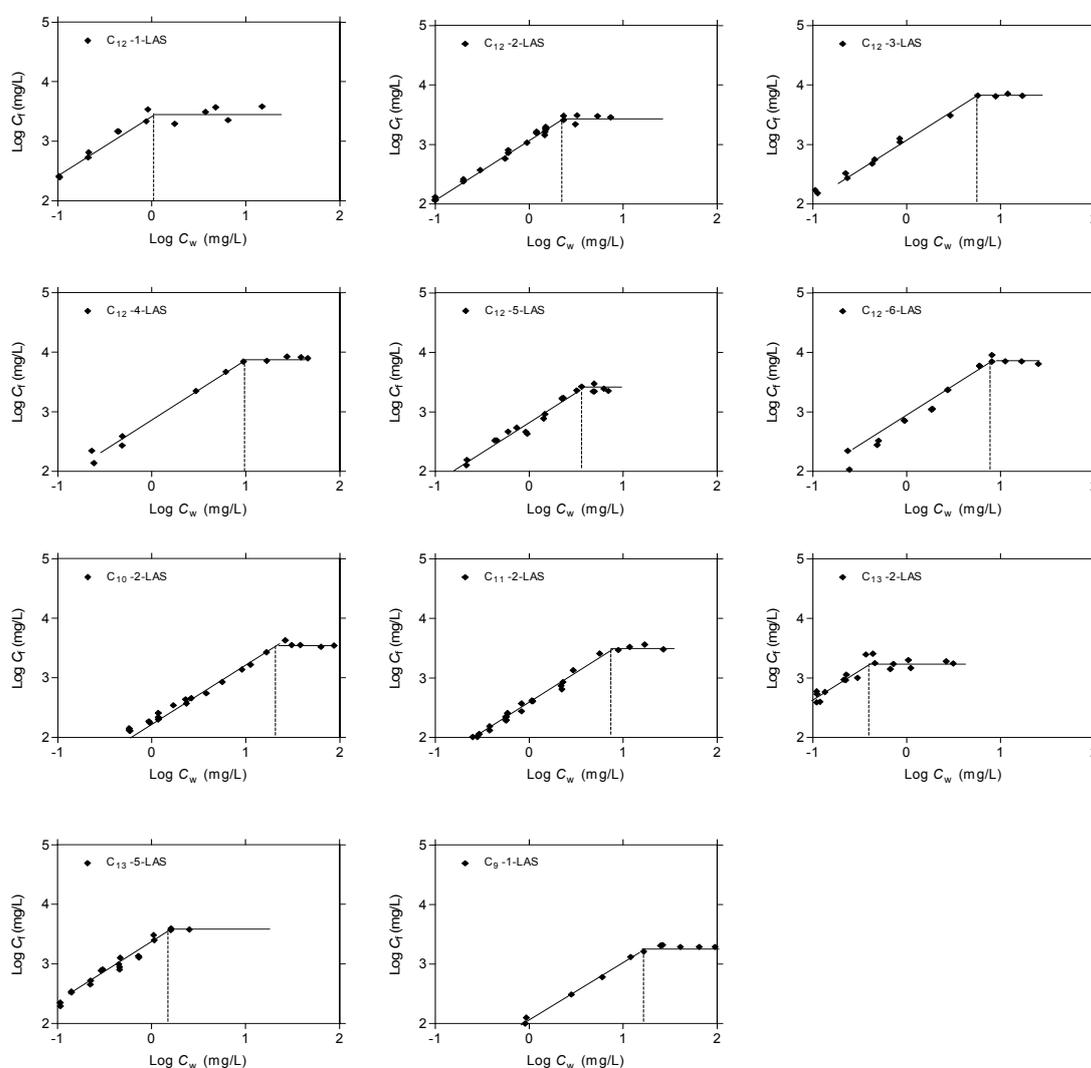


Figure S-4. SPME measurements of freely dissolved LAS compounds up to concentrations above the precipitation point of the compounds in seawater solutions.

Chapter 3

Effect of sediment properties on the sorption of C₁₂-2-LAS in marine and estuarine sediments

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ABSTRACT

Linear alkylbenzene sulfonates (LAS) are anionic high production volume surfactants used in the manufacture of cleaning products. Here, we have studied the effect of the characteristics of marine and estuarine sediments on the sorption of LAS. Sorption experiments were performed with single sediment materials (pure clays and sea sand), with sediments treated to reduce their organic carbon content, and with field marine and estuarine sediments. C₁₂-2-LAS was used as a model compound. Sorption to the clays montmorillonite and kaolinite resulted in non-linear isotherms very similar for both clays. When reducing the organic content, sorption coefficients decreased proportionally to the fraction removed in fine grain sediments but this was not the case for the sandy sediment. The correlation of the sediment characteristics with the sorption coefficients at different surfactant concentrations showed that at concentrations below 10 µg C₁₂-2-LAS/L, the clay content correlated better with sorption, while the organic fraction became more significant at higher concentrations.

INTRODUCTION

Linear alkylbenzene sulfonates (LAS) are high production volume surfactants commonly used in the formulation of industrial and domestic cleaning products. Commercial LAS consist of a mixture of several isomers with an alkyl chain length of 10 to 13 carbon atoms, and a benzene-sulfonate group at the para position, attached to the carbon chain. The average number of carbon atoms in the alkyl chain of commercial LAS is 11.7-11.8 [1].

LAS reach the marine environment by urban and industrial wastewater effluents and can be detected mainly in the sediment, due to their affinity to sorb to the particulate material [2]. Monitoring studies have reported concentrations up to 238 mg/kg of LAS in coastal areas in the Northeast and South of Spain close to waste water effluents (primary or no waste water treatment at all) [3,4]. The sorption of the various LAS homologues and isomers in sediments will likely drive its bioavailability to the endofauna.

The sorption of a compound to sediment depends on the physico-chemical properties of the chemical, the water composition and the sediment characteristics. A number of authors have reported on the effects of the test system (salinity, temperature, etc) or the sediment matrix characteristics (organic carbon content, particle size, metal oxides, etc) on sorption, but a mechanistic model for the sorption of LAS is not yet available. LAS sorption increases with the number of methyl groups in the alkyl chain and with the position of the phenyl group [5,6] likely because the strength of van der Waals interactions increases with an increase in the chain length. Isomers with the phenyl position closer to the end of the alkyl chain have more available surface for Van der Waals forces [5] leading to higher sorption affinities. An increase in sorption with the salinity was observed in marine sediments [7], and with the increase in H⁺ concentration in riverine sediments [5].

Several studies have shown that LAS sorption to sediments and soils is correlated with the organic carbon or the clay content [5,8-10]. Considering that organic carbon and clay content are usually well related to each other in sediments, it is not clear yet which of these two components control LAS sorption. Sorption of LAS to soil and sediment has also been related to aluminium oxides content [5,9,11], or the concentration of iron oxides [11]. Amorphous iron oxides showed a higher sorption affinity than crystalline iron oxides due to the larger surface area of the first ones [11]. In summary, sorption of LAS to sediment has been related to both non-specific (Van der Waals) and specific (polar) interactions and this is not surprising because the LAS molecule has both a non polar and polar functionality.

The understanding of the key parameters that characterize the bioavailability of chemicals in marine sediments is still limited. To our knowledge, few studies have been performed on the sorption of LAS in marine sediments [7,8], and these studies were focused on the effect of aqueous chemistry rather than sorbent characteristics. Sorption studies with riverine sediments are a helpful first approach but do obviously not consider the specifics of seawater composition. Seawater composition is characterized by a high salt content and a relatively constant pH. Sorption coefficients for LAS in seawater media are often higher than in the freshwater environment [2]. Higher concentrations of divalent cations, especially Ca²⁺, will affect the solubility of LAS in the seawater as well as the intensity of its sorption to the

marine sediments. The effect of Ca^{2+} on the sorption of anionic surfactants has already been investigated in studies where the ion concentration was changed [5,12]. The increase in sorption was explained by a decrease in the negative charge of the sediment particles due to the adsorption of Ca^{2+} to the particle surface that consequently favours electrostatic interactions.

The objective of this work is to determine which characteristics of marine sediments influence the sorption of LAS. Besides the testing of field marine and estuarine sediments, this work presents for the first time the contribution of individual sediment constituents under seawater conditions. For this purpose, sorption experiments with pure clays, sea sand, sediments, and sediments treated to reduce their organic carbon fraction, were performed with the model compound 2-n-(p-sulfophenyl)dodecane (C_{12} -2-LAS). Next, several field sediments were characterized in terms of organic carbon, clay content, specific surface area and iron and aluminium oxides. Sediment characteristics were then correlated with the distribution coefficients at different surfactant concentrations in order to understand the main denominators that affect the sorption in the complex sediment matrix.

MATERIALS AND METHODS

Chemicals

C_{12} -2-LAS (97.4% purity) was synthesized in our laboratory by Dr. J.Tolls. Surfactant stocks were made in analytical methanol obtained from Lab-Scan (Dublin, Ireland). Artificial Seawater GP2 medium (pH= 8.3) [13] was made from deionized water prepared with Millipore Milli-Q purification system (resistivity of circa 18 M Ω cm) by adding the salts NaCl, Na_2SO_4 , KCl, $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$ and NaHCO_3 (all from Merck, Darmstadt, Germany) and KBr and KCl salts (Fluka, Buchs, Switzerland). 10 mM NaN_3 (Merck, Darmstadt, Germany) was used as a biocide to avoid biodegradation during the experiments. NaOCl and HCl used for the reduction of the organic fraction were obtained from Fluka (Buchs, Switzerland) and Merck (Darmstadt, Germany), respectively.

Collection and characterization of the sediments and materials properties

Marine and estuarine sediments were collected from the North Sea "Friese Front" area (North Sea 1, 2, 3) and the Eastern Scheldt estuary (Oesterput) in The Netherlands and from four different locations in the Southwest of Spain (La Antilla and Espigón areas in Huelva, Cádiz and San Fernando in the Bay of Cádiz). Freshwater sediment from the Drontermeer Lake (The Netherlands) was only used for the study of the effect of the organic carbon content on the sorption of LAS to sediments and was supplied by Aquasense (Grontmij, The Netherlands). Sediments were sieved (500 μm mesh) and stored at 4°C until their use. The background concentrations of LAS in all areas were relatively low (< 0.2 mg LAS /kg). Sea sand purified by acid and calcined was obtained from Merck (Darmstadt, Germany). Standard kaolinite (K Ga-1) obtained from Washington County (Georgia, USA) and Ca-montmorillonite (SAz-1) from Apache County (Arizona, USA) was kindly provided by the

Geochemistry group of the Department of Earth Sciences at Utrecht University. Both clays were first equilibrated in artificial seawater to avoid differences in the composition of the ions in the media during the sorption experiments. The equilibration was performed as follows: 5 g of clay was suspended in 20 mL of seawater and sonicated in two steps of 30 minutes. The samples were then centrifuged at 3000 rpm and the supernatant was carefully removed. The clay samples were homogenized and stored at 4°C until their use.

Physico-chemical characteristics of the sediments and materials were determined according to standard procedures [14] at the Integrated Laboratory of the Faculty of Geosciences/ TNO at Utrecht University, except for the Drontermeer Lake sediment that was characterized by Analytico Milieu B.V. (Barneveld, The Netherlands).

Removal of the organic fraction of the sediments

Two of the marine sediments (Cádiz and San Fernando) and the Drontermeer Lake sediment were treated to remove the organic fraction, following an adapted methodology with sodium hypochlorite as described by Mikutta et al. [15] and Kaiser et al. [16]. Briefly, sediment samples of ca. 1 g (d.w.) were mixed with 45 mL of a solution of 1M NaOCl adjusted to pH 8.0 with concentrated HCl. The suspension was shaken over 6 h, followed by a centrifugation at 3000 rpm during 30 min, and the supernatant was then discarded. The treatment was repeated five times and samples were subsequently washed with deionised water and left to dry in air. The organic fraction before and after this treatment was measured by loss of ignition at 500 °C. With the use of the NaOCl method, the effects of the treatment on the original matrix are minimized since the whole procedure is performed at room temperature and the natural pH of the marine sediments is not affected. Other traditional methods using H₂O₂ or Na₂SO₈ need prolonged heating (>40 °C), causing the transformation of poorly crystalline minerals into more crystalline forms and consequently reducing the surface area of the sediment materials [17], ultimately affecting sorption characteristics.

Determination of the sorption coefficients

Experiments were performed at a density of sediment of 0.1 g (d.w.) in 20 mL seawater solution containing 10 mM NaN₃. 20 mL scintillation vials (Perkin Elmer, Boston, MA, USA) were used. For the sorption experiments with sea sand, 2 g of sand were used because of the low depletion from the aqueous phase by this material. C₁₂-2-LAS was spiked from a stock made in methanol, while keeping the amount of solvent in the sediment suspension below 1% of the total volume of the system. C₁₂-2-LAS aqueous concentration ranged from 0.02 to 2 mg/L, which are well below the critical micelle concentration (105 mg/L, [18]). The system was left to equilibrate on a rolling device for 24 h at room temperature (20 ± 1 °C). Preliminary tests demonstrated that equilibrium was reached within this time period. The amount of NaN₃ added was adequate to suppress microbial degradation during the experiment. After equilibration, phases were separated by centrifugation (3000 rpm, 30 min) and analyzed (see below). The mass balance was tested for several concentrations and resulted 96.9 (± 8.4) % showing that sorption to the walls of the used scintillation vials was negligible.

In most cases, the distribution coefficient (K_d) was calculated from the measured aqueous and sediment concentrations. For the rest of the samples, C_{12-2} -LAS concentration in the sediment was estimated from the decrease in the concentration of the aqueous phase assuming a 100 % mass balance.

Sample treatment and chemical analyses

The clean up procedure of the water samples was performed by Solid Phase Extraction (SPE) using octadecyl reversed-phase silica (C18) columns (Supelclean™ ENVI™-18, 0.5 g, Supelco, Bellefonte, PA, USA), using a similar procedure as developed and tested by Rubio et al. [7]. The columns were preconditioned with 4 mL of analytical methanol, followed by 5 mL of Millipore water. Next, the whole sample (ca. 20 mL) was transferred and the column was then rinsed with 15 mL of Millipore water. 8 mL of methanol were used to elude the C_{12-2} -LAS from the column.

Freeze-dried sediment samples (ca. 0.1 g d.w.) were extracted with 10 mL of methanol in an ultrasonic bath in two steps of 1 and 2 h. The methanol aliquots were collected in 20 mL scintillation vials and evaporated under a nitrogen gas flow. The dry extracts were then redissolved in 20 mL of Millipore water, and purified by SPE treatment as for water samples.

All samples were analyzed by Flow Injection Analysis (FIA) on a LC-MS/MS system, API 3000 triple quadrupole analyzer (MDS Sciex-Applied Biosystems, Foster City, CA, USA). The interface was a Turbo ion Spray source used in the negative mode, the source temperature was 450 °C and the source gas flow was 7 to 8 L/min. Injection volume of the samples was 20 μ L and the flow rate was 0.12 mL/min. C_{12-2} -LAS was measured by screening of the molecular ion $[M-H]^-$ 325.20 m/z and the daughter ion 183 m/z .

In order to avoid the effects of the matrix components on the detection and quantification of C_{12-2} -LAS, a standard addition method was applied. Detection limit approached 1 μ g/L for all measurements. Quantification was done using the software Analyst 1.4.1 (AB/MDS Sciex Instruments).

Data analyses

Experimental sorption data were fitted to the Freundlich isotherm:

$$\text{Log}C_s = \text{Log}K_F + n \cdot \text{Log}C_w \quad (1)$$

where C_s (mg/kg) and C_w (mg/L) are the concentrations of the surfactant in the sediment and in the aqueous phase at equilibrium, respectively. K_F (L/kg) is the Freundlich constant and n provides information about the linearity of the isotherm. Freundlich isotherms were fitted using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA, USA). Ordinary least square (OLS) regression was performed to relate the single sediment characteristics to the sorption coefficients. Principal component analysis (PCA) was used in order to group the different sediment characteristics. OLS and PCA were performed using Scan software Version 1.1 (Minitab, Coventry, UK).

RESULTS AND DISCUSSION

Sediment characteristics

A list of sediment characteristics is given in Table 1. Figure 1 represents the PCA score and loading plot for all the marine and estuarine sediments and their characteristics. This plot shows that the sediments collected from the North Sea and from Cádiz have relatively similar properties. These sediments show similarities in extractable metal composition (iron and aluminum), clay content and specific surface area. They furthermore contain low organic carbon content. On the other hand, the marine and estuarine sediments from Oesterput, La Antilla and San Fernando are similar regarding organic carbon, clay content and specific surface area, although their extractable aluminium and iron is relatively different. The Espigón sediment is clearly different from the other sediments and this is probably due to the very high clay and iron and aluminium contents of this sediment.

Table 1. Characteristics of the sediments and materials. *d*, *o* and *p* are the dithionite, oxalate and pyrophosphate extractions for aluminium and iron respectively. OC% is the organic carbon fraction of the sediments, SSA is the specific surface area.

Sediment	Al <i>d</i> (g/Kg)	Al <i>o</i> (g/kg)	Al <i>p</i> (g/kg)	Fe <i>d</i> (g/kg)	Fe <i>o</i> (g/kg)	Fe <i>p</i> (g/kg)	OC %	Clay %	SSA (m ² /g)
Marine or estuarine sediments									
Oesterput	0.19	0.26	0.19	3.52	3.74	1.11	1.38	6.99	46.02
Cádiz	0.07	0.04	0.06	0.45	0.11	n.d.	0.06	0.25	5.41
La Antilla	0.67	0.43	0.80	6.29	2.16	1.14	0.84	8.85	60.22
San Fernando	0.19	0.26	0.56	1.44	1.25	0.78	1.24	5.08	67.72
Espigón	1.87	1.66	0.32	16.12	9.87	1.60	0.99	11.90	75.55
North Sea 1	0.19	0.15	0.23	2.87	1.44	0.19	0.28	0.98	18.62
North Sea 2	0.20	0.22	0.15	3.28	2.17	0.10	0.27	1.21	18.83
North Sea 3	0.23	0.27	0.19	3.58	2.87	0.14	0.54	1.19	25.25
Lake sediment									
Drontermeer	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	9.52	15.00	82.00
Specific materials									
Kaolinite	0.34	0.27	0.28	0.51	0.23	n.d.	n.d.	n.a.	24.16
Montmorillonite	0.85	0.88	0.77	4.04	0.78	0.51	n.d.	n.a.	535.05
Sand	0.04	0.01	0.03	0.01	n.d.	n.d.	n.d.	n.a.	2.60

n.d: not detected; n.a: not analyzed

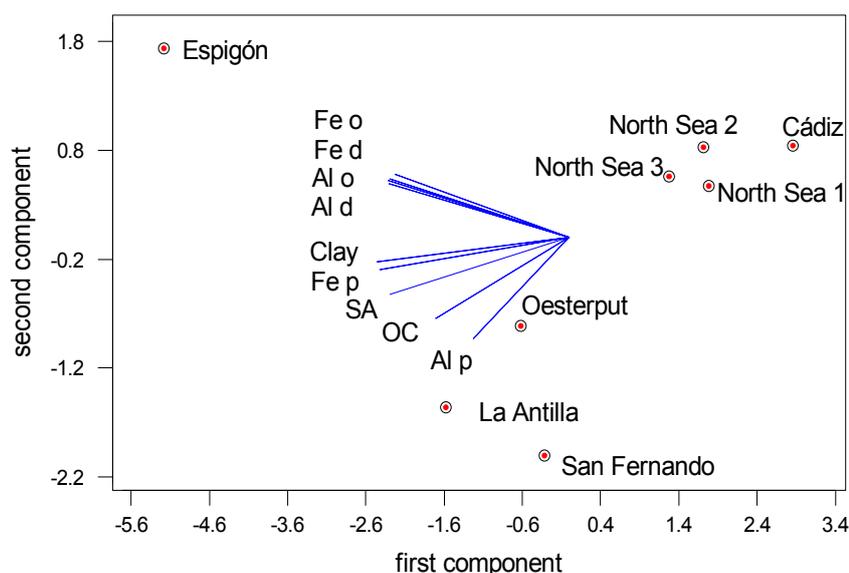


Figure 1. Principal Component Biplot for all the marine and estuarine sediment characteristics. Al p and Fe p are the pyrophosphate extractions for aluminium and iron, Al o and Fe o the oxalate extractions and Al d and Fe d the dithionite extractions. OC is the organic carbon content and SSA is the specific surface area.

Table 2. Correlation matrix (R^2) between the sediment properties for the marine and estuarine sediment.

	Al d (g/Kg)	Al o (g/kg)	Al p (g/kg)	Fe d (g/kg)	Fe o (g/kg)	Fe p (g/kg)	OC %	Clay %	SSA (m ² /g)
Al d (g/Kg)	1.00	0.98	0.07	0.97	0.84	0.57	0.09	0.65	0.45
Al o (g/kg)		1.00	0.03	0.96	0.91	0.57	0.13	0.63	0.46
Al p (g/kg)			1.00	0.04	0.00	0.30	0.23	0.33	0.50
Fe d (g/kg)				1.00	0.91	0.56	0.10	0.64	0.40
Fe o (g/kg)					1.00	0.54	0.18	0.57	0.37
Fe p (g/kg)						1.00	0.65	0.98	0.84
OC %							1.00	0.54	0.70
Clay %								1.00	0.82
SSA (m ² /g)									1.00

The correlation between the characteristics is shown in Table 2. The correlation matrix shows that most of aluminium and iron extracted fractions are related to the clay content of the sediment. Oxalate and dithionite extraction processes (Al o, Al d and Fe o, Fe d in Tables 1 and 2) are supposed to extract predominately Al and Fe oxy(hydr-)oxides. Iron and aluminium oxides occur attached to clay minerals as a consequence of clay mineral weathering. Organic carbon and clay content, which are the main fractions usually associated to LAS sorption to sediments [5,19], are correlated by an R^2 of 0.54. Specific surface area is significantly correlated with both organic carbon and clay content. This correlation between the sediment properties is one of the main problems to differentiate the

contribution of each sediment parameter to the sorption of LAS to the entire sediment matrix. In this study, the contribution of key parameters (e.g., OC, SA) was experimentally investigated using materials with different surface area or by experimentally removing organic carbon from selected sediments.

Sorption of C₁₂-2-LAS to sea sand and clays

The parameters (K_F and n) of the Freundlich isotherms for sorption of C₁₂-2-LAS to the natural sediments, the clays montmorillonite and kaolinite and the purified sea sand are shown in Table 3. Sorption of C₁₂-2-LAS to pure sand was two orders of magnitude lower than to marine sediments collected from the field at the test concentration range. It is therefore expected that the freely dissolved – hence bioavailable fraction – of LAS in sand would be up to two orders of magnitude higher than in field marine sediments. As a consequence, the bioavailability of LAS in toxicity tests run with organisms exposed in acid-washed sand [20] is expected to be significantly different from the one in field sediments, and natural field sediment is therefore recommended.

Hand and Williams [6] reported that sorption of LAS to sediments decreased for sediments with a higher sand content. This is not unexpected because a high sand content is also typically related to a low content of the most reactive constituents for LAS in sediments, organic carbon and clay content. Comparable K_d values to those obtained here have been reported (0.43-0.89 L/kg) for soils with organic matter contents lower than 0.01% [10]. The sorption of C₁₂-2-LAS isotherms to kaolinite and montmorillonite are represented in Figure 2. These two types of clays, typically present in sediments and soils, were chosen for their different structure and properties.

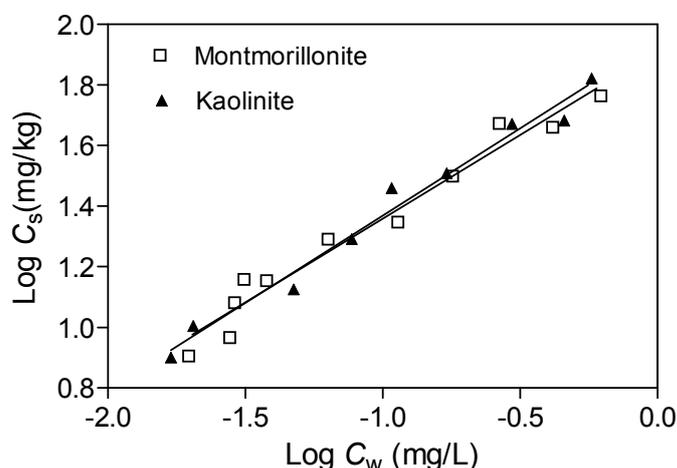


Figure 2. Freundlich isotherms for sorption of C₁₂-2-LAS to Montmorillonite (□) and Kaolinite (▲) clays.

Kaolinite belongs to the 1:1 sheet silicates with a layer of tetrahedral silica connected to a layer of octahedral aluminate. The surface charge of kaolinite is predominately controlled by charge determining reactions of surface groups, in particular acid/base reactions of the aluminol and silanol groups. At sea water pH, both types of surfaces are expected to carry a

net negative charge [21]. Montmorillonite is a 2:1 silicate, which carries, in contrast to kaolinite, a permanent negative layer charge. The expandable layers of montmorillonite confer a considerably larger specific surface area ($535 \text{ m}^2/\text{g}$) respect to kaolinite ($24 \text{ m}^2/\text{g}$). The layer charge is caused by isomorphic substitution of Al^{3+} by divalent cations and Si^{4+} by Al^{3+} . The surface charge of montmorillonite depends only to a minor extent on protonation and deprotonation of surface sites due to the negative layer charge and adsorption of negatively charged ions onto the montmorillonite surface is typically electrostatically unfavorable.

Sorption of LAS to these two clays in seawater showed non-linear isotherms (see Table 3) with n values of 0.55 and 0.58 for kaolinite and montmorillonite, respectively. The Freundlich coefficients were very similar for these two clay types. The similarity of the isotherms suggests that C_{12} -2-LAS only sorbs to the surface of the clays and it does not reach the interlayer spaces of montmorillonite. These results may facilitate the prediction of LAS sorption to natural sediments, where more than one type of clay is present. Specific clay characterization is likely not necessary and information about the total clay fraction is likely sufficient. A recent study of the sorption of LAS to two types of montmorillonite (calcium and sodium montmorillonite) [22] has confirmed that LAS was not present in the interlayer space. Mathijs and De Henau [9] reported a non-linear isotherm ($n=0.5$) and a relatively low Freundlich sorption coefficient ($\text{Log } K_F=1.3$) for the sorption of an LAS mixture to kaolinite. A study of the sorption of LAS to kaolinite using a 0.01M KHCO_3 solution showed a sorption coefficient for C_{12} -LAS (mixture of isomers) of ~ 35 ($\text{Log } K_F=1.5$) when traces of organic matter were removed [23]. The higher sorption coefficient obtained here can be due to the higher electrolyte concentration in seawater. Unfavorable electrostatic interactions between LAS and the clay minerals are diminished at high ionic strength and, in particular, adsorption of divalent cations onto the clay surface may positively influence sorption of LAS.

Effect of the removal of the organic carbon content on the sorption of C_{12} -2-LAS to sediments

Removal of organic matter from the sediments by the NaOCl treatment was incomplete and the organic matter content was reduced by 77%, 60 % and 81% compared with the initial values in the samples from Cádiz, San Fernando and Drontermeer, respectively. Results for the sorption of C_{12} -2-LAS on the three natural and NaOCl treated sediments are shown in Figure 3. In the case of the Cádiz sediment (initial organic carbon content of 0.06%), LAS sorption substantially decreased upon reducing the organic fraction. A possible explanation for this observation is that the small fraction of organic matter of the natural sediment was covering the sand grains before the NaOCl treatment, enhancing the sorption of C_{12} -2-LAS to the sediment, while after the reduction of the organic content, sorption was not enhanced by any of the rest of the constituents from the sediment (mainly sand).

Isotherms for the natural and treated Drontermeer sediment are both relatively close to linearity with n of 1.08 and 0.91 for the untreated and treated sediment, respectively. This suggests that hydrophobic interactions are probably the driving force of LAS sorption in these samples. This is not strange considering that the organic carbon content of this sediment is

still relatively high after the NaOCl treatment (from 9.5% down to 1.8 %). The observed decrease of the K_F coefficient between the treated and the untreated Drontermeer sediment is proportional to the difference in the organic fraction. K_F (considering $n \approx 1$) decreased by 70 % when reducing the organic content of the sediment by 81 %. A proportional decrease in sorption was also observed for the San Fernando sediment, with K_F of 380 and 105 L/kg for the treated and untreated sediment, respectively (72% decrease in K_F value for 60% decrease in organic carbon content). The fact that K_F changed proportionally to the organic carbon reduction in Drontermeer and San Fernando sediments, clearly shows the importance of the organic carbon content in the sorption process.

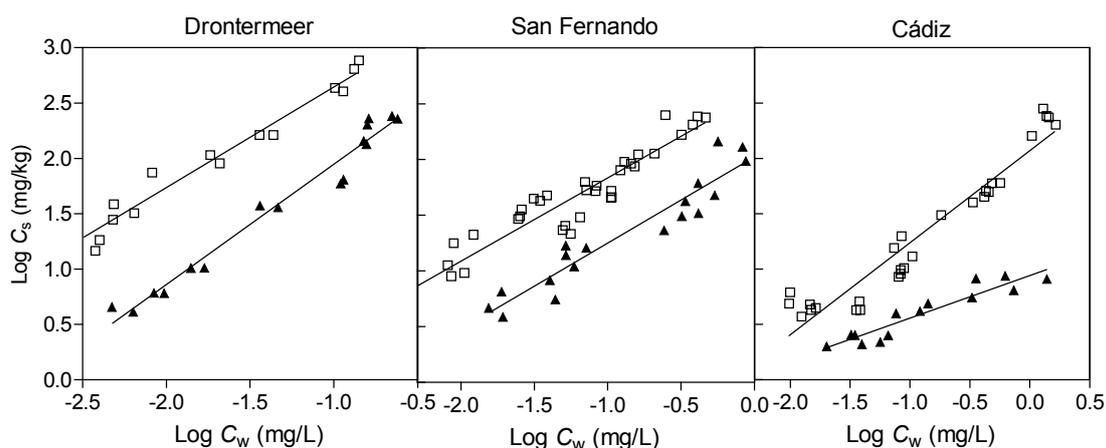


Figure 3. Freundlich isotherms for the natural (□) and NaOCl treated sediments (▲) for the three selected sediments.

Table 3. Freundlich parameters (Log K_F and n) for C₁₂-2-LAS with all the tested sediments and materials. N is the total number of samples used for the isotherm.

	n	Log K_F	R ²	N
Marine or estuarine sediments				
Oesterput	0.87 ± 0.04	2.72 ± 0.06	0.96	29
Cádiz	0.83 ± 0.05	2.06 ± 0.05	0.92	28
La Antilla	0.66 ± 0.02	2.50 ± 0.04	0.97	24
San Fernando	0.75 ± 0.04	2.58 ± 0.05	0.92	37
Espigón	0.61 ± 0.04	2.30 ± 0.06	0.89	33
North Sea 1	0.78 ± 0.04	2.07 ± 0.06	0.94	24
North Sea 2	0.73 ± 0.04	2.09 ± 0.05	0.92	26
North Sea 3	0.80 ± 0.05	2.24 ± 0.06	0.96	14
Lake sediment				
Drontermeer	0.91 ± 0.05	3.55 ± 0.09	0.96	15
Specific materials				
Kaolinite	0.55 ± 0.03	1.91 ± 0.04	0.96	11
Montmorillonite	0.58 ± 0.03	1.95 ± 0.03	0.98	9
Sand	1.23 ± 0.17	0.05 ± 0.10	0.85	12

Sorption of C₁₂-2-LAS to estuarine and marine sediments

The Freundlich parameters of the isotherms for C₁₂-2-LAS with all the selected marine and estuarine sediments are shown in Table 3. Isotherms are non linear with $n < 1$ in all cases, suggesting that other kinds of interactions than only hydrophobic interactions occur. Sorption isotherms did not show the same degree of non-linearity and the Freundlich parameter n varied from 0.61 to 0.87. In the case of Oesterput, San Fernando, La Antilla and Espigón sediments, which are the sediments with the highest organic carbon and clay contents, n value tends to be lower when the clay content increases. Although some trends can be observed for the rest of the sediments, it is not easy to relate the Freundlich parameter n with the organic carbon or clay content. The highest K_F values were observed for the Oesterput and San Fernando sediments, which are also the sediments with the highest organic carbon content. However, this tendency was not observed for the rest of the sediments, confirming the multivariate nature of sorption of LAS in marine sediments. Because the distribution coefficient (K_d) is not a constant, correlations between K_d values and sediment properties were investigated at several aqueous concentrations of LAS varying from 0.001 to 0.5 mg/L. Distribution coefficients were estimated using the Freundlich parameters from Table 3. The correlation coefficients (R^2) between the distribution coefficients at different aqueous concentrations and each single sediment characteristic are given in Table 4. In general, organic carbon, clay, specific surface area and some extractable metals (aluminium and iron pyrophosphate extractions, Al p and Fe p) correlate well with C₁₂-2-LAS sorption to sediments. However, these correlations depend on the surfactant concentration. Far better correlations with the iron, clay fraction and specific surface area are observed ($R^2 > 0.8$) for concentrations below 0.01 mg/L, while K_d values correlate much better with the organic carbon content at higher concentrations. This trend is graphically shown in Figure 4, where correlation coefficients for the relationships of K_d versus the organic carbon content and clay fraction are given as a function of the aqueous concentration.

In the coastal environment, the expected range of LAS concentrations (all isomers and homologues) downstream of a waste water plant with secondary or with no treatment are $< 1 - 10 \mu\text{g/L}$, or $10 - 1000 \mu\text{g/L}$, respectively [24]. The organic carbon content has therefore significant effects on the sorption of LAS (and its bioavailability) in coastal sediments receiving untreated wastewater. It is also in these sites that the organic carbon content is high [4]. In contrast, the clay and the iron pyrophosphate (Fe p) fractions are more important factors in marine/estuarine sediments of regions with appropriate (secondary) wastewater treatment.

The values of the Freundlich constant ($\text{Log } K_F$) for C₁₂-2-LAS as measured for the eight natural marine sediments can be categorized into three groups that correspond to those identified by PCA of the physico-chemical characteristics of the sediment samples (Figure 1), i.e., (1) Espigón ($\text{Log } K_F = 2.3$; very high clay and metal content), (2) North Sea 1, 2, 3, and Cádiz ($\text{Log } K_F = 2.1 - 2.2$, low organic carbon content), (3) Oesterput, La Antilla and San Fernando ($\text{Log } K_F = 2.5 - 2.7$, high organic carbon and clay contents).

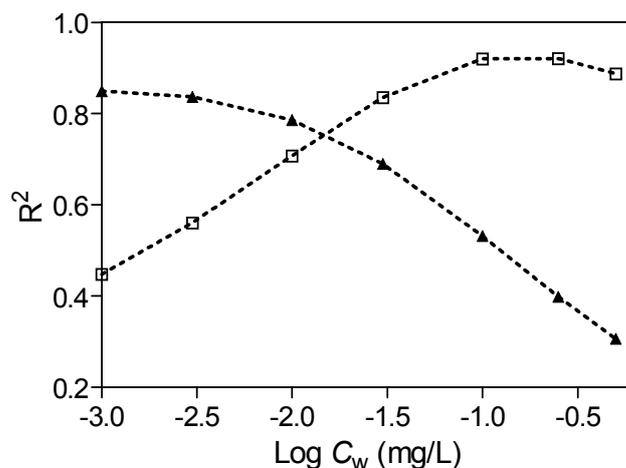


Figure 4. Correlation coefficient (R^2) of the relation between sorption coefficients for all the marine and estuarine sediments and the organic carbon content (\square), and the clay fraction (\blacktriangle) at different aqueous concentrations. Sorption coefficients were calculated using the Freundlich parameters from Table 2.

Good correlations between the organic fraction and sorption of LAS to sediment and soils has already been shown in several studies [5,8-10]. The sorption of LAS to natural sorbents can be related to hydrophobic interactions, and to specific chemical and electrostatic interactions of its functional ionic group. A recent study described the sorption of LAS and perfluoroalkyl surfactants in sediments based on the fraction of the organic matter that is accessible for the surfactant sorption [25]. This model is based on electrostatic and hydrophobic interactions of surfactant molecules with the organic fraction of the sediments. However, organic carbon content is not always clearly related to the sorption of LAS to sediments. The relation of LAS sorption with the content of iron and aluminium oxides has often been observed for freshwater sediments and soils [5,11,26]. Interactions of LAS with these materials were explained by specific surface interactions. In a soil sorption study [11], LAS showed stronger affinity in the presence of amorphous oxides, likely due to their higher surface area. The sorption of LAS to the sediment constituents might furthermore be enhanced in the seawater media, because cations present in solution can adsorb to the particles surface, facilitating interactions with the functional sulfonate group. Besides, the high content of salts will also diminish the negative surface charge of the sediment, enhancing LAS absorption. Research with another anionic surfactant, Sodium Dodecyl Sulfate (SDS), on estuarine sediments in freshwater and seawater media [12] showed no differences between sorption of SDS in river water and seawater solutions using a sediment with a relatively high content in organic carbon (2%). However, after a treatment of this sediment to remove the organic fraction, a higher sorption was observed in seawater than in freshwater media. This phenomenon was explained by the reduction of the negative charge on the surface when removing the organic material, making it more favorable for electrostatic interactions in the case of seawater experiments.

CONCLUSIONS

The results obtained in this work provide relevant data for the understanding of the influence of single sediment materials on the sorption process of LAS to sediments. The challenge is to identify the range of the sediment properties that affect the sorption at the different surfactant concentrations. This study shows that at low concentrations (0.001-0.01 mg/L) C₁₂-2-LAS sorption was mainly dependent on iron and aluminium content, and clay fraction, suggesting a relatively high importance of adsorption processes (interactions at the surface of the sediments). For higher concentrations (> 0.01-0.05 mg/L) a better relation was observed with the organic carbon content, suggesting absorption in the organic fraction. Because concentrations in field sediments are relatively low in comparison with those used in the sorption studies, it is likely that particle size, clay and iron and aluminium oxides contents are more relevant for the predictions of LAS sorption to field marine and estuarine sediments than organic carbon content.

However, we must be prudent when selecting a dominant parameter to predict the sorption of LAS to sediments at environmental LAS concentrations. The work here performed was done for one model compound, while LAS in commercial mixtures is composed of molecules with different alkyl chains and phenyl positions. Furthermore, because water composition affects the sorption affinity of LAS to sediments, the salinity of the medium, together with the specific sediment constituents, may have an important contribution to the whole sorption process.

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Chapter 4

Predicting sediment sorption coefficients from polyacrylate-water partition coefficients for LAS congeners at different aqueous composition

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ABSTRACT

The effect of the molecular structure and the aqueous composition on the sorption of the anionic surfactant linear alkylbenzene sulfonate (LAS) has been studied. The analysis of several individual LAS constituents in seawater and of one specific LAS congener at different aqueous dilutions was carried out by solid phase microextraction (SPME). Sorption isotherms for the sediment sorption data for the tested LAS constituents and at different aqueous media were all non linear with a constant Freundlich exponent (n) of 0.78. Differences between LAS congeners for the Freundlich coefficients (K_F) from the sediment sorption experiments were similar to the differences among the partition coefficients (K_{fw}) observed for the polyacrylate SPME fibers. The aqueous composition significantly affected the sorption of LAS to both the sediment and the SPME fiber. However, sediment sorption coefficients of a single LAS congener were less significantly related to the K_{fw} values at varying aqueous media, likely because different sorption mechanisms are taking place in both phases. The effect of the presence of specific cations in solution (Na^+ and Ca^{2+}) on the sorption to sediment and fiber was further investigated. In the absence of experimental data for octanol-water coefficients (K_{ow}) of LAS constituents at different medium compositions, the use of the K_{fw} as a simple tool to predict sorption and other hydrophobicity related processes is suggested.

INTRODUCTION

Sorption is one of the most relevant processes that determine the fate, distribution and toxicity of contaminants in the aquatic environment. Sorption of ionic organic compounds, such as the anionic surfactant linear alkylbenzene sulfonate (LAS), is related to their molecular structure, the sediment constituents, and the specific media conditions [1-3]. The sorption behaviour of LAS depends on the hydrophobicity, as indicated by the increase of sorption with increasing alkyl chain length [4,5]. Also the relation between the sorption affinity and the organic carbon content of the natural sorbents shows that hydrophobic interactions are important [6,7]. On the other hand, several studies have revealed that specific chemical and/or electrostatic interactions affect the sorption of LAS [6-8].

Additionally to the sorbent and sorbate properties, the aqueous solution chemistry also influences the sorption of ionisable organic compounds, because processes such as ionic exchange or ion pair formation are directly influenced by the media composition [1,2,9]. Consequently, the sorption of LAS will be different in fresh water, estuary or seawater conditions, and sorption coefficients of LAS in the marine environment are generally higher than in fresh water media [5]. The effect of the aqueous composition on sorption is of particular interest in estuaries. These ecosystems are characterized by large salinity variations, and they often are the receiving areas for urban, harbour and industrial effluents, that may contain relatively high concentrations of surfactants [3,10]. While the effect of the sorbent properties on the sorption of LAS has been extensively studied, the effect of the solution chemistry has been mainly tested with simple electrolyte solutions [6]. Information about the effect of the complex composition of aqueous solutions on the sorption of LAS and other anionic organic compounds is rather scarce.

Commercial LAS consist of a mixture of molecules with different alkyl chain length (10 to 13 carbon atoms) and isomers that vary in the position of the benzene sulfonate moiety on the alkyl chain. A typical product contains more than 20 different LAS congeners [11]. Because it is desirable to avoid sorption testing of all compounds every time, acceptable estimation methods are needed. Therefore sorption is often related to the chemical structure of surfactants. Several studies have shown that LAS molecules with long alkyl chain are the most sorptive [4,6,12,13]. Another study has shown that the isomers with the most external phenyl positions have a higher sorption affinity to sediments than isomers where the phenyl is attached to the more internal carbon atoms of the alkyl chain [4]. Although information about the sorption of individual isomers is clearly relevant for the understanding of the overall sorption process, sorption data for individual LAS constituents have only been reported for a few compounds [4,6], and most of the sorption studies have used LAS mixtures of homologues or isomers.

In this study, we have used polyacrylate (PA) solid phase microextraction (SPME) fibers for the analysis of LAS pore water concentrations in the sediment sorption tests. The SPME method has been extensively applied for the analysis of freely dissolved concentrations of organic compounds in complex matrices (see, for instance refs [14-16]), and it is considered a practical passive sampling tool, because phase separation and purification steps are not

needed [17-19]. Application of the SPME procedure to ionic organic compounds is more complicated than an SPME analysis of non-ionized chemicals, because a more specific optimization of the method is required [17,20,21]. The analysis of LAS compounds in seawater was performed using the fiber-water coefficients that are reported elsewhere [22].

The main aim of the present work was to investigate the effect of the molecular structure of a series of LAS congeners and the solution chemistry on sorption to sediment. The effect of the molecular structure on the sorption of LAS to a natural sediment was studied in artificial seawater media using nine individual LAS constituents with chain lengths varying from 10 to 13. To our knowledge, this is the first study reporting sorption coefficients for several single LAS compounds at seawater conditions. For one LAS molecule (C₁₂-2-LAS), the effect of the composition of the medium on sediment sorption was investigated. For that purpose, sorption was measured in a series of dilutions of both fresh and seawater.

In order to use the SPME method at different aqueous composition, sorption isotherms for the polyacrylate fiber were generated for several seawater and fresh water dilutions to obtain the appropriate fiber-water partition coefficients (K_{fw}). Because the presence of cations in solution considerably affects the sorption of LAS to natural sorbents [6,23], a second objective was to investigate the effect of Ca²⁺ and Na⁺ on the fiber-water distribution using simple electrolyte solutions. Na⁺ is the most abundant cation in natural waters. On the other hand, Ca²⁺ is typically the cation influencing most of the environmental processes of anionic surfactants in aquatic systems [6,23,24]. Sorption data to the polyacrylate fiber as well as to sediment were then compared at varying concentrations of these two cations.

A third objective was to investigate whether the fiber-water partition coefficients for the several congeners and different media composition could be used as parameter to predict the sediment sorption. If such an approach is successful, the fiber-water partition coefficient would represent a very valuable and simple tool in structure-activity relationships, as an alternative to the octanol-water partition coefficient (K_{ow}). Experimental data for K_{ow} 's of ionic surfactants are not available and hardly measurable [25,26].

MATERIALS AND METHODS

Chemicals, sediment, and chemical analysis

Linear alkylbenzene sulfonates are abbreviated as C_n-m-LAS, where n is the length of the alkyl chain and m represents the position of the phenyl group on the alkyl chain. C₁₀-2-, C₁₁-2-, C₁₁-5-, C₁₂-2-, C₁₂-3-, C₁₂-5-, C₁₂-6-, C₁₃-2- and C₁₃-5-LAS (purity > 97%) were synthesized by Dr. J. Tolls as sodium salts in our lab [27]. Chemical stocks were made in analytical methanol obtained from Lab-Scan (Dublin, Ireland). Seawater –GP2 medium- (SW) [28], and Dutch standard freshwater, NEN 6503 – (DSW) [29], both containing 10 mM NaN₃ (Merck, Darmstadt, Germany), were prepared according to standard procedures in Millipore water from a Milli-Q purification system (resistivity of circa 18 MΩ cm). The ion composition of both stock solutions is presented in Table 1.

The sediment used was collected from the North Sea “Friese Front” area. Physico-chemical characteristics of the sediment were analyzed at the Integrated Laboratory of the Faculty of Geosciences/TNO at Utrecht University. Organic carbon content was 0.27 %, and particle size distribution 1.2 % clay (<2 μm), 11.0 % fine silt (<8 μm), 19.2 % silt (2<63 μm) and 79.5 % sand (63<1000 μm). Additional measured parameters for this sediment have been reported before (see sediment “North Sea 2” in ref [7]).

Extraction of LAS from water and sediment and clean up steps were performed as described elsewhere [7]. Millipore water and ammonium acetate were added to the final solid phase extracts (C_{18} -SPE) to make the final composition similar to the LC/MS/MS eluents, which consisted of 80 % Methanol, 20% Millipore water and 10 mM ammonium acetate. Samples were analyzed in an LC/MS/MS system as reported earlier [22].

Table 1. Ion composition of the artificial seawater (SW) and Dutch standard water (DSW).

	SW mmol L^{-1}	DSW mmol L^{-1}
Na^+	418	11
Mg^{2+}	46.73	0.30
Ca^{2+}	8.98	1.36
K^+	8.92	0.20
Sr^{2+}	0.08	
Cl^-	480	2.70
SO_4^{2-}	24.80	0.30
HCO_3^-	2.02	1.40
Br^-	0.74	
$\text{B}_4\text{O}_7^{2-}$	0.09	

SPME procedure

SPME fibers were obtained from Polymicro Technologies (Phoenix, AZ) and consisted of glass fibers of 108-109 μm core with a polyacrylate (PA) coating of 7.5-7.6 μm thickness. Fibers were cut in pieces of 40 ± 0.1 mm length, thermally conditioned in a gas-chromatography (GC) oven overnight at 120 $^\circ\text{C}$, and kept in Millipore water for at least 24 h. Details about the conditioning and extraction procedure of the SPME fiber is given elsewhere [22]. All experiments were conducted at room temperature (19 ± 1 $^\circ\text{C}$). Surfactant solutions were made by adding aliquots of LAS stock in methanol (<1% of the total volume) in 20 mL glass scintillation vials (Perkin Elmer, Boston, MA, USA). Vials were then completely filled with the correspondent aqueous solutions and one SPME fiber per vial was placed. Test systems were equilibrated and SPME fibers were extracted and analysed as explained elsewhere [22]. Aqueous solutions at the lowest and the highest nominal concentrations were extracted by SPE in order to check if biodegradation or adsorption of LAS to the glass vial should be considered. For all cases, recoveries were above 93 %. For the rest of the vials, the nominal aqueous concentrations were considered.

Experiments to obtain the extraction isotherms for SPME fibers at different solution composition were performed with C₁₂-2-LAS and several dilutions of artificial seawater and fresh water. Seawater dilutions were prepared by mixing the seawater with the freshwater stock at different ratios. For the freshwater dilutions, the freshwater stock was mixed with Millipore water. All solutions contained NaN₃ 10 mM to avoid LAS biodegradation. Separate experiments were conducted to evaluate the effect of Ca²⁺ and Na⁺ on the fiber extraction. Test solutions were made by adding the reagent grade salts CaCl₂·H₂O or NaCl from Merck (Darmstadt, Germany) to a 10 mM NaN₃ MilliQ water solution. Concentrations ranged from 0.14 to 8.98 mM for Ca²⁺ and from 10 to 418 mM for Na⁺, which correspond to the Ca²⁺ and Na⁺ concentrations for the seawater and freshwater dilutions tested. The pH of every equilibrated solution was determined with a 761 Calimatic pH-Meter (Knick, Germany).

Sorption experiments

Sorption experiments were carried out in 20 mL scintillation vials by adding 0.2 g (ww) of the sediment to the LAS solutions. The system was equilibrated for 24 h, as it has been previously described [7]. Thereafter, one fiber was placed in each vial. The test systems were then placed on a shaking device for at least 48 h to equilibrate the fibers with the sediment suspension. Next, fibers were extracted and aqueous concentrations were determined. Controls without sediment were used to confirm initial aqueous concentrations. For at least two samples from every test, extraction of the water (by SPE) and sediment phase (methanol extraction by sonication followed by SPE, as previously described [7]) were also conducted and concentrations were measured. Mass balance was close to 100 % (96 ± 8 %). For the rest of the test systems, sediment concentrations were determined by SPME, by deriving the aqueous concentration from the concentration in the fiber. Sorption to sediment in seawater was measured for nine LAS congeners. C₁₂-2-LAS was used to test the effect of the solution chemistry on the sorption of LAS to sediments.

RESULTS AND DISCUSSION

Influence of the molecular structure on the sorption of LAS to sediment: Correlation between sediment-water and fiber-water sorption coefficients

Sorption data resulted from the sediment suspension experiments were fitted to the Freundlich isotherm:

$$\text{Log}C_s = \text{Log}K_F + n \cdot \text{Log}C_w \quad (1)$$

where C_s (mg/kg) and C_w (mg/L) are the surfactant concentration in the sediment and in the water phase, respectively. $\text{Log} K_F$ is the Freundlich constant or distribution coefficient at a fixed C_w of 1 mg/L, and n is the slope or Freundlich exponent.

The sediment sorption isotherms for all the tested LAS compounds in seawater are represented in Figure 1, and the parameters fitted to the Freundlich equation are listed in

Table 2. Sorption isotherms were clearly non linear for all tested compounds. The Freundlich exponents (n) do not differ significantly from each other with a mean value of 0.78 ± 0.05 . In general, the magnitude of the Freundlich coefficient (K_F values) was notably influenced by the alkyl chain length and the position of the benzenesulfonate moiety. The effect of the alkyl chain length on the sorption of LAS to fresh water sediments has been shown earlier [4,6,12] and also bioconcentration factors in fish show similar trends [9,30]. Besides, data from marine monitoring studies also show accumulation of longer alkyl chain lengths compared to the aqueous phase [31-33].

Not only the length of the alkyl chain, but also the position of the phenyl group plays a significant role in the sorption affinity of the LAS isomers to sediment. The most external isomers (2-phenyl) of each n -alkylbenzene sulfonate series had a similar sorption as the most internal isomers of its homologue with an additional methyl group. This is the case of the K_F values obtained for C_{13} -5-LAS, and C_{12} -2-LAS, and for C_{11} -5-LAS and C_{10} -2-LAS. The same trends are also reported for the K_{ow} and vapor pressure of linear alkylbenzenes [34]. Similar effects of the position of the phenyl group were also observed for partitioning into polyacrylate SPME fibers of the same set of nine LAS congeners as used in this study [22].

Figure 2 shows the logarithm of the sediment sorption coefficient (K_F) for all tested LAS compounds against their respective fiber-water partition coefficients (K_{fw}) in seawater. A good correlation between these two parameters is obtained, as shown by the small standard errors and the slope close to the unity (regression coefficient, $r^2= 0.9$). This strong correlation indicates that differences in the sediment sorption coefficients K_F of the tested LAS compounds are well predicted by the fiber-water partition coefficients (K_{fw}). The effect of the alkyl chain length in the LAS congeners, as well as the position of the phenyl group in the isomers on sediment sorption are very similar to these effects on the partition to the polyacrylate fiber. This also suggests that the differences in sorption are mainly related to the hydrophobicity of the LAS molecules.

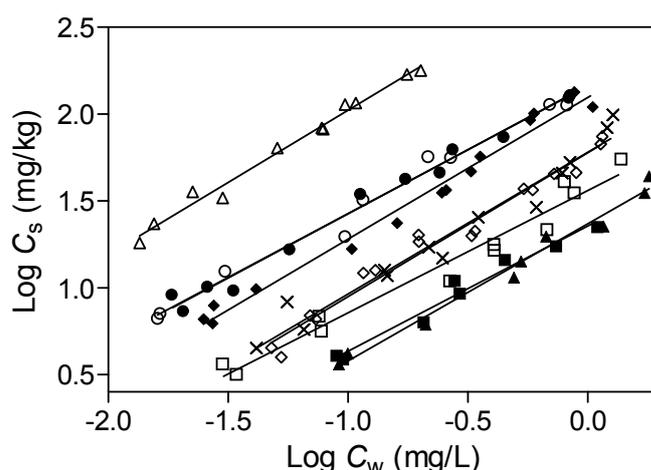


Figure 1. Sediment-water sorption isotherms for the LAS compounds tested at seawater salinity: C_{10} -2-LAS (■), C_{11} -2-LAS (□), C_{11} -5-LAS (▲), C_{12} -2-LAS (○), C_{12} -3-LAS (◆), C_{12} -5-LAS (◇), C_{12} -6-LAS (x), C_{13} -2-LAS (△) and C_{13} -5-LAS (●).

Table 2. Freundlich parameters of the sediment-water isotherms for the tested LAS compounds in seawater.

Compound	Log K_F	n
C ₁₀ -2-LAS	1.4 ± 0.03	0.72 ± 0.05
C ₁₁ -5-LAS	1.4 ± 0.02	0.79 ± 0.04
C ₁₁ -2-LAS	1.6 ± 0.04	0.70 ± 0.04
C ₁₂ -6-LAS	1.8 ± 0.04	0.82 ± 0.05
C ₁₂ -5-LAS	1.8 ± 0.03	0.83 ± 0.04
C ₁₂ -3-LAS	2.1 ± 0.03	0.82 ± 0.03
C ₁₂ -2-LAS	2.2 ± 0.04	0.73 ± 0.03
C ₁₃ -5-LAS	2.2 ± 0.03	0.74 ± 0.03
C ₁₃ -2-LAS	2.9 ± 0.04	0.83 ± 0.03

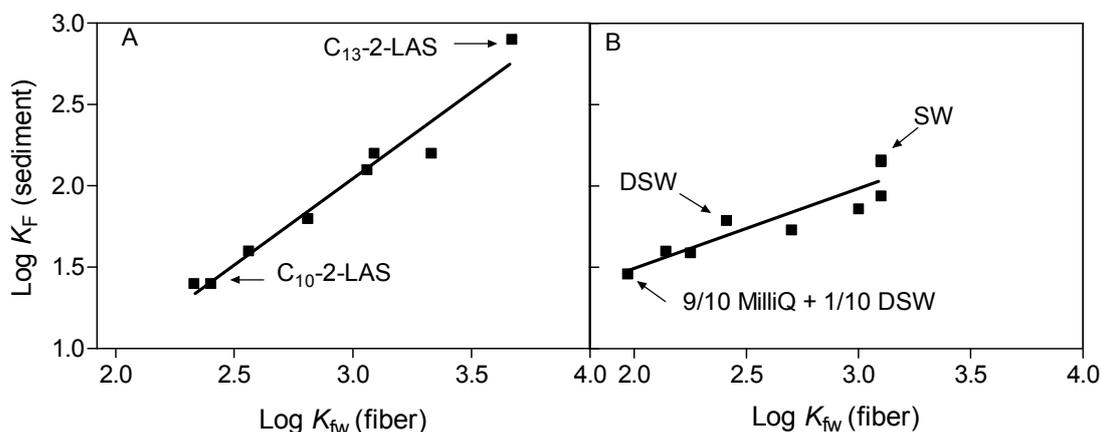


Figure 2. Sediment-water distribution coefficient (K_F , at 1 mg/L) plotted against the fiber-water partition coefficient (K_{fw}) for all the LAS tested compounds (A), and for C₁₂-2-LAS at different aqueous composition (B). The fitted regression lines are $\text{Log } K_F = 1.1 (\pm 0.08) K_{fw} - 1.15 (\pm 0.24)$, $R^2=0.96$ for the LAS congeners, and $\text{Log } K_F = 0.5 (\pm 0.08) K_{fw} + 0.5 (\pm 0.22)$, $R^2=0.84$ for C₁₂-2-LAS for varying aqueous composition.

Sorption of C₁₂-2-LAS to polyacrylate fibers and sediment at various aqueous media

Linear sorption isotherms were fitted to the fiber-water sorption data for C₁₂-2-LAS at different seawater and freshwater solutions (Figure 3). Table 3 lists the calculated log K_{fw} values, for which small standard errors were obtained in all cases (≤ 0.03 log units). The observed linear isotherm suggests that the sorption is an absorption process, as discussed in ref [22]. Only small differences in K_{fw} values were observed for the several seawater dilutions. However, K_{fw} values were substantially lower for the freshwater solutions as compared to the seawater solutions.

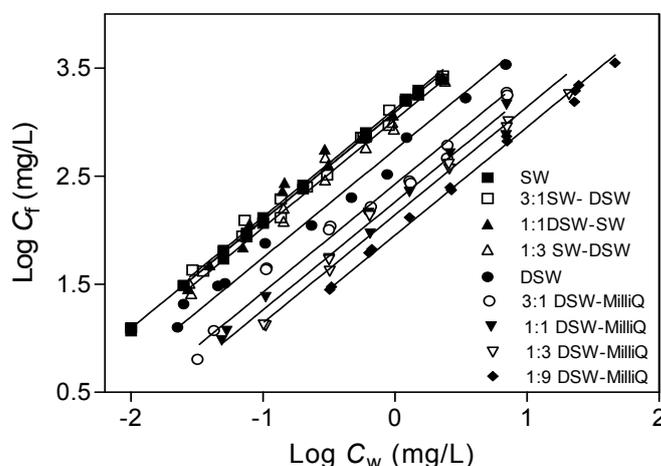
Table 3. Freundlich parameters of the sediment-water isotherms (K_F and n) and fiber-water sorption coefficients (K_{fw}) for C₁₂-2-LAS at the dilutions tested.

Solution Composition	Sediment		SPME fiber		
	Log K_F	n	Log K_{fw}^a	Log K_{fw}^b (CaCl ₂)	Log K_{fw}^c (NaCl)
SW	2.2 ± 0.04	0.73 ± 0.03	3.0 ± 0.02	2.9 ± 0.06	3.0 ± 0.08
3:1SW-DSW	2.2 ± 0.03	0.79 ± 0.04	3.1 ± 0.03	3.0 ± 0.03	2.9 ± 0.04
1:1 SW-DSW	1.9 ± 0.01	0.76 ± 0.02	3.1 ± 0.02	2.9 ± 0.06	3.0 ± 0.09
1:3 SW-DSW	1.9 ± 0.04	0.79 ± 0.05	3.1 ± 0.01	2.8 ± 0.04	2.9 ± 0.05
DSW	1.7 ± 0.03	0.80 ± 0.05	2.7 ± 0.03	2.7 ± 0.02	2.5 ± 0.05
3:1 DSW-MilliQ	1.8 ± 0.05	0.78 ± 0.05	2.4 ± 0.03	2.5 ± 0.02	
1:1 DSW-MilliQ	1.6 ± 0.04	0.85 ± 0.06	2.3 ± 0.03	2.4 ± 0.03	
1:3 DSW-MilliQ	1.6 ± 0.04	0.71 ± 0.11	2.1 ± 0.03	2.3 ± 0.03	
1:9 DSW-MilliQ	1.5 ± 0.03	0.85 ± 0.07	2.0 ± 0.01	2.2 ± 0.05	

^a K_{fw} from the complex seawater and freshwater dilutions

^b K_{fw} from the experiments with CaCl₂ solutions at the same salt concentration as for the seawater and freshwater dilutions.

^c K_{fw} from the experiments with NaCl solutions at the same salt concentration as for the seawater and freshwater dilutions. No uptake was obtained for the NaN₃ 10 mM solution.


Figure 3. Fiber-water sorption isotherms for C₁₂-2-LAS at different seawater and freshwater dilutions.

Linear isotherms were also obtained when exposing the polyacrylate fibers to solutions of CaCl₂ or NaCl at electrolyte concentrations comparable to the seawater and freshwater dilutions (Figure 4, Table 3). Although no buffer was added, the pH did not change dramatically, varying from 7.31 to 7.5 and from 7.02 to 7.51 from the highest to the lowest CaCl₂ and NaCl concentrations, respectively. Therefore, differences in K_{fw} values are mainly due to the media composition. Obtained K_{fw} values for the sodium solution systems were not different at concentrations equal or higher to 112 mM Na⁺. The selected Na⁺

concentration corresponds to the Na^+ concentration in the seawater dilutions. Furthermore, the average K_{fw} value for the higher Na^+ concentrations was very close to the value obtained for the complex seawater dilutions. K_{fw} was notably different for the solution containing 11 mM Na^+ .

Isotherms corresponding to the Ca^{2+} solutions showed a similar pattern to that obtained for the complex sea and freshwater dilutions. For the lower Ca^{2+} concentrations, the K_{fw} increases with the concentrations, while at Ca^{2+} concentrations equal or higher to 3.27 mM, the K_{fw} remains constant. The K_{fw} values at high Ca^{2+} concentrations are again very similar to the K_{fw} for the reported seawater dilutions and the single electrolyte systems with the highest Na^+ concentrations.

Figure 5 represents the sediment sorption isotherms for the experiment at different aqueous compositions. In contrast to the fiber-water isotherms, sediment-water isotherms were all non linear, with $n < 1$ in all cases, suggesting that adsorption processes play a role in the sorption of LAS to sediment. Isotherms showed a very similar degree of non-linearity, as the Freundlich exponent n did not significantly differ among the isotherms, with a mean value of 0.79 ± 0.05 .

Sorption of C_{12} -2-LAS to sediment systematically increased with the salt concentration in solution. The increase of sorption to natural sorbents with increasing salt content in solution has been reported for LAS and other anionic surfactants [3,6,12,24]. In Figure 6, the relation between K_{fw} (fiber) and the Freundlich coefficient K_F (sediment) and the Ca^{2+} concentration in the seawater and freshwater dilutions is presented. An average increase of $0.37 \pm 0.04 \log K_F$ per $\log [\text{Ca}^{2+}]$ unit is obtained. This factor is not far from the value of 0.23 given by Westall et al. [6] when using only CaCl_2 solutions at a range between $10^{-3.8}$ and $10^{-2.7}$ M, and almost identical to the one obtained by García et al. [35] for LAS homologues sorption to sludge. Moreover, this factor is also comparable to the one obtained for the effect of Ca^{2+} concentration on the sorption of perfluorinated surfactants to fresh water sediments [24]. Consequently, the increase of the sorption of LAS to sediment when increasing the salt concentration seems to be well predicted by the Ca^{2+} content in solution.

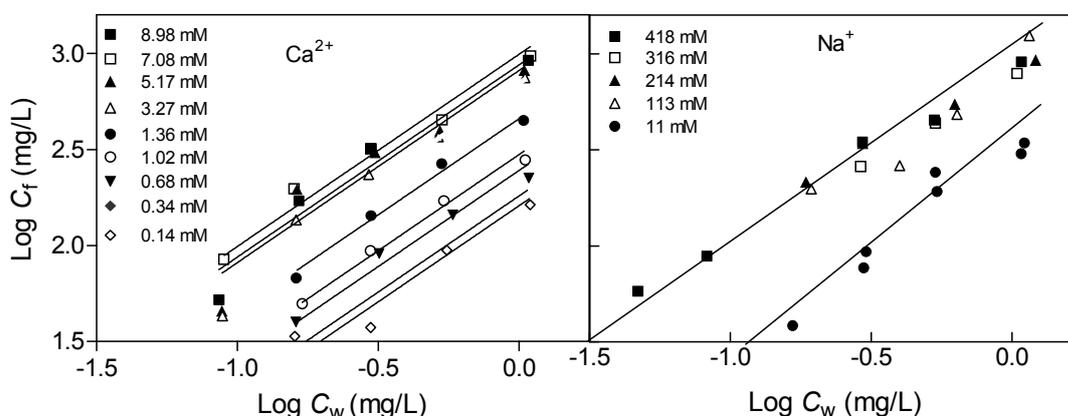


Figure 4. Fiber-water isotherms for C_{12} -2-LAS at different Na^+ and Ca^{2+} concentrations.

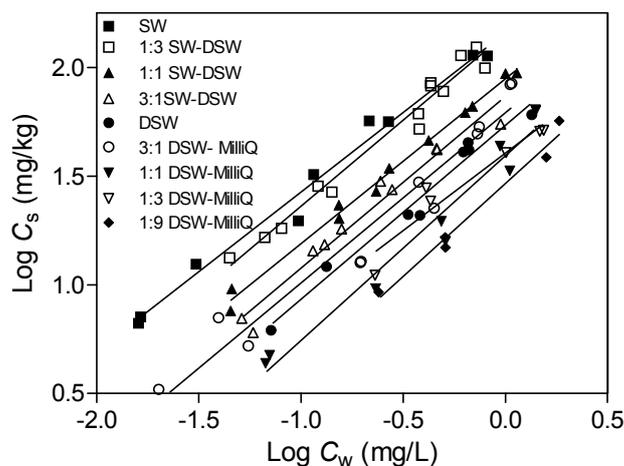


Figure 5. Sediment-water isotherms for C_{12} -2-LAS at different seawater and freshwater dilutions.

The effect of the aqueous composition on K_{fw} and K_F

The effect of the ion concentration (Ca^{2+} , Na^+ , K^+ , etc) on sorption processes of ionizable compounds has been observed in several studies with sediment [2,6,24], octanol [2] and membranes [36,37]. Two mechanisms can explain this effect. The first one is ion pair formation of LAS molecules with the cations present in solution. Variation of the sorption affinity at different electrolyte concentration is therefore related to the predominance of the neutral species, since ion pair partitioning is considered to be much higher (up to 10 000 times) than ion partitioning [36]. A second explanation is related to the effect of the cations on the sorbent phase itself. The cations present in solution (such as Ca^{2+} and Na^+) may interact with the negative groups of the sorbing phase, creating a positive charge at the fiber surface, and thereby increasing the affinity for negatively charged ions such as LAS. Alternatively, reduction of the negative charge of a sorbing phase, due to the increasing presence of cations, might also reduce the repulsion of the LAS anion, enhancing the adsorption of LAS to the fiber.

The first mechanism (ion pairing) is probably the most plausible explanation for the effects of the different ionic composition on K_{fw} values, though still poorly understood because the second mechanism will only occur in an adsorption process. The linear isotherms are already an indication that absorption is the process dominating the sorption of LAS to the fiber. An earlier study that focused on the calibration of SPME fibers for LAS compounds has furthermore shown that the sorption of LAS to the fiber is mainly governed by diffusion into the fiber polymer, and adsorption to the fiber surface is not significant [22]. In addition, the relationship between K_{fw} and the Ca^{2+} concentration from data in the fresh and seawater solution as well in the $CaCl_2$ solutions (Figure 6), are similar to typical complexation curves of ionic compounds [2,20]. This curve shape was not observed for Na^+ , likely because the tested concentration range does not cover the entire LAS- Na^+ “complexation curve” (Figure 6 b).

According to Jafvert et al. [2] the partitioning of an anionic organic compound to the octanol phase in a single electrolyte solution that contains divalent cation can be mainly described by the following reaction:



Where M^{2+} represents the divalent cation and A^{-} is the organic anion. In this reaction, the anionic organic compound is acting as a counter ion to keep the electroneutrality. When fitting the K_{fw} values and the $\text{Log } [Ca^{2+}]$ concentration from the simple electrolyte solution experiment to a sigmoidal curve of a fix slope of 1, an apparent formation constant K_{Ca-LAS}^{+} of 2.5 log value is obtained. This value might be considered as an indication of the affinity of the partitioning of LAS at different Ca^{2+} concentration, but we cannot state if this is reflecting the real ion pair formation or not, since to our knowledge, no formation constant of this complex is yet reported.

The fact that all the sediment-water isotherms are non-linear is a first indication that partitioning is not the only process occurring, and the functional sulfonate group might therefore contribute by either specific or non-specific chemical interactions of an adsorption process. Contrary to the pattern observed for the fiber-water coefficient K_{fw} , the sediment water Freundlich constant (K_F) systematically increases when increasing the concentration of Ca^{2+} (Figure 6). The relation between K_F and Ca^{2+} does not follow the shape of a complexation curve. It remains unclear if the observed dependence of the sorption on the cation concentration is only related to a change in the sorbing phase and it may as well be a combination of this mechanism and ion pair formation.

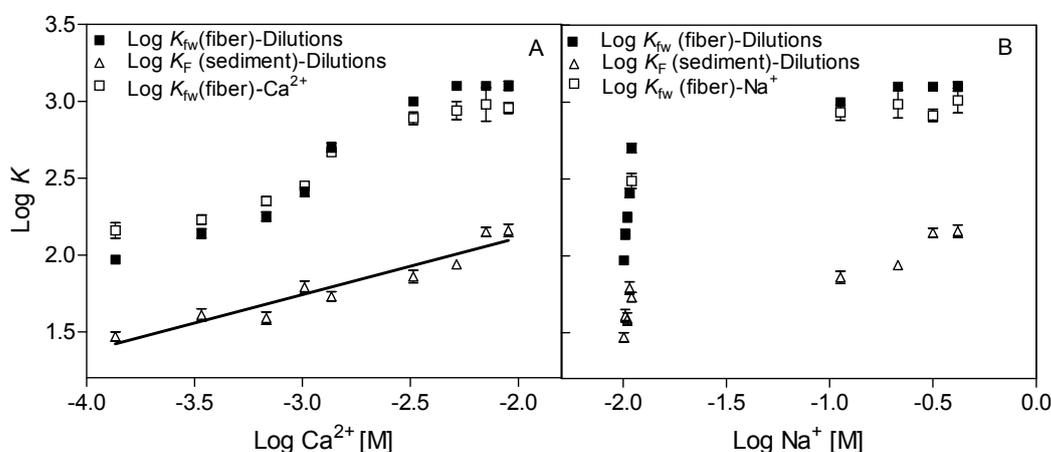


Figure 6. Sediment-water distribution coefficient (K_F , at 1 mg/L) or fiber-water partition coefficient (K_{fw}) as a function of the Ca^{2+} concentration (A) and Na^{+} (B) for the seawater and freshwater dilutions experiment and for the experiment with $CaCl_2$ and $NaCl$.

Fiber–water partition coefficients as a measure for hydrophobicity and parameter to predict sediment sorption: a brief evaluation

The effect of hydrophobicity on sorption processes is often modeled with the octanol-water partition coefficient (K_{ow}), and numerous studies have been reported showing a relation between the sorption coefficient (K_p) and K_{ow} [38-40]. Although the octanol-water partition

coefficient K_{ow} is used for the estimation of the hydrophobicity of organic compounds, the measurement or estimation of this parameter is not straightforward for ionic compounds like LAS. The experimental determination of K_{ow} for surfactants is complicated, because of the tendency of these compounds to accumulate at surfaces and readily emulsify both phases. Other parameters such as the critical micelle concentration (cmc) are used as hydrophobicity measure for surfactants, but this is not a good parameter when comparing different surfactant classes [41]. Roberts et al. [42] have proposed an algorithm for the calculation of K_{ow} values for LAS compounds, and also the KowWIN software from the EPI-suite (V3.20) package supplies estimates of K_{ow} for LAS homologues. It is questionable whether octanol or any other phases encapture the molecular interactions between ionized compounds and organic matter. Obviously, the mentioned estimation routines for K_{ow} are furthermore far from ideal, since (i) both estimation routines are not based on really measured experimental data, (ii) the estimates from both methods differ more than 0.5 log units, and (iii) no isomer specific K_{ow} are calculated by the KowWIN program. Moreover, as it is well known, the hydrophobicity of surfactants as LAS depends on the aqueous solution chemistry and estimation programs do not take that into account. The fiber-water partition coefficient might represent a suitable parameter for hydrophobicity differences of LAS constituents and correlates very well with the sediment sorption coefficient (see Figure 2). The advantage of the optimized SPME method is that it is the first hydrophobic phase for which distribution coefficients have been consistently determined for individual LAS congeners, both single and in mixtures, over broad concentration ranges and at varying salinity. Besides being easy to perform, it reduces analytical steps which might introduce systematic errors and background contamination.

The relation between the logarithms of the sediment sorption coefficients (K_f) versus the fiber-water partition coefficient (K_{fw}) for all the tested solution ratios is shown in Figure 2. Although there is a significant correlation ($r^2= 0.8$) between the sediment sorption coefficient and the fiber-water partition coefficient at different ionic composition of the aqueous phase, this correlation is not as good as the one obtained for the several LAS congeners in seawater. As mentioned above, the weaker correlation may be related to the fact that the sorption mechanisms to the fiber and the sediment are not completely similar. For a first screening, such a correlation may be useful, but for more precise estimates of sediment sorption coefficients at a certain aqueous solution, we would suggest to test one congener as a reference chemical in that particular solution. The sorption for other congeners in the same solution can then be predicted from a correlation with the fiber-water partition coefficient.

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Chapter 5

The effect of the molecular structure and water composition on the distribution of the anionic surfactant linear alkylbenzene sulfonate to humic acid

ABSTRACT

The sorption of LAS to Aldrich and Leonardite humic acid has been studied for different individual LAS compounds and at variable aqueous composition using polyacrylate (PA) SPME fibers. Linear sorption isotherms were obtained for all compounds. Solution chemistry did not affect the sorption affinity of LAS to the humic acid at the water dilutions tested. Sorption coefficients increase with increasing alkyl chain length, and they also depend on the position of the phenyl group on the alkyl chain. Besides the utility of the SPME method as a tool to measure the freely dissolved concentration of LAS compounds in humic acid solutions, the fiber-water partitioning coefficient values (K_{fw}) appears to be a good predictor for the sorption of LAS compounds to humic acids.

INTRODUCTION

The anionic surfactant linear alkylbenzene sulfonate (LAS) is one of the most extensively used synthetic surfactants in the formulation of detergents and household cleaning products. LAS is produced as a mixture of molecules with different alkyl chains (10 to 13 methyl groups) and different position of the benzene-sulfonate moiety on the alkyl chain. Literature about the effect and behaviour of LAS in the terrestrial and aquatic environment is very extensive due to its extensive use during the past three decades. Regarding the sorption of LAS to natural sorbents, both non polar and specific interactions are considered to contribute to the overall sorption process [1-4]. Sorption of LAS depends on the sorbent characteristics as well as the structure of the LAS congener or isomer [1,5-7].

Whereas several studies have been published on the sorption of LAS to environmental sorbents such as sediments and soils, less information is available about the sorption of LAS molecules to dissolved humic acids. It has been demonstrated that the sorption of organic pollutants to dissolved organic matter (DOM) affects the freely dissolved concentration of these compounds in the environment, and hence, their bioavailability to organisms [8-13]. Humic acids are one of the main constituents of DOM, and they consist of polydisperse molecules that contain hydrophilic and hydrophobic units, with a variable molecular weight and structural conformation [13-15]. Only Traina et al. [16] have studied the sorption of LAS isomer mixtures to different types of dissolved humic acids. This study also showed that the toxicity of LAS to fathead minnows was considerably reduced at humic acid concentrations between 8 and 24 mg C/L. The sorption of individual congeners or isomers was nonetheless not tested. In order to get a more accurate prediction of the fate and final distribution of these compounds in the environment, sorption coefficients of the individual LAS compounds to humic acids are needed.

Solution chemistry variables, such as pH or salinity, may also affect the sorption of ionisable compounds to humic acids, because both the compound and the sorbent can interact with the ions present in solution, and they consequently change their sorption characteristics [15,17]. Distribution of quinolones to dissolved humic acids changed when varying the solution pH [18], but this could not be predicted using the pH and the ionization constants of the compounds, likely because the sorption mechanism involved various types of interactions. Another example is the decrease of sorption of phenanthrene to humic acids with increasing ionic strength in the solution [19]. This effect was explained by conformational changes of the humic substance.

There are several experimental techniques for the determination of the binding of organic compounds to dissolved sorbents, including techniques such as fluorescence quenching [9,16], headspace partitioning, dialysis [20], etc. For this study, we have chosen solid phase micro extraction (SPME) fibers. SPME is a relatively simple tool for the analysis of freely dissolved concentration of non-polar and polar organic compounds. The SPME technique is specially appropriated for systems in which the sorbent phase is difficult to separate from the aqueous phase [10,21-25]. The SPME method can be designed to extract only a negligible fraction (< 5 % of the amount in solution) without significantly affecting the

equilibrium of the bound and free form of a chemical. [26]. The concentration of the analyte in the aqueous phase can then be derived from the concentration in the fiber polymer and the appropriate partition coefficient (K_{fw}). The optimization of the SPME analysis of individual LAS compounds in a wide range of seawater and freshwater matrices has been already performed [5,27].

The main objective of the present work was to elucidate the effect of the molecular structure and the solution composition on the sorption of LAS compounds to dissolved humic acids. Two types of humic acids were used, commercial Aldrich and the standard Leonardite humic acid. Additionally, Aldrich humic acid was tested as received by the supplier and after a purification treatment. Given that fiber-water coefficients (K_{fw}) for LAS compounds are dependent of the aqueous chemistry [5]. We have first calibrated the SPME method for all the test compounds at the specific composition of the aqueous test medium in this study. In several cases we could use K_{fw} values that are already published [27]. The effect of the solution chemistry and pH on the sorption of LAS to dissolved humic acid was further tested for the individual compound C₁₂-2-LAS. Humic acid sorption coefficients are related to fiber water partition coefficients (K_{fw}), where K_{fw} can be regarded as a measure for the hydrophobicity of LAS congeners and isomers. The implications of DOC binding to the behaviour of LAS in the environment are here discussed.

MATERIALS AND METHODS

Chemicals, solutions and SPME fibers

Individual LAS compounds are abbreviated as C_n-m-LAS, where n is the length of the alkyl chain and m is the position of the benzene-sulfonate group on the alkyl chain. C₁₀-2-LAS, C₁₁-2-LAS, C₁₂-1-LAS, C₁₂-2-LAS, C₁₂-3-LAS, C₁₂-4-LAS, C₁₂-5-LAS, C₁₂-6-LAS and C₁₃-2-LAS with a purity > 97% were synthesized as sodium salts by Dr. J.Tolls [28]. Surfactant stocks were kept in analytical methanol (Lab-Scan, Dublin, Ireland). Freshwater solution was prepared according to standard procedures [29] by adding the salts CaCl₂·2H₂O, MgSO₄·7H₂O, NaHCO₃ and KHCO₃ from Merck (Darmstadt, Germany) to Millipore water, at a final concentration of 200, 180, 100 and 20 mg/L, respectively. NaN₃ (10 mM) was added to all the test solutions to inhibit biodegradation. Experiments were performed at different dilutions of standard freshwater (FW) with MilliQ water (9:1, 3:1, 1:1, 1:3, 1:9 volume-volume ratio FW-MilliQ).

Polyacrylate (PA) SPME fibers (7.5-7.6 μm polyacrylate thickness) were obtained from Polymicro Technologies (Phoenix, AZ). Fibers of a length of 40 ± 0.1 mm were thermally conditioned in a GC oven and kept in Millipore water as reported elsewhere [27].

Humic acids (HA)

Two types of humic acids were used. One was the Aldrich humic acid sodium salt, and the other the Leonardite humic acid standard (catalogue no. 1S104H), acquired from the International Humic Substance Society (IHSS; www.ihss.gatech.edu). Aldrich humic acid was

first planned to be used as received by the supplier, without any modification. Hence, the humic acid was dissolved in Millipore water at a nominal concentration of 1000 mg/L and adjusted to pH 10 with the aid of NaOH to accelerate the dissolution; the solution was shaken overnight and thereafter adjusted to pH 8. However, small grains were observed at the bottom of the final solution after 2-3 days. This solution was then filtered using an ultra-filtration membrane (0.45 μm HVLP Durapore[®] Membrane Filters, Ireland). In the present work, we refer to this treatment as the “non purified-humic acid”. The second treatment (“purified-humic acid”), consisted on the purification procedure described by Koopal et al. [8], with the exception of the removal of the metal traces. Leonardite humic acid was dissolved in Millipore water with NaOH at pH 10, and readjusted to pH 8 after the complete dissolution. No filtration was needed for the Leonardite humic acid solutions. Dissolved organic carbon content (DOC) was determined at the integrated laboratory of the Faculty of Geosciences/TNO –Geolab- at Utrecht University (the Netherlands). Humic acids stocks solutions in Millipore (ca. 1000 mg/L on a nominal basis) were kept at 4 °C until their use.

SPME-freshwater isotherms for different LAS constituents

Fiber-water coefficients (K_{fw}) for most dilutions and for the different test solutions are available from an earlier study [5], but not for the 9:1 (volume-based) artificial freshwater-MilliQ water stock. Therefore, K_{fw} was measured for the 9:1 dilution according to the procedure as previously reported [5]. Briefly, solutions were made in 20 mL scintillation vials with polyethylene-lined screw caps (Perkin Elmer, Boston, MA) by spiking a weighed aliquot (<1% of the total volume) of the individual LAS stocks in methanol to the freshwater solution. All experiments were performed at room temperature (19 ± 1 °C). Equilibration of the fiber-surfactant solution system (48 h) and fiber extraction was performed as described earlier [27]. LAS concentrations in solution were determined for at least two of the test systems (low and high concentration) by Solid Phase Extraction –SPE- with C18 SPE columns (Supelclean[™] ENVI[™]-18, 0.5 g, Supelco, Bellefonte, PA), following the procedure described elsewhere [3]. Measured concentrations were close to 100 % of the nominal concentration for all compounds, with the lowest recovery obtained for C₁₀-2-LAS ($93 \pm 8\%$). Accordingly, adsorption of LAS to the walls of the vial and depletion by the SPME fiber was considered negligible, and a 100 % mass balance was thus considered for the calculations in the rest of the tests.

Because the pH slightly changes with the dilution factor (from 7.8 to 8.3), we also performed a study for one of test chemicals (C₁₂-2-LAS) at a fixed pH of 7.7. The buffer was prepared with NaH₂PO₄ (Merck, Darmstadt, Germany). The fiber-water sorption coefficient was not affected by the difference in pH. The experiments with other test chemicals were therefore performed without buffer.

Humic acid-water sorption experiments

Aliquots of the Aldrich or Leonardite humic stocks were added to Erlenmeyer flasks that contained the freshwater standard solution. Except for the experiments to test the effect of the different aqueous dilutions, all test systems contained 9:1 (volume-based) FW:MilliQ

solutions. Humic acid stocks were therefore diluted in MilliQ water to achieve the humic acid concentration and solution ratio required. Surfactant aliquots in methanol (<1% of the total volume) were added to the 20 mL vials that contained the humic acid solutions, and systems were equilibrated during 24 h, which corresponds to the equilibration period used for sorption experiments with sediments [3]. A study of the sorption of LAS homologues to humic acids in a similar system have reported that 2 h is sufficient to reach the equilibrium [16]. Next, SPME fibers were introduced in the vials and equilibrated for at least 48 h. After equilibration, fibers were extracted and aqueous concentrations were measured. K_{DOC} was calculated from the difference between the measured aqueous concentration and the nominal concentration, assuming 100 % mass balance. In order to get accurate measurements, only experiments in which LAS depletion by the humic acid was higher than 20 % of the total amount in the system, were used for the calculations. Blank and control systems were included to check the possible background concentration and to check the 100 % mass balance assumption. Duplicate samples were used for all tests.

Sorption experiments with Aldrich humic acid

One of the purposes of this study was to test the sorption of LAS to dissolved humic acid at a wide range of salinities. With this objective, aliquots of purified-HA stocks were initially added to artificial seawater –GP2- prepared according to a standard procedure [30] and to freshwater-seawater dilutions, resulting in ionic strengths from 0.1 to 0.6 M. However, flocks and precipitated particles were detected for all the seawater dilutions tested here. Sorption experiments were thus only performed for the freshwater stock and further freshwater-MilliQ dilutions, for which no flocculation or precipitation was observed. Literature also reports visual detection of coagulates in humic acid solutions at ionic strengths from 0.05 to 0.5 M [31], and no coagulation at ionic strengths from 0.001 to 0.01 M for solutions of Sodium, Aluminium or Calcium [19].

Sorption experiments with freshwater dilutions were then performed with and without the buffer salt NaH_2PO_4 . Freshwater stocks were diluted at different ratios with MilliQ water, resulting in ionic strengths varying from 0.006 to 0.011 M. pH measurements in the absence of buffer showed a difference of 0.5 log units in the pH from the first to the most diluted solution (pH=7.8-8.3). Buffered solutions had a constant pH of 7.8. Every test system had a final humic acid concentration of 50 mg/L (nominal concentration) and an initial C_{12} -2-LAS concentration of 100 $\mu\text{g/L}$.

Thereafter, distribution coefficients for the four C_n -2-LAS homologues to Aldrich humic acid were obtained for the non purified- and purified-humic acids at a fixed surfactant concentration of 300 $\mu\text{g/L}$, and varying humic acid concentrations between 2 and 150 mg of C/L.

Sorption experiments with Leonardite humic acid

Sorption of LAS to Leonardite humic acid in artificial freshwater was studied for the four C_n -2-LAS homologues and for all the C_{12} -m-LAS isomers at a humic acid concentration of 50 mg/L in non buffered solutions, and initial surfactant concentrations ranging from 30 to 3100 $\mu\text{g/L}$.

All experiments were performed in the 9:1 FW-MilliQ dilution. Sorption experiments and SPME fiber extraction were performed following the procedure explained above.

Chemical analysis

Details on the extraction and LC/MS/MS analysis of the SPE and SPME samples are reported elsewhere [27]. Millipore water and Ammonium acetate were added to the SPE samples to make the solution equal to the eluent composition (80 % MeOH, 20 % H₂O, 10 mM NH₄⁺).

RESULTS AND DISCUSSION

Determination of the fiber-water coefficient (K_{fw})

The fiber-water sorption coefficient values (K_{fw}), for the tested individual LAS compound in the 9:1 FW-MilliQ solutions are presented in Table 1. Sorption isotherms are represented in Figure S-1 of the Appendix. All fiber-water sorption data was fitted to a linear equation of a slope of 1. The linear isotherms indicate that an absorption process is governing the sorption of the LAS compounds to the SPME fiber. The differences obtained between the K_{fw} values for C_n-2-LAS homologues and C₁₂-m-LAS isomers, are similar to the differences among the K_{fw} values for LAS in seawater. However, K_{fw} values in freshwater are 0.4-0.6 log units lower than in seawater, because LAS partitioning into the fiber is significantly affected by the amount of salts in solution [5].

The distribution of C₁₂-m-LAS at different freshwater dilutions to the SPME fiber was also tested at a fixed pH. Extraction isotherms for the same compound and freshwater dilutions, but at a variable pH (7.8-8.3) have been reported in Chapter 4 [5]. No differences were observed among the K_{fw} values at a constant and variable pH, indicating that the differences are likely due to other factors than pH variation (e.g. differences of Ca²⁺, as discussed in Chapter 4 [5]). An example is shown in Figure S-1 (Appendix) for the isotherm of C₁₂-2-LAS, in which data points from the 9:1 FW: MilliQ solution without buffer and with the buffer salt fit the same isotherm.

Influence of the solution chemistry on the sorption of C₁₂-2-LAS to humic acid

K_{DOC} is the sorption coefficient that relates the concentration of the compound in the humic acid (as dissolved organic carbon) and the aqueous solution. K_{DOC} values for C₁₂-2-LAS at the freshwater dilutions tested did not significantly differ along the dilutions. Figure 1 shows the determined distribution coefficients at the different dilutions tested versus the [Ca²⁺] in solution at a constant and variable pH. Previous literature research on the sorption of LAS and other anionic surfactants to natural sorbents, have shown that Ca²⁺ is the cation that enhances LAS sorption [1,32,33]. Besides, an earlier study using the same dilution ratios has shown that the sorption of C₁₂-2-LAS to natural sediment is significantly different among the dilutions [5]. Based on these findings, differences in K_{DOC} between the first freshwater

solution (9:1 dilution, corresponding to 1.22 mM Ca^{2+}) and the most diluted solution (0.14 mM Ca^{2+}) were here expected as well.

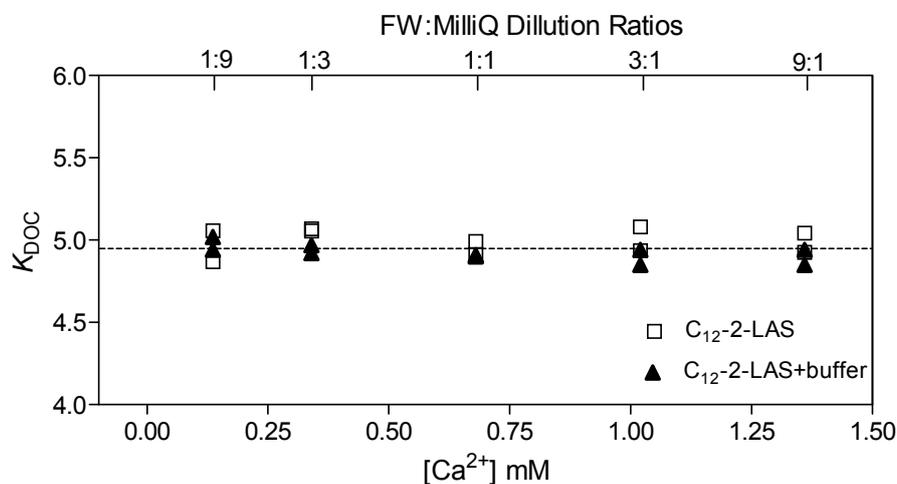


Figure 1. Calculated K_{DOC} (purified Aldrich HA) values for C_{12} -2-LAS at the different dilution ratios and expressed as the Ca^{2+} concentration in solution at a variable (\square) and constant (\blacktriangle) pH.

Changes in the conformation of the humic acid molecules due to changes in the ionic composition can explain an effect of Ca^{2+} . Humic acid molecules can form complexes with some of the cations in solution (Na^+ , K^+ , Ca^{2+} , etc) and then change the initial sorption characteristics [15,19,25]. Jones and Tiller [19] has shown that the sorption of phenanthrene to humic acid was significantly influenced by the effect of the ionic strength, and the maximum variation was obtained for NaNO_3 solutions at ionic strengths of 0.005 and 0.01 M; which are very similar to the ionic strengths of the present study. The same study reported that K_{DOC} values were considerably lower for solutions with only divalent and trivalent species.

The apparent lack of an effect of the solution composition on LAS binding to humic acid might also be due to two counteracting processes. For the low ionic strength solutions, the humic acid structure is more expanded, and more surface is available for surfactant-humic acid interactions [14,19]. At higher ionic strength, the humic acid conformation is probably more condensed, because divalent cations such as Ca^{2+} will favour cationic bridges between different parts of the humic acid molecule [14]. On the other hand, the presence of cations in the humic acid molecule might also neutralize the charge of the humic substance, facilitating the sorption of LAS by either hydrophobic or electrostatic interactions.

Sorption of LAS compounds to Aldrich humic acid: Effect of the purification treatment and DOC amount in the system

Figure 2 shows the variation of K_{DOC} at different DOC concentrations for the purified-Aldrich humic acid. Table 1 shows the K_{DOC} values obtained for both humic acid treatments (purified and non purified Aldrich humic acid). In general, the purification step does not seem to affect

the affinity of LAS compounds to DOC. For both humic acid treatments, K_{DOC} values were constant when varying the DOC concentration, suggesting that absorption is mainly the process governing the sorption of LAS to humic acids. Besides, as it will be discussed further on, sorption coefficient values increased with the number of methyl groups in the alkyl chain.

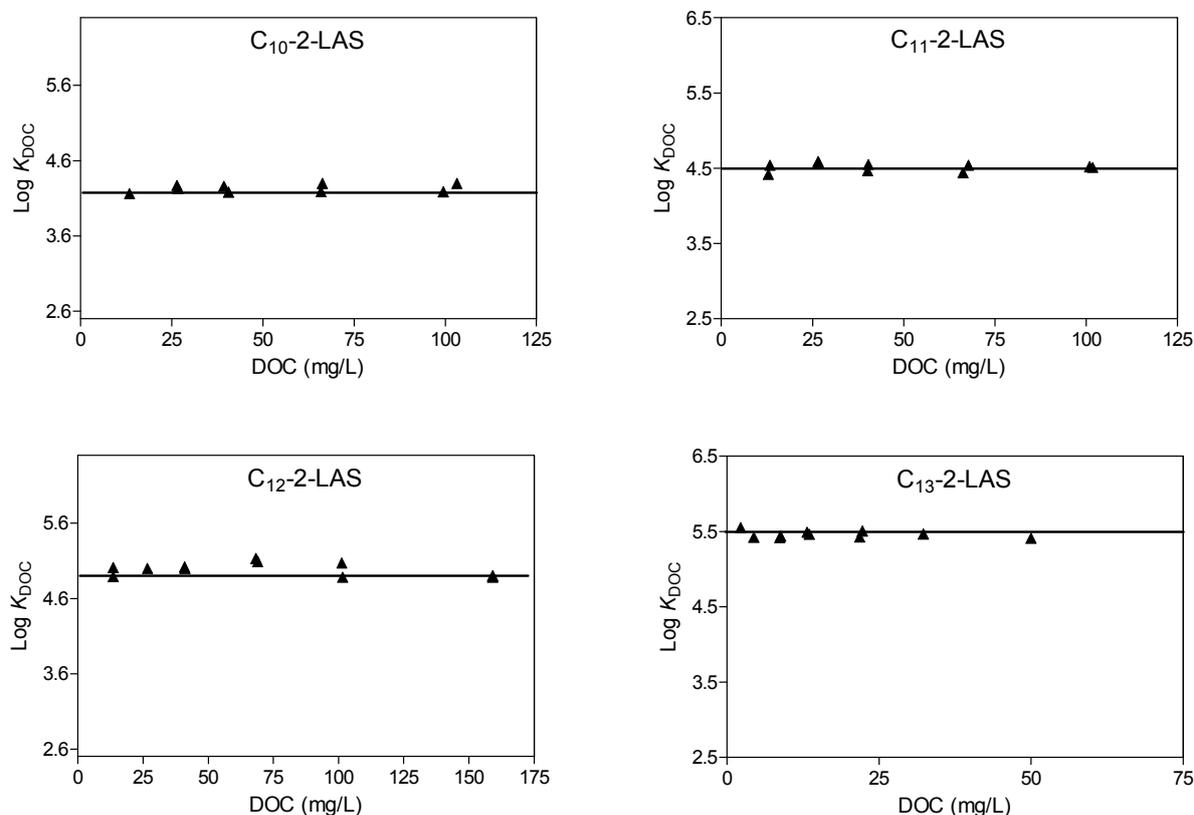


Figure 2. Calculated K_{DOC} (Aldrich HA, non buffered solution) values for the four C_n -2-LAS homologues at the different DOC concentrations for the purified humic acid. The lines represent the average of the K_{DOC} values.

Influence of the compound characteristics on the sorption of LAS to Aldrich and Leonardite humic acid

The sorption isotherms for Leonardite humic acid for the four C_n -2-LAS compounds and the six C_{12} -m-LAS isomers in the 9:1 FW-MilliQ dilution are represented in Figure 3. Sorption data were fitted to the Freundlich isotherm:

$$\text{Log}C_{\text{DOC}} = \text{Log}K_{\text{F-DOC}} + n \cdot \text{Log}C_w \quad (1)$$

where C_{DOC} (mg/kg) and C_w (mg/L) are the LAS concentration in the dissolved organic carbon and in the aqueous phase at equilibrium, respectively. $K_{\text{F-DOC}}$ (L/kg) is the Freundlich constant and n is the slope of the linear regression equation. The Freundlich parameters ($K_{\text{F-DOC}}$ and n) for all the tested LAS compounds are shown in Table 1.

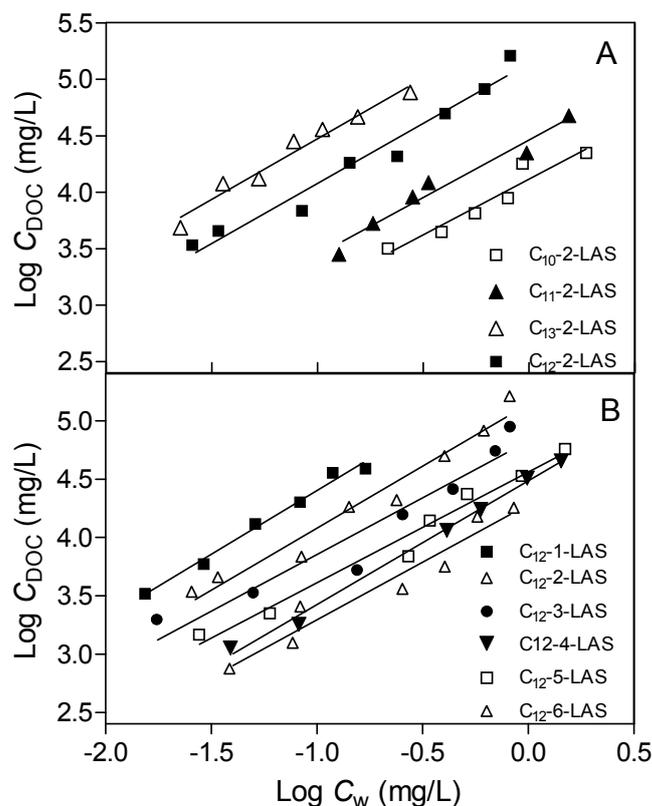


Figure 3. Sorption isotherms to Leonardite HA for the C_n -2-LAS homologues (A) and C_{12} -m-LAS isomers (B) in 9:1 (v:v) freshwater-MilliQ solution.

The slope n was very close to the unity in all cases. As observed for the Aldrich humic acid, sorption coefficients values increase when increasing the alkyl chain length, which is suggested to be mainly the result of a higher hydrophobicity [1,4]. Respect to the isomers, the highest K_{F-DOC} values are observed for the compounds with the most external phenyl position. Figure 4A represents the K_{F-DOC} values for each of the individual LAS compounds against the number of carbon atoms in the alkyl chain. Similar differences among the isomers have been already reported for bioconcentration factors [34] and sediment sorption coefficients [4,5]. Figure 4B shows the logarithm of the humic acid sorption constants (K_{F-DOC}) for all tested LAS compounds against their respective fiber-water partition coefficients (K_{fw}) measured in the same freshwater solution. A reasonably good linear relationship between these two parameters is obtained ($n= 0.9$; $r^2=0.8$), indicating that K_{fw} values can be an optimal parameter to predict the sorption of LAS compounds to humic acids. A good relationship between the K_{fw} and the sediment sorption coefficient values for the tested compounds and at different salinities for one specific isomer has also been obtained earlier [5]. The use of the K_{fw} values as experimental values to predict sorption of LAS compounds to environmental sorbents is advantageous over the application of the octanol-water coefficients (K_{ow}). Reliable experimental K_{ow} measurements for surfactants are difficult, because of the amphiphilic character of the molecule, while K_{fw} measurements are relatively

easy to perform. In addition to the application of SPME in measuring DOC sorption coefficients, the K_{fw} can also be used as a parameter to estimate DOC sorption coefficients for LAS.

Table 1. Fiber-water coefficients (K_{fw}), Freundlich parameters for the sorption of LAS to Leonardite humic acid (K_{F-DOC} , n), and dissolved organic carbon-water coefficients for the Aldrich humic acid experiments (K_{DOC}) for all the LAS tested compounds.

Compound	SPME fiber	Leonardite Humic Acid		Aldrich Humic Acid
	Log K_{fw}	Log K_{F-DOC}	n	Log K_{DOC}
C ₁₀ -2-LAS	1.6 ± 0.03	4.1 ± 0.14	0.9 ± 0.14	4.2 ± 0.05 ^b 4.1 ± 0.14 ^c
C ₁₁ -2-LAS	1.9 ± 0.03	4.5 ± 0.06	1.0 ± 0.09	4.5 ± 0.06 ^b 4.4 ± 0.17 ^c
C ₁₂ -1-LAS	3.0 ± 0.04	5.5 ± 0.08	1.1 ± 0.06	
C ₁₂ -2-LAS	2.6 ± 0.02 2.6 ± 0.03 ^a	5.1 ± 0.08	1.1 ± 0.08	4.9 ± 0.09 ^b 4.9 ± 0.21 ^c
C ₁₂ -3-LAS	2.6 ± 0.06	4.8 ± 0.12	0.9 ± 0.13	
C ₁₂ -4-LAS	2.3 ± 0.02	4.5 ± 0.03	1.1 ± 0.04	
C ₁₂ -5-LAS	2.3 ± 0.05	4.6 ± 0.05	0.9 ± 0.07	
C ₁₂ -6-LAS	2.4 ± 0.05	4.3 ± 0.1	1.0 ± 0.11	
C ₁₃ -2-LAS	3.0 ± 0.03	5.6 ± 0.11	1.1 ± 0.09	5.5 ± 0.05 ^b 5.4 ± 0.14 ^c

^aC₁₂-2-LAS isotherm using the buffer NaH₂PO₄.

^bCalculated as the average of K_{DOC} at all the DOC concentrations tested for the purified and filtered Aldrich Humic acid.

^cCalculated as the average of K_{DOC} at all the DOC concentrations tested for non purified Aldrich Humic acid.

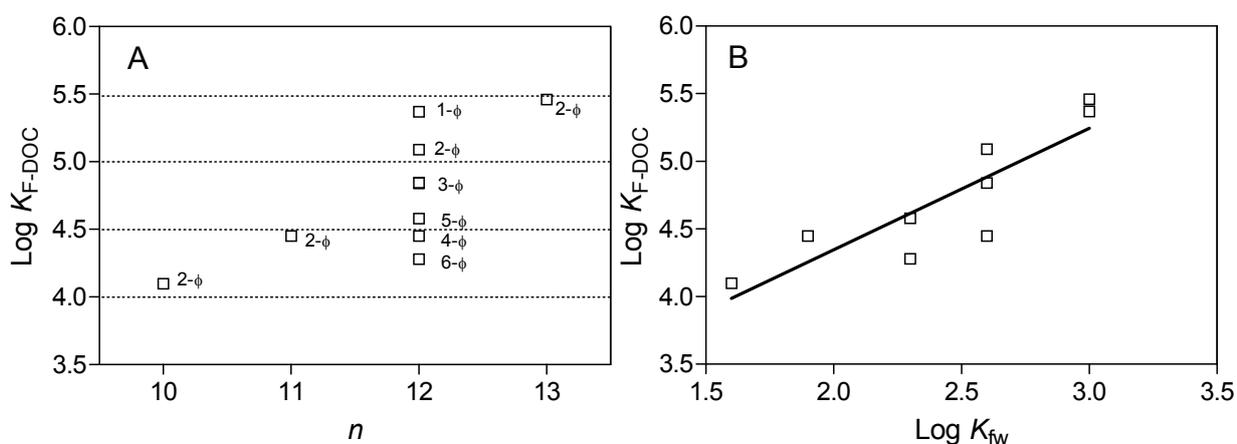


Figure 4. K_{F-DOC} values (Leonardite humic acid) of LAS congeners as a function of the number of methyl groups in the alkyl chain (A) and as a function of the fiber-water sorption coefficient (K_{fw}) (B). The fitted regression line is $\text{Log } K_{F-DOC} = 0.9 (\pm 0.19) K_{fw} + 2.55 (\pm 0.48)$.

Environmental and practical implications of DOC binding of LAS

The present investigation has shown that the presence of DOC in the aquatic environment may considerably decrease the freely dissolved LAS concentration, and that K_{DOC} values are clearly dependent of the molecular structure of the LAS compounds. Although Aldrich humic acid is not always comparable with natural humic acids [13], the obtained results indicate that LAS compounds have similar affinities to the untreated and purified Aldrich humic acid, and K_{DOC} values for the four C_n -2-LAS homologues were very similar to those obtained for the Leonardite humic acid. The obtained K_{DOC} values are also in the same order of magnitude as those obtained by Traina et al. [16] for LAS homologues (as a mixture of isomers) using Aldrich and other reference and natural humic acids.

Figure 5 shows the influence of the DOC concentration on the free fraction of the different LAS congeners tested. Curves were obtained from the $K_{\text{F-DOC}}$ values obtained for the Leonardite humic acid and the equation:

$$1 - \alpha = \frac{1}{(1 + K_{\text{F-DOC}} \cdot [\text{HA}])} \quad (2)$$

Where α is the bound fraction of the LAS compound at the different humic acid concentrations ($[\text{HA}]$, kg/L). For an environmental relevant humic acid concentration of 15 mg/L, 20-35 % of C_{12} -LAS compounds with the phenyl group in the central position of the molecule (C_{12} -4- C_{12} -5 and C_{12} -6-LAS) will be associated to humic acid, versus 50-65 % of C_{12} -LAS compounds with the phenyl group in the most external positions (2- and 3-phenyl). These differences will directly affect the LAS dissolved concentrations in the aquatic environment. In the HERA report for LAS [35], it is listed that commercial LAS is a mixture of isomers and homologues with the mole ratio $C_{10}: C_{11}: C_{12}: C_{13}=13:30:33:24$, an average carbon number of 11.6, and 18-29% content of the 2-phenyl isomers. However, environmental fingerprints differ from those of the commercial products, because processes such as sorption affect more the longer alkyl chain homologues and the molecules with the most external phenyl groups.

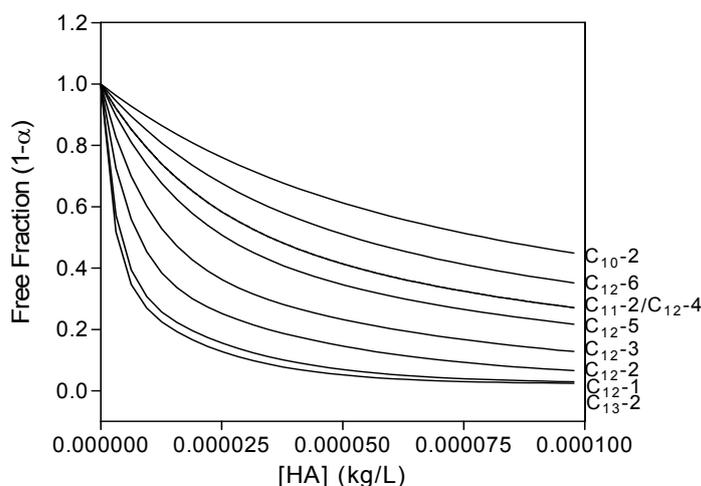


Figure 5. Variation of the freely dissolved fraction of LAS versus the humic acid concentration for the LAS congeners tested with Leonardite humic acid (for explanation see text).

Indeed, the same report estimates that the ratio of the homologues in the aquatic environmental samples is C10:C11:C12:C13 = 45:30:23:2 with an average carbon number of 10.8. Although the average isomer fraction is not reported, it is expected that the fraction of the 2- and 3-phenyl isomers will also be smaller. In spite of this, the final composition of LAS compounds reaching the environment will also depend on the location, type of effluent, and wastewater treatment received.

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APPENDIX

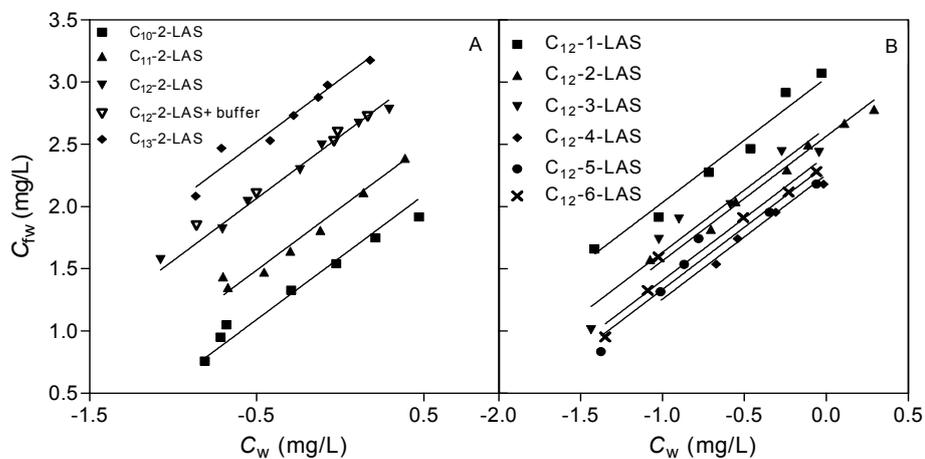


Figure S-1. Fiber-water isotherms for the C_n -2-LAS homologues (A) and C_{12} -m-LAS isomers (B) in 9:1 (v:v) freshwater-MilliQ solution.

Chapter 6

Equilibrium partitioning theory to predict the sediment toxicity of the anionic surfactant C₁₂-2-LAS to *Corophium volutator*

ABSTRACT

The study of the effect of the sorption of linear alkylbenzene sulfonates (LAS) on the bioavailability to marine benthic organisms is essential to refine the environmental risk assessment of these compounds. According to the equilibrium partitioning theory (EqP), the effect concentration in water-only exposure will be similar to the effect concentration in the sediment pore water. In this work, sorption and desorption experiments with two marine sediments were carried out using the compound C₁₂-2-LAS. The effect of the sediment sorption on the toxicity of benthic organisms was studied in water-only and in sediment bioassays with the marine mud shrimp *Corophium volutator*. In addition, three common spiking methods were tested for its application in the toxicity tests, as well as the stability of the surfactant during the water-only and sediment-water test duration. LC50 values obtained from water-only exposure showed a good correspondence with the pore water concentrations calculated from the sorption and desorption isotherms in the spiked sediments.

INTRODUCTION

Coastal ecosystems can contain traces of synthetic surfactants, which are the active constituents of commercial detergents, personal care and industrial cleaning products. Among these compounds, linear alkylbenzene sulfonates (LAS) are one of the most produced surfactants, with a total world consumption of 2.9 million tons in 2003 [1]. The presence of these compounds in the marine environment is mostly due to discharges of treated or untreated wastewaters. Although it has been shown that LAS is removed up to 99% in treatment plants [2], and that it is highly biodegradable under aerobic conditions in seawater [3], and under anaerobic conditions in marine sediments [4], monitoring studies have shown the presence of these compounds in coastal sediments close to points of municipal and industrial discharges [5]. Insight into the sorption processes of LAS in marine sediments and the effects of sorption on the bioavailability is thus required to improve the risk assessment to marine benthic organisms.

For non-polar organic compounds, it has been shown that toxicity in the sediment, expressed as the concentration in sediment pore water, is often the same as toxicity measured in water only exposure. This aqueous pore water concentration can be estimated from the concentration in the sediment, and the sediment sorption coefficient. These two aspects form the basis for the Equilibrium Partitioning approach (EqP) [6]. The octanol-water partition coefficient (K_{ow}) is often applied to estimate the sorption coefficient to sediment as well as the bioconcentration factor and the acute toxicity of non-polar contaminants. This theory has been developed and applied to non polar contaminants such as PAH's [7,8]. Application of EqP to more complex structures and polar or ionized compounds is less straightforward because the information about their sorption is often not available. In addition, partitioning of surfactants between two phases is different from most non-polar organic compounds because of their amphiphilic properties. Sorption processes of polar and ionized organic compounds like LAS in sediments do not only involve hydrophobic interactions, but specific or electrostatic interactions may also play an important role, making it difficult to predict the sorption to different environmental matrices [9]. Most of the sorption studies with LAS have been performed with fresh water sediments and materials [9-13] and only few studies have reported sorption values on marine sediments [14-16]. In general, sorption of LAS increases with the alkyl chain of the homologue. Sorption of LAS also depends on the characteristics of the medium (salinity, pH, [Ca²⁺]) and the sediment (organic carbon, clay content, extractable Fe and Al). Nevertheless, the specific contribution of these factors has not been fully characterized yet.

The other aspect of the EqP theory is the toxicity in both sediment and the aqueous phase. Sediment toxicity data for LAS are rather scarce and most of the studies have been focused on exposure via the water column. Comber et al. [17] suggested that the LAS dissolved fraction was the main fraction responsible for the toxicity of organisms with different feeding strategy in freshwater sediments. However, pore water concentrations were not reported. Hampel et al. [18], stated that different feeding habits may change the sensitivity of the organisms to sorbed LAS in seawater sediments. Although effect

concentrations are well above concentrations in the field, testing the EqP theory to this class of chemicals is of interest for the development of quality criteria. Cano et al. [19] studied the effect of one sediment variable, the organic carbon content, on the toxicity of highly branched alkylbenzene sulfonate (ABS) to fresh water amphipods. The organic carbon content was artificially changed by varying the amount of peat moss in a sandy sediment. The toxicity data corresponded within a factor of 10 with predictions from the EqP approach with organic carbon normalized partition coefficient, and the surfactant sorption model by Di Toro et al. [10]. However, in natural sediments, other factors than organic carbon influence the bioavailability of LAS in sediments due to the complexity of these matrices.

The aim of the present study was to test the validity of the EqP theory for the single LAS constituent C₁₂-2-LAS (2-(*p*-sulfophenyl)-dodecane) in marine sediments. For this purpose, sorption and desorption studies were first performed with the marine sediments that have been used in the bioassays. Subsequently, toxicity tests with the marine amphipod *Corophium volutator* were carried out in water only exposure as well as in spiked sediments. *Corophium volutator* are sediment-dwelling amphipods living in the upper 5 cm of intertidal estuarine sediments in the North Sea, and they are commonly used in hazard assessments of sediment contaminants [20,21]. Spiking of the sediments for their use in toxicity tests is not a straightforward procedure, in particular because of the low solubility of LAS in seawater. This low solubility complicates the spiking with natural seawater (the most realistic way) at the high concentrations needed in a toxicity bioassay. In general, spiking methods should be evaluated in terms of homogeneity and changes of the sediment that may affect the bioavailability of the compound in the test. These topics have already been discussed in detail by Northcott and Jones [22] and several recommendations have been presented. For this study, three spiking methods were tested: glass-coating, solvent spiking (via methanol) and direct spiking via the aqueous phase. In addition, the stability of the chemical was tested under the same conditions as those used in the bioassays. Short-term sediment toxicity tests were performed that allowed to work at exposure concentrations.

MATERIALS AND METHODS

Chemicals, organisms, sediments and sediment characterization

The compound 2-n-(*p*-sulfophenyl)-dodecane sodium salt (C₁₂-2-LAS), with a purity of 97.4%, was synthesized by Dr. J.Tolls at our laboratory. Stock solutions were kept in analytical methanol from Lab-Scan (Dublin, Ireland). Deionized water used for the spiking of the sediments was prepared in a Millipore Milli-Q purification system with a resistivity of circa 18 MΩ cm. Natural seawater originating from the Eastern Scheldt, an estuary in the south of The Netherlands, was filtered over a sand-bed filter that withdraws the particles larger than 10 μm.

Sediments were collected from a depth of approximately 5 cm from the Eastern Scheldt estuary (Oesterput, The Netherlands) and the Cádiz bay (southwest of Spain). Both

areas are relatively clean with C₁₂-LAS background concentrations of 23.3 (± 8) µg/kg and 301 (± 29) µg /kg for Oesterput and Cádiz sediments, respectively. Sediments were sieved (500 µm mesh) and stored at 4°C until their use (no more than 4 weeks storage) as recommended by standard protocols [23-25]. Sediment particle size distribution and organic carbon content were determined in the Geochemistry department of the University of Utrecht. The grain size distributions were determined in a Laser Particle sizer (Malvern, Mastersizer MS17). For the measurement of the organic carbon content, sediments were first treated with hydrochloric acid to remove carbonates and then analyzed in a sulfur-carbon analyzer at 1300°C. The Cádiz Sediment was a sandy sediment with 99.30 % sand size particles and low organic carbon content (0.06%). The grain size distribution of Oesterput sediment was 46.6% sand, 46.4 % silt and 6.99% clay, and the organic carbon content was 1.38 %. These sediments were chosen because the sorption of LAS to them was expected to be different, and this is an important aspect for testing the equilibrium partitioning approach.

Amphipods (*Corophium volutator*) were collected from the same site in the Eastern Scheldt estuary from the top 5 cm sediment and sieved (500 µm mesh). The organisms were acclimated and maintained in the laboratory in buckets with clean sediment and seawater, and aerated continuously at 17 (±1) °C for at least 4 days before testing.

Sorption and desorption studies of C₁₂-2-LAS in the sediments

Sorption experiments were performed at a density of sediment of 0.1 g (d.w.) in 20 mL of C₁₂-2-LAS natural seawater solution, containing 10 mM NaN₃ and at the same concentration range as used for the toxicity studies (0.01 to 2.5 mg C₁₂-2-LAS /L in the aqueous phase). All LAS concentrations are well below the Critical Micelle Concentration for LAS (105 mg/L in 0.01 M Na₂SO₄ solution [26]). The effect of the NaN₃ concentration to the sorption of LAS to sediment has been previously evaluated [9] and it showed no effect at 10 mM or higher concentrations. All treatments were carried out in triplicate. A detailed description of the experimental procedure for sorption experiments is given by Rico-Rico et al. [27], and this study also presents sorption isotherms. However, the isotherms are different because the experiments were carried out using different water matrices (artificial versus natural seawater) and at a different concentration range.

For the desorption experiments, sediment was equilibrated with the C₁₂-2-LAS solution in seawater. Phases were then separated by centrifugation and the aqueous fraction was removed and analyzed. Subsequently, 20 mL of a seawater solution 10 mM NaN₃ was transferred and the system was left to equilibrate for 48 h (apparent equilibrium was reached after 20 h). The samples were subsequently processed as described for the sorption experiments.

Testing three different spiking methods

Three of the most common spiking procedures applied in sediment toxicity bioassays were tested in terms of recovery and homogeneity (for two of the methods) and aqueous concentration at equilibrium were measured in these systems. All experiments were done in triplicate.

“Coating method”. 5 mL of a C₁₂-2-LAS solution in methanol of 20 mg/L was transferred to 20 mL vials. The solvent was evaporated until dryness leaving a coating of the compound on the walls of the vials. 5 g of wet sediment with 1 mL of seawater, to facilitate mixing, were then added and the system was placed on a two-dimensional shaker, at a vigorous intensity, during 20 h. Next, two fractions were taken from every vial. The first fraction of ca. 1 g of sediment was freeze-dried and extracted. A second fraction of 2 g was mixed with 8 mL of seawater in a similar (volume: volume) ratio as in the bioassay (1:4). The system was left to equilibrate for 24 h in a climate-controlled chamber at 4 °C to limit biodegradation of the compound. Phases were separated by centrifugation (3000 rpm, 30 min) and the overlying solution was used for C₁₂-2-LAS analysis.

“Solvent-matrix method”. 4 g of wet sediment was mixed with 4 mL of a C₁₂-2-LAS solution in methanol of 100 mg/L on a two-dimensional shaker during 2 h. The spiked sediment was then placed in fumehood to evaporate the solvent (process controlled by weighing). 4.5 g of clean sediment and 0.5 g of the spiked sediment were then added to every test vial. The sediment system was shaken during 20 h. The content of the different vials were then separated in two fractions as described above. In order to study the homogeneity of the method, the same procedure was used with 200 g of wet sediment and a final nominal concentration of 20 mg/kg (d.w.). This experiment was performed in triplicate. Three sediment samples were taken from each beaker for C₁₂-2-LAS analysis.

“Water spiking method”. 40 g of wet sediment were mixed with 20 mL of a 20 mg C₁₂-2-LAS/L Millipore water solution. The system was shaken for 24 h to reach equilibrium (equilibrium time had been tested in sorption experiments) and left to set for another 24 h. Next, the overlying water was removed and sediment fractions were taken as reported above. Homogeneity was also studied with 200 g of wet sediment.

Stability study

The experiments to test the stability of C₁₂-2-LAS were performed in seawater and in sediment-seawater systems in similar conditions as used in the bioassays (aeration, photoperiod 16 h light/ 8 h dark, 17 ±1 °C). All experiments were carried out in triplicate.

For the study of the stability in seawater, solutions of 0.5 and 1.2 mg/L of C₁₂-2-LAS were made by adding an aliquot of the C₁₂-2-LAS stock solution in methanol to 500 mL of seawater. The beakers were then covered and aeration was provided during the experiment. An aliquot of 10 mL was taken from each beaker at every sampling time (0, 1 h, 7 h, 25 h, 48 h and 3, 4, 6, 8, 11 days).

For the sediment-water system experiments, ca. 200 g of wet sediment was spiked as in the "water spiking" procedure at two concentrations of 2.5 mg/kg and 650 mg/kg (d.w.) on a nominal basis. 500 mL of seawater were added and the system was left to equilibrate and to settle for 24 h at 4 °C. The test beakers were then placed in the 17 °C chamber. Sediment samples of 2 g were taken directly after the spiking step, and at 0, 1, 3, 5, 7 and 10 days once the seawater was added.

Acute toxicity assays in water-only exposure

The acute toxicity in water-only exposure was tested with *Corophium volutator* for three and five days. Nominal concentrations ranged from 0.2 to 1.6 mg/L and controls with the methanol carrier and with seawater were also included. C₁₂-2-LAS solutions were made by adding an aliquot of the stock solution in methanol to beakers containing 500 mL of the filtered seawater. The concentration of the solvent in the aqueous phase was always kept below 1 % of the total volume. Due to the decrease in the concentration of the test compound after 2-3 days, solutions were renewed on the second day in the five-day exposure experiment and mortality was also counted at that time.

Organisms were carefully sieved from the original sediment and kept in a beaker with clean seawater for 4 h before they were added to the test beakers to ensure the absence of sediment particles in the system. 20 organisms were gently placed in every beaker with a plastic Pasteur pipette and the beakers were closed and connected to the aeration system. Mortality (absence of movement when gently disturbed) was used as the endpoint. Bioassay conditions were similar as those mentioned in the stability tests.

Aliquots of 10 mL were taken from every test beaker and aqueous concentrations were measured in both assays at the beginning and at the end of the test (see below for sample treatment). For the experiment conducted over five days, samples were also taken before and after the renewal of the solution at the second day.

Acute toxicity assays in sediment-water exposure

For the sediment exposure, 200 g of wet sediments were spiked individually as in the water spiking procedure. After the spiking, the Millipore water was removed and 500 mL of seawater was added. The system was left to equilibrate and to settle for 24 h at 4 °C. The test beakers were then placed in the 17 °C chamber and the amphipods (20 organisms per test beaker) were added. Preliminary range finding test were performed to select the exposure concentrations. All exposure concentrations were prepared in triplicate.

The range finding study was carried out with the Oesterput sediment at two concentrations of 50 and 500 mg/kg. Separate test beakers were used for each exposure time (1, 3, 5, 7 and 10 days). Oesterput and Cádiz sediments were used for the five days exposure assay. Nominal concentration ranged from 60 to 600 mg/kg. Controls systems with the natural sediment and with sediment treated with Millipore water but without the surfactant were tested as well to check the effect of the spiking procedure on the organisms' response. Amphipods were sieved from the storage sediment and carefully transferred to the test.

At the end of the test, sediments were gently sieved to count the organisms. Missing organisms were assumed to be dead and mortality was used as the endpoint. Sediment and overlying water samples were taken in triplicate at the beginning (right before the introduction of the organisms) and end of the test.

Chemical analysis

Sediment samples (ca. 0.1 g) were extracted using 20 mL of methanol in an ultrasonic bath. Methanol extracts were dried and redissolved in 10 mL of Millipore water. Water and

sediment samples from all the experiments were cleaned up and concentrated by Solid Phase Extraction (SPE) using octadecyl reversed-phase silica (C18) columns (Supelclean™ ENVI™-18, 0.5 g, Supelco, Bellefonte, PA, USA). Recoveries for the sediment extraction method were 92 (± 19) %. Final extracts were analyzed by Flow Injection Analysis (FIA) on an LC-MS/MS system API 3000 triple quadrupole analyzer (MDS Sciex-Applied Biosystems, Foster City, CA, USA). A detailed description of these procedures is given by Rico-Rico et al. [27].

Data analysis

The experimental sorption data were fitted with a Freundlich isotherm:

$$\text{Log}C_s = \text{Log}K_F + n \cdot \text{Log}C_w \quad (1)$$

where C_s (mg/kg) and C_w (mg/L) are the concentration of the surfactant in the sediment and in the aqueous phase at the equilibrium, respectively. K_F (L/kg) is the Freundlich constant and n provides information about the linearity of the isotherm.

LC50 values were calculated with the sigmoidal dose-response curve with variable slope (four parameter logistic equation) using GraphPad Prism version 3.00 for Windows, (GraphPad Software, San Diego, CA, USA):

$$Y = \frac{\text{Bottom} + (\text{Top} - \text{Bottom})}{(1 + 10^{((\text{LogEC50} - X) \cdot \text{HillSlope}))}} \quad (2)$$

where Y is the response (percent of mortality), X is the logarithm of the concentration of the compound, Bottom is the response at the bottom plateau; Top is the response at the top plateau, and Log LC50 is the logarithm of the concentration when the response is halfway between Bottom and Top. The variable HillSlope is related to the slope of the curve. Background concentrations of 330 µg/kg and 1 µg/L (maximum measured LAS background concentrations) for the sediment and water-only tests, respectively, were used as the control concentrations for the data analysis.

RESULTS AND DISCUSSION

Coating, solvent-matrix and water spiking methods

Results for the three tested spiking methods are reported in Table 1. Actual concentrations in sediments are close to the nominal one for the water and solvent spiking methods. For the glass-coating method, actual concentrations were lower than estimated (<50%) and standard deviations were relatively larger. The homogeneity was studied for the “solvent” and “water” spiking procedures by taking sub samples from the same beaker. The standard deviations were slightly lower for the “water spiking method” (1.8 %) than for the “solvent-matrix spiking

method” (4.1 %). Previous studies have recommended the avoidance of solvents due to a potential effect on the bioavailability of the compound [22]. Results from concentrations measured in the overlying water suggest an easier desorption from the “solvent” spiked sediment than from the sediment spiked via water. The spiking method via water seems to be the most adequate procedure, also because it is closer to the real world situation, and it was therefore adopted for further experiments.

Table 1. Mean (±SD) nominal and actual sediment concentrations during the three spiking methods tested. Data are from three replicates.

Spiking method	C _s Nominal (mg/kg)	C _s Actual (mg/kg)	Overlying Water (µg/L)
Coating	40	18.6 (±12.2)	-
Solvent-Matrix	20	16.1 (±4.55)	57 (±18)
Water	20	24.6 (±2.19)	19 (±7)

Sorption and desorption of C₁₂-2-LAS in Oesterput and Cádiz sediment

Sorption and desorption isotherms of the compound C₁₂-2-LAS measured with Oesterput and Cádiz sediments are shown in Figure 1.

Sorption isotherms for both sediments were reasonably linear in the test concentration range ($n \approx 1$). Sorption was higher for the Oesterput sediment. However, sorption coefficients for the Cádiz sediment were considerably higher than expected considering that it is very sandy with low clay and organic fraction.

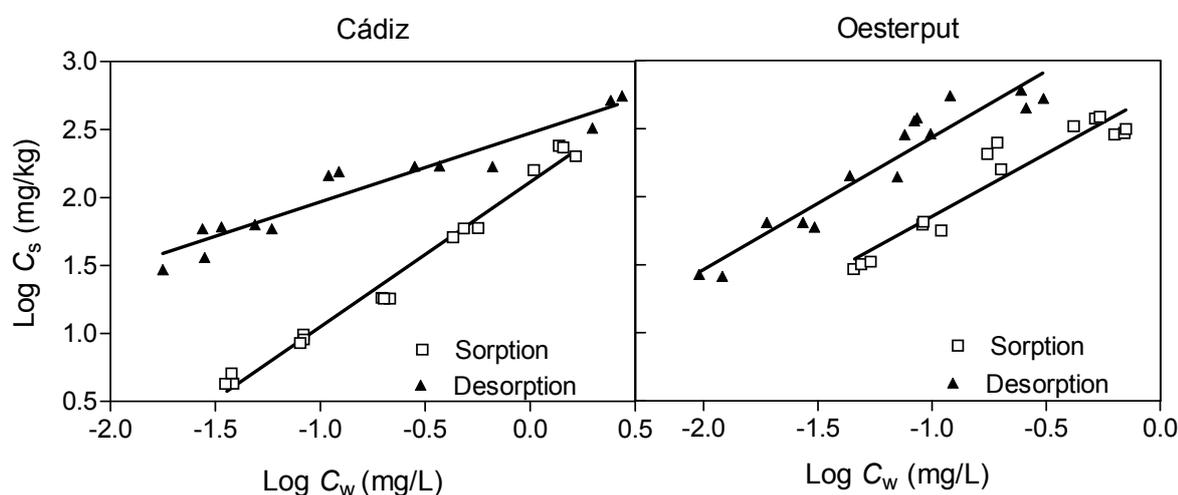


Figure 1. Isotherms for the sorption and desorption of C₁₂-2-LAS on the Oesterput and the Cádiz sediment. Freundlich equations are as follows (±SE): Oesterput sediment: $\text{Log } C_s = 2.77(\pm 0.07) + 0.92(\pm 0.08) \text{Log } C_w$ for sorption, and $\text{Log } C_s = 3.41(\pm 0.11) + 0.97(\pm 0.08) \text{Log } C_w$ for desorption; Cádiz sediment: $\text{Log } C_s = 2.11(\pm 0.03) + 1.06(\pm 0.03) \text{Log } C_w$ for sorption, and $\text{Log } C_s = 2.47(\pm 0.04) + 0.50(\pm 0.04) \text{Log } C_w$ for desorption.

The desorption isotherm for the Oesterput sediment was almost linear ($n=0.97$) and clearly non linear for the Cádiz sediment ($n=0.50$). For both sediments, the intercept value $\text{Log } K_F$ was significantly larger for the desorption than for the sorption isotherm. Differences between sorption and desorption of LAS in sediments and soils have been reported by other authors [11,14,28], and an irreversible adsorption of the compound to a fraction of the sediment matrix has been suggested as a potential cause of this phenomenon. Another possible explanation is related to slow desorption kinetics as it has been reported for some organic compounds [29]. However, this explanation is unlikely the cause of the difference in sorption in this study because an apparent equilibrium was observed after 20 h.

In the case of toxicity bioassays using contaminated sediment, desorption is the process that controls the concentration of the compound in the water phase and, therefore, the desorption coefficients were also used for the calculation of the pore water concentrations to assess the exposure of the test organisms in sediment toxicity tests.

Stability in water-only and sediment

Figure 2 illustrates the stability of C_{12} -2-LAS concentration in seawater and sediment under the bioassay conditions. It appears that the concentration of the surfactant in seawater remained almost constant for the first two days for the 0.5 mg/L system and three days for 1 mg/L, and decreased significantly thereafter. This lag phase is probably related to an adaptation of the micro-flora in the system after this period. Only 1% of the initial amount of C_{12} -2-LAS remained in the seawater system after eight days. A similar pattern was observed for the concentration in sediments, where concentrations were significantly lower after five days. At day 10, measured surfactant concentrations in the sediment were less than 40 % of the initial concentration. Results from the stability test in the aqueous phase are in agreement with literature data from biodegradation studies in seawater with C_{12} -LAS [3], where 99% primary degradation was observed after 100 h of exposure at two temperatures (10 and 25 °C). From the results obtained in the present study, it can be concluded that the removal of C_{12} -2-LAS from the water-only system is faster than from the sediments. Previous studies have reported similar biodegradation rates in water and sediment when aerobic conditions are ensured [30-32]. In the present work, the test systems were aerated, but the settled sediment was not disturbed. It is then possible that at a depth of few millimeters, the conditions in the sediment stratum were already anoxic, slowing down the degradation process.

The objective of this experiment was to assess the stability of the test compound in the bioassay with *Corophium volutator* in sediment or water-only exposures. The toxicological effects of LAS are expected to occur relatively fast and, therefore, the concentrations at the beginning of the toxicity tests will likely be the relevant ones. In order to have relatively constant exposure concentrations during the bioassay, organisms should not be exposed more than two-three days in static conditions in water-only systems, and no more than five days in the sediment test.

A shorter-term sediment bioassay than the standardized acute test (ten days duration) [24] was therefore designed to ensure a more stable exposure concentration.

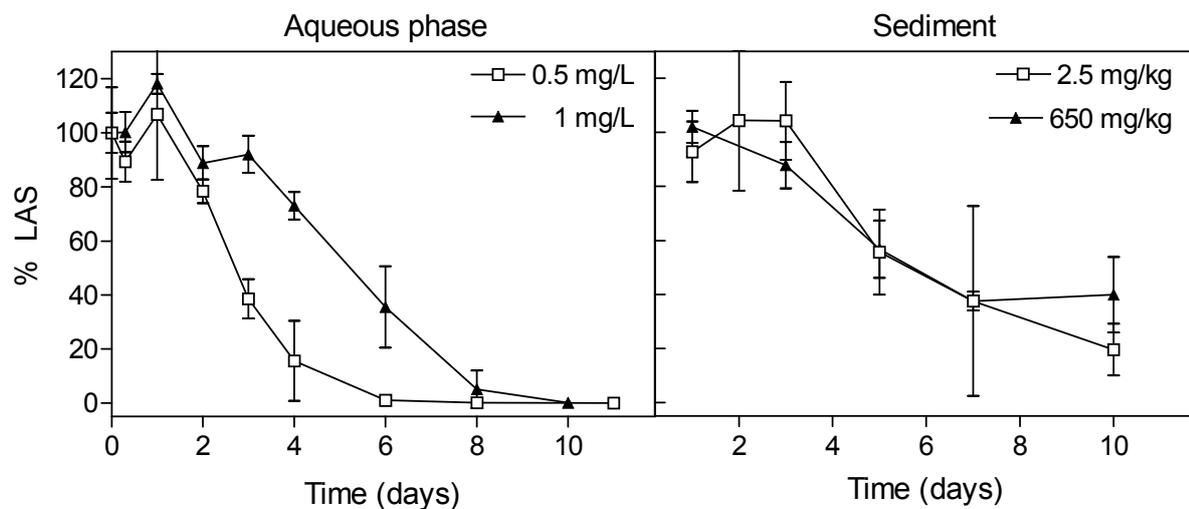


Figure 2. Decrease in concentration of C₁₂-2-LAS (mean \pm SD) with time in Oesterput sediment and natural seawater. Values are given in percentages of nominal concentrations.

Acute toxicity in water only

The dose-responses curves for the effect of LAS on the mortality of *Corophium volutator* during two, three and five days exposure based on the mean actual as well as nominal concentrations are reported in Figure 3. The 2-day dose-response curve corresponds to the mortality on the second day before the renewal of the media of the test performed over five days, the reported actual concentrations are the average values of the concentrations measured at the start of the test and just before the renewal of the media. For the dose-response curve from the 3-day exposure data, actual concentrations are the average values of the concentrations measured at the start and at the end of the test. Finally, for the five days exposure test, actual concentrations are calculated as the average of the values of the measured concentrations at the beginning, before renewal and end of the test. The measured concentrations at every sampling occasion during the tests are given in the Appendix (Table S-1). LC50 values based on the nominal and the mean actual concentrations are shown in Table 2. LC50 values from the three and five days exposure tests did not show a clear effect of the exposure duration. The slight variation (factor 2.5) in the LC50 values seems to be more related to slight differences in the organism's sensitivity as commonly reported for various organisms exposed to various toxicants [33]. Exposure durations of three to four days are commonly used for *Corophium volutator* water-only exposure bioassays with reference toxicants [24,25]. The LC50 values for the water-only exposure here reported for the specific isomer C₁₂-2-LAS are in agreement with literature data on the aquatic toxicity of homologues commercial mixtures of LAS to marine crustaceans and other invertebrates [34,35]. However, because toxicity tests with surfactants are often carried out with a complex mixture, a direct comparison with literature data is not straightforward.

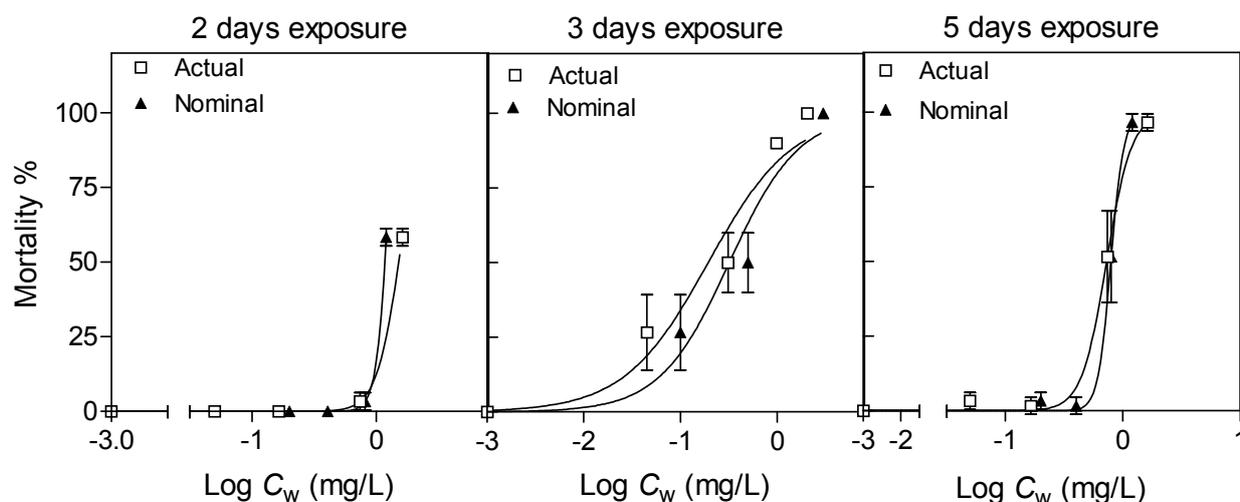


Figure 3. Dose responses curves at different exposure times for water-only toxicity tests based on nominal and mean value of the actual concentrations. Data points correspond to the mean \pm SD.

Table 1. Decrease in concentration of C₁₂-2-LAS (mean \pm SD) with time in Oesterput sediment and natural seawater. Values are given in percentages of nominal concentrations.

Time (days)	LC50 Nominal (mg/L)	LC50 Actual (mg/L)
2	1.08 (0.74-1.62) ^a	1.00 (0.8-1.2) ^a
3	0.31 (0.22-0.46)	0.19 (0.12-0.29)
5	0.79 (0.76-0.82)	0.73 (0.67-0.79)

^a concentrations for which the minimum and the maximum effect have been observed when 100% mortality did not occur.

Acute toxicity in sediments

The effect of the exposure duration on the mortality of *Corophium volutator* has been investigated for one of the sediments in a pilot test (Figure 4). The data indicate that 60 – 80 % of the final mortality was observed as of 3 days of exposure. Based on the outcome of the stability study of C₁₂-2-LAS in sediments, showing that the surfactant concentration in the sediment significantly decreased after five days and based on this pilot test, a five days exposure test was selected for the sediment toxicity test with *Corophium volutator*.

The short-term five days toxicity tests were performed with Oesterput and Cádiz sediments. The outcome of these tests is presented in Figure 5 and Table 3. Although *corophium volutator* is not an original organism of the Cádiz area, the organisms performed very well in the control experiment (non spiked sediment) in comparison to the exposure in the original sediment. Sediment and overlying water concentrations are given in Table S-2 of the Appendix. For comparison purposes, an LC50 value based on measured concentration and averaged over 3 and 5 days was calculated and was 0.47 mg/L. Pore water LC50 values

were derived from the sediment LC50 values using the Freundlich equations for both sorption and desorption. In general, calculated LC50 values were of the same order than LC50 values measured in water-only bioassays. For the Oesterput sediment, both estimations of the pore water effect concentrations are in accordance with the LC50 results from the three and five days water-only tests. Pore water effect concentration (0.11 ± 0.02 mg/L) calculated from the desorption coefficient seems to be slightly lower than the LC50 values from the water-only tests. However, confidence intervals still overlap. In the case of the Cádiz sediment, for which sorption and desorption isotherms were considerably different, LC50 pore water values calculated from the sorption coefficient were slightly higher and outside the confidence intervals obtained for water-only exposure, while values obtained from the desorption expression corresponded better with the LC50 values from the water-only tests. The results obtained here with the sediment from Cádiz suggest that the desorption coefficient slightly better estimate the pore water effect concentrations. This observation did not apply to the Oesterput sediment. The importance of the desorption process on the bioavailability of xenobiotics to benthic organisms has already been reported in previous studies with hydrophobic organic contaminants [36,37], where it is suggested that the use of desorption values is an appropriate tool in bioavailability studies. The correspondence of the pore water effect concentration with the effect concentration from the water only test suggests that organisms are exposed mainly via the pore water. However, a recent study of Droge et al. [38] with alcohol ethoxylates has shown that the overlying water is also an important exposure route for *Corophium volutator* because of the circulation of the water in its burrow. In our study, overlying water measurements of C₁₂-2-LAS showed that, although a considerable decrease was observed, surfactant concentration in the overlying water compartment was still significant at the end of the experiment.

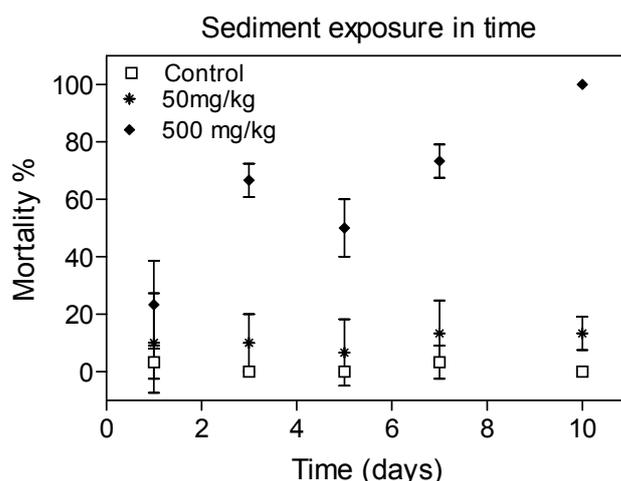


Figure 4. Percent of mortality of *Corophium volutator* versus time in the sediment at two C₁₂-2-LAS concentrations and the control sediment (non-spiked). Data points correspond to the mean \pm SD.

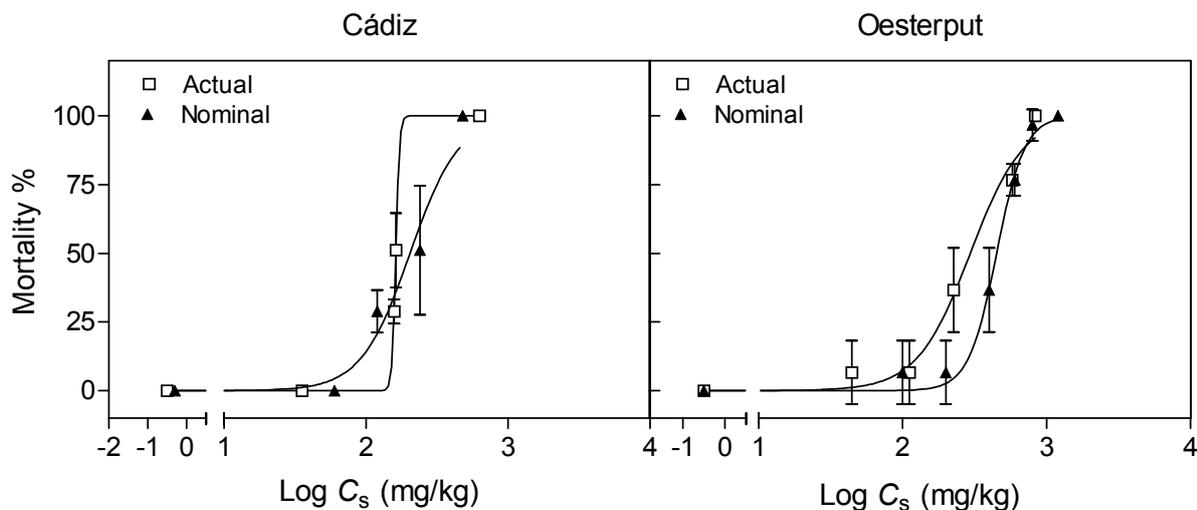


Figure 5. Dose responses curves for sediment toxicity tests based on nominal and average values of the actual concentrations at the start and end of the test. Data points correspond to the mean \pm SD.

The results obtained here agree with the conclusion of a study on fresh water sediment organisms using a highly branched alkylbenzene sulfonate (ABS) [19] where toxicity values based on the pore water concentrations were similar to the toxicity values in water-only exposure. In that study, the applicability of the partitioning theory was tested using a sandy sediment for which the organic carbon content was changed by adding peat moss. The increase in the organic carbon content influenced the pore water surfactant concentration and decreased the toxicity. Later studies, focused on the sorption of LAS to sediments at lower concentrations [9], have demonstrated that organic carbon content is not the only factor that accounts for sorption to sediments, and other parameters may also influence the bioavailability of these compounds in sediment and soil matrices. Recently, a study on the chronic and acute toxicity of surfactants in fresh water sediments [39] has also demonstrated that organic fraction and granulometry affect the toxicity of LAS in sediments. In that case, the Critical Body Residual (CBR) approach was proposed as a dose metric for chemical exposure. However, in the case of the CBR approach, other mechanisms like the biotransformation (involving kinetics of the process) of these compounds in the organisms should be considered in more detail. Environmental concentrations of LAS (mixture) in marine sediments are in general far below the concentrations we have used here for the acute toxicity studies. Recent monitoring studies in the South of Spain [40] have reported pore water concentrations for different LAS homologues with a maximum of 15 $\mu\text{g/L}$ for C_{12} -LAS in areas close to wastewater discharges, considerably below the lethal concentrations for water-only exposure we have obtained. According to Rico-Rico et al. [27], organic carbon content (OC) is the main parameter to affect sorption in marine sediments at water concentrations $> 10 \mu\text{g/L}$. In this study, the LC50 values were 100 – 700 $\mu\text{g/L}$. It is therefore expected that the organic fraction was the most important factor to control the bioavailability of LAS to *Corophium volutator* in the tests reported here. However, the mortality of this test organism appeared too variable to account for the difference in LAS bioavailability expected

from the characterization of the two tested sediments (Oesterput, 1.38% OC; Cádiz, 0.06% OC). In the field, the range of LAS concentrations and of OC content can be much larger.

Table 3. LC50 (C.I.) values based on nominal and average of the actual concentrations for sediment toxicity tests at the start and end of the test, estimated LC50 pore water concentrations from the nominal, and actual sediment concentrations and the sorption and the desorption coefficients calculated from the Freundlich equations.

Sediment test	LC50 Sediment nominal (mg/kg)	LC50 Sediment actual (mg/kg)	LC50 Pore water nominal by K_{sorption} (mg/L)	LC50 Pore water actual by K_{sorption} (mg/L)	LC50 Pore water nominal by $K_{\text{desorption}}$ (mg/L)	LC50 Pore water actual by $K_{\text{desorption}}$ (mg/L)
Oesterput	450 (416-487)	295 (248-351)	0.75 (0.69-0.81)	0.47 (0.39-0.57)	0.17 (0.15-0.18)	0.11 (0.09-0.13)
Cádiz	204 (167-250)	162 (160-165)	1.54 (1.28-1.87)	1.24 (1.23-1.26)	0.48 (0.32-0.72)	0.30 (0.29-0.31)

CONCLUSIONS

The aim of the present paper was to assess if the toxicity of the surfactant C₁₂-2-LAS in marine sediments is related to the pore water concentrations using the marine benthic amphipod *Corophium volutator* as a test organism. The spiking of the sediment has been performed via water because of the good recoveries and homogeneity obtained with this method. Exposure test duration has been adapted to ensure a stable concentration during the water and sediment bioassays. Results have shown that estimated pore water effect concentrations in the two tested sediments agree with the LC50 values obtained from the water-only exposure tests. These results therefore suggest that predictive assessment of LAS toxicity in sediments could be made from water-only exposure toxicity values when the sorption/desorption coefficients have been characterized. Further studies to understand the key parameters that affect the sorption processes will help to better predict the bioavailability of LAS in marine sediments.

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APPENDIX

Table S-1. Nominal and mean (SD) actual concentrations of C₁₂-2-LAS at the start and end of the test in the water-only exposure toxicity tests for the different exposure times. Data are from three replicates.

3 days water-only exposure test				
Nominal	C _w day 1 (mg/L)		C _w day 3 (mg/L)	
0	-		-	
0.1	0.07 (0.03)		0.02 (0.04)	
0.5	0.46 (0.14)		0.16 (0.10)	
1	1.15 (0.17)		0.82 (0.21)	
3	2.13 (0.31)		1.97 (0.48)	
5 days water-only exposure test				
Nominal	C _w day 1 (mg/L)	C _w before renewal day 2 (mg/L)	C _w after renewal day 2 (mg/L)	C _w day 5 (mg/L)
0	-	-	-	-
0.2	0.08 (0.04)	0.05 (0.02)	0.09 (0.02)	0.03 (0.01)
0.4	0.17 (0.017)	0.16 (0.03)	0.30 (0.02)	0.29 (0.17)
0.8	0.66 (0.13)	1.11 (0.44)	0.47 (0.43)	0.43 (0.23)
1.2	1.28 (0.57)	1.16 (0.20)	1.05 (0.34)	1.52 (0.72)

Table S-2. Nominal and mean (SD) actual concentrations of C₁₂-2-LAS at the beginning and end of the test in sediment (C_s) and overlying water concentrations (C_{ow}) in the sediment exposure bioassays. Data are from 3 replicates.

Sediment Test	C _s nominal (mg/kg)	C _s actual day 1 (mg/kg)	C _s actual day 5 (mg/kg)
Oesterput Sediment test	0	-	-
	100	54.1 (11.6)	34.8 (14.9)
	200	169.5 (65.8)	53.2 (16.9)
	400	284.7 (50)	167.1 (34.9)
	600	704.8 (36.2)	450 (337.2)
	800	842 (167.7)	822 (245.4)
Cádiz Sediment test	0	-	-
	60	41.7 (4.8)	29.1 (5.2)
	120	171.4 (11.6)	144.9 (33.1)
	240	202 (2.2)	123.3 (2.6)
	480	561.9 (71.4)	701 (29.2)
Sediment Test	C _s nominal (mg/kg)	C _{ow} actual day 1 (mg/L)	C _{ow} actual day 5 (mg/L)
Overlying water Oesterput sediment test	0	-	-
	100	0.09 (0.03)	0.008 (0.007)
	200	0.28 (0.12)	0.02 (0.01)
	400	0.33 (0.11)	0.022 (0.02)
	600	0.61 (0.22)	0.108 (0.09)
	800	1.21 (0.46)	0.332 (0.26)
Overlying water Cádiz sediment test	0	-	-
	60	0.20 (0.02)	0.02 (0.010)
	120	0.48 (0.06)	0.05 (0.02)
	240	1.16 (0.18)	0.12 (0.01)
	480	2.03 (0.92)	0.78 (0.49)

Chapter 7

Summary and general discussion

SUMMARY

The sorption of contaminants to sediments and dissolved organic matter affects the transport, distribution and bioavailability of these compounds in the aquatic environment.

This thesis is focused on the study of the sorption of the anionic surfactant linear alkylbenzene sulfonate (LAS) in relation to the compound, the sorbent and the aqueous media characteristics, and the implications of this process in sediment toxicity.

Chapter 2 has covered the testing and validation of the use of the polyacrylate (PA) solid phase microextraction (SPME) fibers as a passive sampling tool to analyze the freely dissolved concentration of LAS compounds in seawater matrices. The use of the SPME method at different aqueous media has been additionally tested in chapters 4 and 5. Chapters 3 to 5 explored the effect of the system variables (sorbent and aqueous characteristics) and the compound molecular structure on the sorption of LAS to sediments and humic acids. Finally, Chapter 6 has shown that, in conformity with the Equilibrium Partitioning approach –EqP–, the toxicity of LAS to a marine benthic organism is mainly due to the aqueous phase exposure. A summary of the main results of this thesis is presented here.

nd-SPME analysis of LAS compounds in different aqueous matrices

The solid phase microextraction method (SPME) allows to extract the freely dissolved concentration of a compound in solution, avoiding phase separation and clean-up steps before chromatographic analysis. Chapter 2 has shown that 7 μm polyacrylate coated fibers can be used for the analysis of LAS compounds in seawater. However, the original disposable fibers have to be thermally conditioned before its use for LAS compounds. With the optimized method, concentration and co-solute independent K_{fw} values were obtained for a large number of LAS constituents, and these values varied for the different LAS homologues and isomers. The method was applied to study the solubility of LAS compounds in seawater. Results indicated that the solubility could be well predicted by the formation of precipitates with Ca^{2+} in the aqueous solution. In Chapters 4 and 5, the PA SPME fibers were used to analyze the LAS aqueous concentration in sediment and humic acid suspensions at different aqueous chemistry. The aqueous composition strongly affected the sorption of LAS compounds to the fiber, and the relationship between K_{fw} and the specific electrolyte concentration in the media (Na^+ and Ca^{2+}), indicated that ion pairing was likely the main mechanism governing the sorption of LAS to the fiber. Although detection limits of the method are limited by the background concentration from many sources, its simplicity and sensitivity makes it a promising tool for laboratory-based studies, where extraction of the dissolved fraction is complex.

Besides the analytical application of the SPME method, Chapter 4 and 5 explored the utility of the K_{fw} values as a predictor of the hydrophobicity of LAS compounds. A good correlation was found between the K_{fw} values and the sorption coefficients obtained from the experiments with sediment (Chapter 4) and humic acids (Chapter 5). The correlation between sediment sorption coefficients and fiber-water partition coefficient at different

aqueous composition of the medium was less significant, probably because different sorption mechanisms are taking place in both the sediment and the fiber. Nonetheless, good estimates of the sorption of the different LAS constituents could be derived from the testing of one congener at the specific conditions, and the K_{fw} values.

Sorption of LAS: Effect of the sorbent, aqueous composition, and compound characteristics

Sorption of LAS compounds to natural sediments was clearly non linear at seawater and freshwater conditions. The role of the sediment properties on the sorption of LAS to marine and estuarine sediments is shown in Chapter 3. The correlation of the different sediment characteristics with the sorption coefficients showed that the sorption of the model compound used (C₁₂-2-LAS) depends on both clay (grain fraction < 2 μm diameter) and organic carbon content, and the importance of each parameter differs with the surfactant concentration. At the low microgram per liter range, the clay fraction of the sediment was the most important parameter that correlated with sorption, while the organic carbon content became more significant at higher concentrations.

In relation to the aqueous composition, sorption was significantly different in seawater and freshwater conditions, and sorption coefficients gradually increased with the amount of salts in solution (Chapter 4). In addition, sorption was very well correlated with the Ca²⁺ content of the complex solutions. The sorption coefficients varied similarly to other studies with anionic surfactants using simple CaCl₂ solutions.

Commercial LAS consist of more than 20 different constituents that behave differently according to their alkyl chain length and the position of the benzene-sulfonate group on the alkyl chain. In Chapter 4, sediment sorption data for individual LAS constituents showed that the differences in sorption are mainly related to the hydrophobicity of the LAS molecules. The most sorptive LAS compounds are those with longer alkyl chains and most external phenyl positions. As discussed above, this sorption behavior is well predicted by the K_{fw} values.

Chapter 5 explored the effect of the presence of dissolved humic acid on the freely dissolved concentration of LAS. The sorption of LAS to the two different humic acids tested (Aldrich and Leonardite) was concentration independent. High sorption coefficients (Log K_{DOC} = 4.1-5.6) were obtained for all compounds, and sorption affinity varied according to their molecular structural differences as for sediments and SPME fibers. However, opposite to the trend observed for sediment and fiber, the sorption of LAS to humic acids was not dependent of the salt content in solution. It was hypothesized that the effect of the solution composition on the K_{DOC} is masked by changes in the conformation of the humic acid molecule, and more experiments that include mixtures of LAS compounds should be considered to better understand the process.

Toxicity of LAS in marine sediments

One of the main objectives of this thesis was to test the validity of the equilibrium partitioning theory (EqP) for LAS in sediments. According to this theory, the effect concentration in water-only exposure will be similar to the effect concentration in the sediment pore water. The

toxicity of a marine mud shrimp, *Corophium volutator*, in two well-characterized sediments and in water-only exposure was studied in Chapter 6. Moreover, special attention was given to the way of introducing the chemical in the sediment system (spiking procedures), as well as to the stability of the compound during the toxicity test duration. Effect concentrations (LC50 values) showed a good agreement between the water-only exposure results and the pore water concentration derived from the sorption/desorption coefficients.

GENERAL DISCUSSION

Scope

The present thesis has focused on three principal areas:

- (1) Development of the sampling tool for the analysis of freely dissolved concentrations of LAS compounds.
- (2) Understanding of the main parameters affecting the sorption of LAS in the aquatic environment.
- (3) Testing the validity of the EqP approach for the sediment toxicity of LAS.

This chapter discusses the main findings in a more practical perspective. The chapter begins examining the potential applications and obstacles of the developed SPME method in laboratory and field conditions. Furthermore, the use of the fiber-water sorption coefficients as a tool to predict hydrophobicity-related process is discussed. The second part discusses the main environmental parameters that influence the sorption of LAS compounds in the aquatic environment, and reported LAS concentrations in the field are discussed in relation to the results of the sorption studies from this thesis. Finally, the main conclusions are summarized, and future perspectives from the point of view of the author are given.

SPME analysis of LAS compounds

Potential applications and drawbacks of the method

Polyacrylate (PA) SPME fibers (7 μ m diameter coating) can be used for the extraction of LAS compounds in aqueous systems. A simple thermal pre-treatment is nonetheless needed prior the PA SPME fiber use.

One of the advantages of the use of SPME fibers for LAS extraction is that it considerably reduces the number of experimental steps before the chromatographic analysis. The fiber is directly transferred from the sample to the HPLC vials, and therefore, the margin of error due to contamination from different sources is minimal. Furthermore, the SPME procedure leads to very clean extracts for chromatography analyses.

Although limits of detection were in the low microgram per liter range for all compounds, the applicability of SPME in field samples from low contaminated areas is limited, due to the small proportion of each LAS isomer in LAS commercial mixtures. Total LAS concentrations in the sediment pore water are in the low microgram per liter at locations a few kilometers away from the treated wastewater effluent, and maximum LAS concentrations of 48 μ g/L are observed in areas very close to the discharge point (Table 1, Chapter 1).

Nonetheless, detection limits are not only dependent of the SPME fiber extraction capacity. The analytical solvents already contain LAS background in the picogram per liter range, and this becomes problematic when solvent samples (for example solid phase extraction –SPE- extracts) have to be concentrated before analysis. Besides, LAS is also present in many of the new and old laboratory glassware.

The developed SPME method is nevertheless suitable for many laboratory-based studies. In this thesis, SPME analysis was used to study the solubility of LAS compounds in seawater (Chapter 2) and in sorption studies with sediment and humic acids suspensions (Chapters 4 and 5). Besides, PA SPME fibers have been currently used for the analysis of pore water concentrations in a marine sediment bioassay with C₁₂-LAS isomer mixture (A. Mauffret, manuscript in preparation). Other possible applications are measurements of the critical micelle concentration (cmc) in different aqueous matrices, as it has been tested for non ionic surfactants [1], or its use for the analysis of the freely dissolved concentration in *in vitro* tests.

LAS uptake by the SPME fiber showed to be dependent of the salt concentration in solution. Fiber-water sorption coefficient values (K_{fw}) for the different LAS congeners tested in the seawater solutions (with ionic strengths > 160 mM) did not vary; however, these values were different to those obtained in standard fresh water solution and its further dilutions. In Chapter 3, it is suggested that the partitioning of LAS into the SPME fiber can be explained by the ion pair formation of the LAS molecules with cations present in solution. The relation between K_{fw} values and the Ca²⁺ concentration had a sigmoidal shape, and this shape was similar to other processes that are also ion-pair dependent [2]. Besides, different K_{fw} were also obtained in a solution where only the concentration of NaCl was varied, but no clear sigmoidal relationship was observed, probably due the electrolyte concentration range used. This outcome implies that the use of the SPME method in freshwater, or in solutions with relatively low content of salt, requires the determination of the K_{fw} values at that specific ionic composition for at least one LAS isomer. The K_{fw} values for the rest of the LAS compounds can be easily calculated, because the differences among the sorption coefficients of the LAS congeners do not vary with the solution chemistry. Besides, if the partitioning of LAS to the SPME fiber is indeed related to an ion pairing process, the fiber could be potentially used for the study of the association of these compounds with cations present in solution. However, the absence of data on the association constant of LAS compounds with cations makes it difficult to interpret if SPME K_{fw} values are really predicting ion pairing.

The use of fiber-water sorption coefficients to predict LAS hydrophobicity

Hydrophobicity is one of the main parameters governing the distribution of chemicals between the environmental compartments and organisms. The octanol-water partitioning coefficient (K_{ow}) is generally used as the standard hydrophobicity parameter. A good correlation between the K_{fw} and the K_{ow} values has been shown for many non-ionic organics [3-5]. For surfactants, K_{ow} is not sufficiently describing their behavior, due to the amphiphilic character of the surfactant molecules [6]. In addition, for ionisable surfactants such as LAS, the behavior and hydrophobicity will be strongly affected by the aqueous composition. Chapters 4 and 5 have shown the effectiveness of the K_{fw} values to predict the sorption of LAS compounds to environmental sorbents. In Chapter 4, a good correlation was obtained when comparing the K_{fw} values and sediment sorption coefficients constant (K_F) for nine LAS congeners in seawater (Figure 1). Similarly, in Chapter 5, an important correlation was also

obtained between the K_{fw} and the sorption coefficients to dissolved humic acid (as K_{F-DOC}) in freshwater samples.

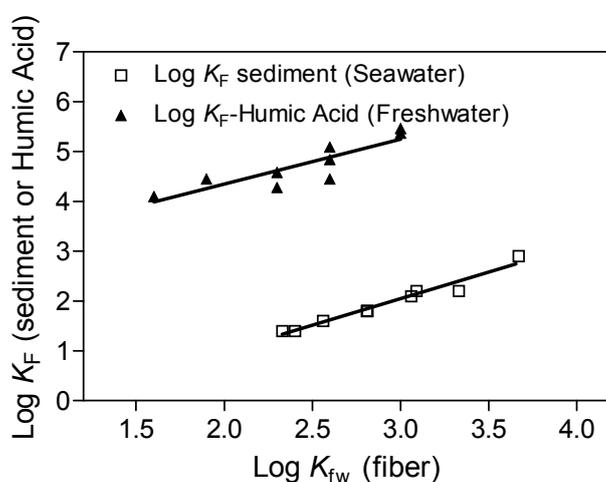


Figure 1. Sediment and humic acid sorption coefficients (Freundlich coefficients K_F) as a function of the fiber-water sorption coefficient (K_{fw}) for the tested LAS compounds in seawater (for sediment sorption data) and freshwater (for the humic acid sorption data). Data belongs to Chapters 2, 4 and 5.

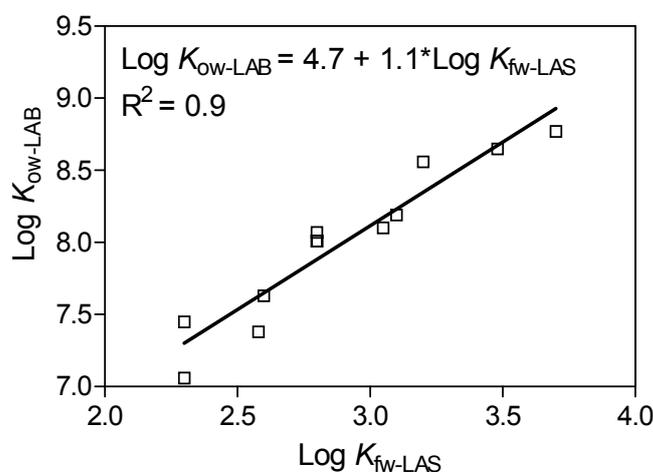


Figure 2. Octanol-water sorption coefficients for linear alkylbenzene sulfonates (K_{ow-LAB}) as a function of the fiber-water sorption coefficient for the tested LAS compounds in seawater (K_{fw} values belong to Chapter 2).

The length of the alkyl chain and the position of the phenyl group play a significant role in the sorption affinity of the LAS isomers to the SPME fiber, sediment and humic acids. Longer alkyl chain compounds and isomers with the most external phenyl groups are the most sorptive compounds. Comparable trends have been already reported for the measured and estimated K_{ow} values of the unsulfonated linear alkylbenzenes (LAB) [7]. As it is illustrated in Figure 2, a good correlation exists between K_{fw} for LAS compounds and K_{ow} for LAB, indicating once again that differences of sorption affinity between the LAS constituents

are mainly due to their differences in hydrophobicity. Hence, if K_{fw} values are available for all the LAS compounds, K_{fw} can be considered a good experimental parameter for the prediction of the sorption and other hydrophobicity driven environmental processes. These excellent agreements reveal that the fiber-water distribution coefficients obtained with the present SPME method are useful for the prediction of sorption mechanisms of LAS compounds in the environment.

A direct application of the SPME method could be the testing of the sorption capacity of a site-specific sediment using a reference chemical, and extrapolation of the sorption results to the rest of congeners by the K_{fw} values.

Fate and distribution of LAS in the aquatic environment

Predicting and modeling the sorption of LAS in the aquatic environment

The understanding of the sorption of contaminants to sediments is important to characterize the risk of these compounds in the environment. Sediments act as a sink of LAS in the aquatic environment [8], and this process is directly influencing their transport and bioavailability.

Non polar interactions but also electrostatic and specific interactions can explain the sorption of LAS [9-11]. The increase of sorption when increasing the chain length shows the importance of the non polar forces. On the other hand, some studies have emphasized the contribution of more specific chemical interactions of the sulfonate group [9,12-14]. This has been mainly supported by the effect of solution parameters such as calcium concentration and pH changes on the sorption of LAS to sediments [9] and by the correlations between metal oxides content and LAS sorption [9,14].

Results obtained from this thesis support the current literature data and give new inputs on the effect of individual constituents and specific environmental parameters. Besides, the present thesis has stressed on marine conditions, for which much lesser studies could be found.

Sorption of LAS structures that vary from 9 to 13 carbon atoms on the alkyl chain and with different positions of the benzene sulfonate group in the alkyl chain were tested with sediment and humic acids (Chapters 4 and 5). The data from Chapter 4 have shown that sorption affinity increases for those compounds with longer alkyl chain and with the phenyl groups in the most external positions. However, modeling of this data should be done with care, because the sorption coefficients did not show a constant variation with the compound structure. Besides, this thesis has shown that the freely dissolved concentration of LAS compounds in the freshwater and marine sediment compartment is affected by the grain size and organic carbon content of the sediment (Chapter 3), the salt content (mainly Ca^{2+}) (Chapter 4), and the dissolved organic carbon concentration (Chapter 5).

The sorption of LAS to natural sediments and specific single materials was discussed in Chapter 3. In this chapter, the sorption isotherms for two different clays (kaolinite and montmorillonite) were very similar, indicating that LAS is only sorbing to the clay surface, and no LAS molecules are present in the interlayer space of the swelling clay montmorillonite.

These results indicate that prediction of LAS sorption to natural sediments, in which different types of clays are expected to be present, could be made with the total clay fraction. The sorption data for the different sediments and materials fitted well to the Freundlich equation. The non linearity of the isotherms obtained in all cases is already an indication of the contribution of more than one mechanism on the overall sorption process. Although specific contribution of the sediment parameters (and therefore the relative importance of each sorption mechanism) on LAS sorption to sediments could not be completely understood, an important correlation was observed between the sorption coefficients at the different surfactant concentrations and the clay and organic fraction of the sediments. The good correlation of the sorption coefficients with the clay content at low surfactant concentrations (<10 µg/L) suggests the importance of the adsorption process at this concentration range, while at higher concentrations, the organic content, and therefore absorption, is more relevant. In a previous work with non-ionic surfactants [15], adsorption is also the process governing the sorption at low surfactant concentrations, while absorption was most typically happening at high surfactant concentrations. In that case, this sorption behavior could be modeled by a combination of both Langmuir and Freundlich equations. However, this model did not fit well with the results obtained in the present thesis.

The presence of dissolved inorganic salts in water affects the solubility and sorption of LAS compounds to sediments, and both processes can be well described by the concentration of Ca^{2+} in solution (Chapters 2 and 4). Thus, for two sediments with identical characteristics, the aqueous composition can strongly affect the affinity of the LAS compounds to the sorbent, and this effect can be easily quantified ($\Delta \log K_d / \Delta \log [\text{Ca}^{2+}] \approx 0.37$).

Although quantification of the effect of some environmental variables such as the salinity was possible, a simple model to predict the effect of the compound and environmental parameters is still difficult. Alternatively, the present work has shown that the SPME method can be considered as a good tool for measuring and prediction purposes.

Comparison of the obtained results with field monitoring data

According to the results obtained in Chapter 3, organic carbon (OC) and clay content are the sediment parameters that describe the sorption of LAS to marine and estuarine sediments. OC is the main parameter to affect LAS sorption (as C_{12-2} -LAS) in marine sediments at aqueous concentrations > 10 µg/L, while the clay fraction is more important for lower concentrations.

In Chapter 6, the LC50 values for *Corophium volutator* were 100 – 700 µg C_{12-2} -LAS/L. It is therefore expected that the OC fraction was the most important factor to control the bioavailability of LAS to *Corophium volutator*. Reported monitoring studies from the last decade have revealed LAS total concentrations of up to 238 mg/kg in marine sediments exposed to effluents with primary or no waste water treatment (Table 1 in Chapter 1). Considering the sorption coefficients for a sediment with similar characteristics and origin as applied in Chapter 3 (San Fernando sediment), LAS pore water concentration (if $K_d \text{ C}_{12-2}\text{-LAS} \approx$

K_d LAS mixture) in such a sediment would be approx. 200 $\mu\text{g/L}$, and therefore, acute toxicity is expected to occur due to LAS.

Nonetheless, sediment and pore water LAS concentrations are very low in areas where wastewater discharges receive a proper treatment [16]. Besides, it is difficult to discern if the reported pore water concentrations are only corresponding to the freely dissolved fraction of LAS, or they are the sum of bound and free LAS, since the particulate phase present in the pore water (e.g. dissolved organic carbon) is usually difficult to separate by conventional techniques.

According to the European Technical guidance for the risk assessment of chemicals [17], the risk characterization of sediments is performed by the sediment-water partitioning coefficient, the Predicted Non Effect Concentration for sediment organisms (PNEC_{sed}), or the PNEC for water column in the absence of PNEC_{sed} . This guidance does not consider the sorption of the chemical to the dissolved organic fraction (DOC) in the pore water. DOC concentrations between 0 and 50 mg/L are typically present in sediment pore water in the environment [18]. Considering the high K_{DOC} values obtained for the different LAS compounds in Chapter 5 (Log K_{DOC} between 4.1 and 5.5), the dissolved organic fraction can significantly affect the bioavailability of LAS compounds. As illustrated in Figure 2 for $\text{C}_{12}\text{-2-LAS}$, a DOC concentration of 8 mg/L will cause a reduction of the 50 % of the initial freely dissolved concentration of this compound in the aquatic compartment. Measurements of the total concentration may lead to overestimation of the freely dissolved (and bioavailable) LAS concentration, or underestimation of sediment-water sorption coefficient, and this will have direct implications for the risk characterization of the sediments.

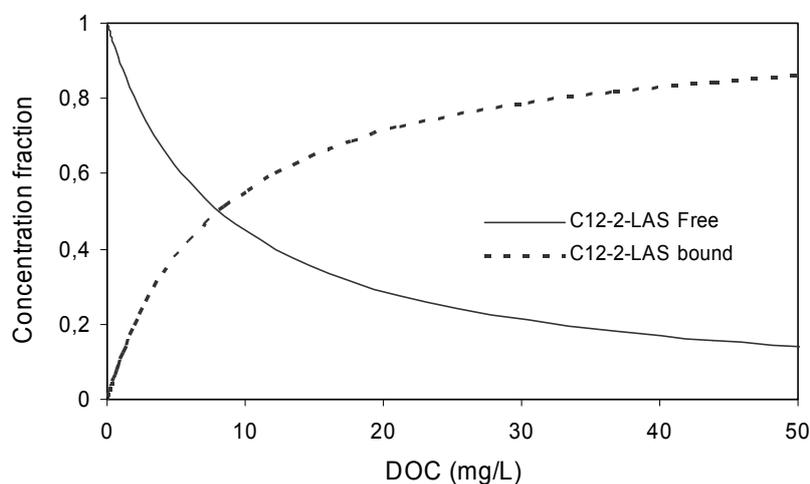


Figure 2. Variation of the freely dissolved and bound fraction of $\text{C}_{12}\text{-2-LAS}$ versus the DOC concentration based on the results obtained for the Leonardite humic acid in Chapter 5.

Future perspectives

The use of the SPME method to measure the freely dissolved concentration of LAS compounds greatly improved the quality of the data in the subsequent sorption studies. Indeed, sorption data from Chapter 4 and 5 are less scattered than in Chapter 3, where solid phase extraction (SPE) was used as the extraction technique. The use of an optimal tool for the analysis of compounds in the test systems will therefore lead to a better understanding of the compound behavior and modeling of the data will be easier.

More sorption studies are needed to understand the fate and behavior of ionisable organic compounds such as LAS in the environment. Because the great variety of sediment types, sediments corresponding to the areas more exposed to contaminants should be considered of main concern. These areas primary correspond to sediments directly exposed to effluents. For the particular case of LAS, the improvement of its analytical detection and separation of the LAS constituents will considerable improve the risk characterization of these compounds.

The successful application of the PA SPME fibers for measurement of LAS concentrations may lead to the possibility of applying the method for other charged organic molecules such as for the increasingly used perfluorinated surfactants compounds, cationics surfactants, etc., from which comparable analytical challenges are expected.

Concluding remarks

- Thermally conditioned polyacrylate SPME fibers are suitable to analyze the freely dissolved concentrations of LAS compounds in complex matrices.
- The use of the SPME method can be potentially extended to a wide variety of laboratory-based studies of LAS compounds, such as precipitation and sorption studies.
- The SPME PA fibers are the first hydrophobic phase for which distribution coefficients have been consistently determined for individual LAS congeners, for a wide concentration range and aqueous media.
- K_{fw} values for LAS can be considered a potential hydrophobicity parameter.
- Fiber-water sorption coefficient (K_{fw}) is a useful parameter to estimate the sorption of LAS compounds to sediments and humic acids.
- K_{fw} values can be applied as a first screening parameter to predict LAS sorption at different aqueous composition. For more accurate estimates, one reference chemical should be tested for each specific solution.
- Sorption of LAS to sediments is described by both the organic carbon and clay fraction, and this dependence varies with the surfactant concentration in the system.
- Sorption of LAS to sediment at different aqueous composition or salinity is well described by the Calcium concentration in solution.
- Water exposure accounts for the toxicity of LAS to the marine benthic organism *Corophium volutator* in spiked sediment tests.

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SAMENVATTING

De sorptie van verontreinigende chemicaliën aan sediment en opgelost organisch materiaal beïnvloedt transport, distributie en biologische beschikbaarheid van deze stoffen in het aquatische milieu.

Dit proefschrift richt zich op de sorptie van het anionisch surfactant lineair alkylbenzeen sulfonaat (LAS) met betrekking tot de eigenschappen van de stof, het sorbent en de waterfase, en de gevolgen van dit proces voor de toxiciteit van het sediment.

Hoofdstuk 2 behandelt het testen en valideren van polyacrylaat (PA) solid phase microextraction (SPME) als een passieve bemonsteringsmethode voor de analyse van vrij opgeloste concentraties van LAS in mariene matrices. Het gebruik van SPME in verschillende aquatische media is verder getest in hoofdstuk 4 en 5. De hoofdstukken 3 tot 5 verkennen de effecten van systeemp parameters (i.e. eigenschappen van sorbent en waterfase) en molecuulstructuur op de sorptie van LAS aan sedimenten en humuszuren. Tenslotte laat hoofdstuk 6 zien dat, in overeenstemming met de evenwichtspartitioneringstheorie (EqP), de toxiciteit van LAS voor mariene, bentische organismen hoofdzakelijk kan worden verklaard door blootstelling aan de fractie in de waterfase. Hier wordt een samenvatting van de belangrijkste resultaten gepresenteerd.

nd-SPME analyse van LAS in verschillende aquatische matrices

De solid phase microextraction methode (SPME) maakt het mogelijk om de vrij opgeloste concentratie van een stof in een oplossing te bemonsteren, zonder dat fasescheiding en clean-up nodig zijn voor chromatografische analyse. In hoofdstuk 2 is gedemonstreerd dat 7 μm polyacrylaat gecoate SPME fibers kunnen worden gebruikt voor de analyse van LAS in zeewater. Echter, de oorspronkelijke wegwerpfibers moeten thermisch geconditioneerd worden voor zij gebruikt kunnen worden voor de analyse van LAS. Met de geoptimaliseerde methode zijn zowel concentraties als mengsel-onafhankelijke PA-water partiticoëfficiënten (K_{fw}) verkregen voor een groot aantal lineaire alkylbenzeen sulfonaten. Deze waarden variëren voor de verschillende LAS homologen en isomeren. De methode is eveneens toegepast, om de oplosbaarheid van LAS in zeewater te meten. De resultaten laten zien, dat de oplosbaarheid goed voorspeld kan worden aan de hand van de vorming van neerslagcomplexen met Ca^{2+} ionen in de oplossing.

In hoofdstuk 4 en 5 zijn PA SPME fibers gebruikt voor het meten van de vrij opgeloste LAS concentratie in sediment- en humuszuur-suspensies met verschillende chemische samenstellingen van de waterfase. Deze samenstelling heeft een sterke invloed op de partitionering van LAS naar de fibercoating en de verhouding tussen de K_{fw} en de specifieke elektrolyt (Na^+ en Ca^{2+}) concentraties in de media geeft aan dat de vorming van een ion-paar (ion pairing) waarschijnlijk het belangrijkste mechanisme is voor de sorptie van LAS naar PA. Hoewel detectielimieten van de methode worden beperkt door achtergrondniveaus afkomstig van uiteenlopende bronnen, maken de eenvoud en de gevoeligheid PA SPME tot een veelbelovend instrument voor laboratoriumstudies, waarin extractie van de opgeloste fractie complex is.

Naast de analytische toepassing van de SPME methode, verkennen hoofdstuk 4 en 5 het nut van K_{fw} waarden als voorspeller van de hydrofobiciteit van LAS congenere. Een goede correlatie is gevonden tussen K_{fw} waarden en sorptiecoëfficiënten verkregen uit experimenten met sedimenten (hoofdstuk 4) en humuszuren (hoofdstuk 5). De samenhang tussen sediment sorptiecoëfficiënten en PA-water partiticoëfficiënten gemeten bij verschillende samenstellingen van de waterfase was minder duidelijk, waarschijnlijk omdat verschillende sorptiemechanismen plaatsvinden in zowel het sediment als in de fibercoating. Toch kon een goede schatting van de sorptie van verschillende LAS homologen worden gedaan op basis van testen, uitgevoerd onder de specifieke omstandigheden met één congener, en de K_{fw} waarden.

Sorptie van LAS: Effecten van sorbent, samenstelling van de waterfase en contaminant eigenschappen

Sorptie van LAS in natuurlijke sedimenten was duidelijk niet lineair in zowel zout- als zoetwater. De invloed van sedimenteigenschappen op de sorptie van LAS in mariene en estuariene sedimenten is gepresenteerd in hoofdstuk 3. De relatie tussen de verschillende sedimenteigenschappen en de sorptiecoëfficiënten laat zien, dat de sorptie van de gebruikte modelstof (C_{12} -2-LAS) zowel van de kleifractie (korrelgrootte < 2 μm diameter) als van de organisch koolstof fractie afhangt en dat het belang van beide parameters verschilt met de surfactant concentratie. Bij concentraties in de orde grootte van microgrammen per liter is de kleifractie van het sediment de belangrijkste determinant van sorptie, terwijl het organisch koolstofgehalte belangrijker wordt bij hogere concentraties.

Met betrekking tot de samenstelling van de waterfase is er een significant verschil tussen sorptie onder zout- en zoetwater omstandigheden en sorptiecoëfficiënten nemen geleidelijk toe met de hoeveelheid opgelost zout (hoofdstuk 4). Bovendien blijkt sorptie zeer goed te correleren met de Ca^{2+} concentratie van de complexe oplossingen. Sorptiecoëfficiënten variëren vergelijkbaar met andere studies, die zich concentreerden op anionische surfactanten en gebruik maakten van eenvoudige $CaCl_2$ oplossingen.

Commerciële LAS bestaat uit 20 verschillende congenere die zich verschillend gedragen, afhankelijk van hun alkyl ketenlengte en de positie van de benzeen sulfonaat groep in de alkylketen. In hoofdstuk 4 laten sediment sorptie data van individuele LAS homologen zien, dat verschillen in sorptie hoofdzakelijk samenhangen met de hydrofobiciteit van de LAS moleculen. De sterkst sorberende LAS componenten hebben langere ketens en meer extern gepositioneerde fenyl groepen. Zoals hierboven besproken, kan dit sorptiegedrag goed voorspeld worden met behulp van de K_{fw} waarden.

In hoofdstuk 5 is nader verkend, wat het effect is van opgelost humuszuur op de vrij opgeloste LAS concentratie. De sorptie van LAS aan de twee verschillende, geteste humuszuren (Aldrich en Leonardite), bleek onafhankelijk te zijn van de concentratie. Hoge sorptiecoëfficiënten ($\log K_{DOC} = 4.1-5.6$) zijn verkregen voor alle stoffen en sorptie varieert met verschil in moleculaire structuur, hetgeen ook het geval is voor sedimenten en SPME fibers. In tegenstelling tot de trend waargenomen voor sedimenten en polyacrylaat, is de sorptie van LAS aan humuszuren niet afhankelijk van de zoutconcentratie in de oplossing.

De hypothese is, dat het effect van de samenstelling van de oplossing op de K_{DOC} gemaskeerd wordt door veranderingen in de moleculaire conformatie van het humuszuur. Om dit proces beter te begrijpen, moeten meer experimenten uitgevoerd worden, waarin mengsels van LAS worden gebruikt.

Toxiciteit van LAS in mariene sedimenten

Eén van de hoofddoelen van dit proefschrift was, om de validiteit de testen van de EqP voor LAS in sedimenten. Volgens deze theorie is de effectconcentratie, bepaald bij blootstelling in een uitsluitend water bevattend systeem, vergelijkbaar met die gemeten in poriewater in sediment. De toxiciteit van LAS voor de mariene slijkgarnaal, *Corophium volutator*, is onderzocht in twee goed gedefinieerde sedimenten en in systemen met alleen water, zoals beschreven in hoofdstuk 6. Daarbij is speciaal gelet op de manier om testchemicaliën aan een sediment toe te voegen (spiking), alsmede op de stabiliteit van de stoffen gedurende de toxiciteitstest. De effectconcentraties (LC50s) laten een goede overeenkomst zien tussen de resultaten van de blootstellingstesten in de systemen met alleen water en de concentratie in poriewater, afgeleid met behulp van de sorptie/desorptie coëfficiënten.

RESUMEN

Los procesos de adsorción de contaminantes en sedimentos y materia orgánica disuelta tienen consecuencias directas en su transporte, distribución y biodisponibilidad en el medio acuático.

La presente tesis está enfocada en el estudio de los procesos de adsorción del tensioactivo aniónico sulfonato de alquilbenceno lineal (LAS) en relación a las características del compuesto, las propiedades del sustrato y la composición del agua, así como de la implicación del proceso de adsorción del LAS en la toxicidad en sedimentos.

El capítulo 2 trata sobre el estudio y desarrollo de un método de extracción pasiva para el análisis de la concentración disuelta del LAS en agua de mar. Este método es conocido como micro extracción en fase sólida (SPME). Los capítulos 4 y 5 estudian el uso de las fibras SPME en otros medios. Los capítulos 3, 4 y 5 exploran el efecto de las principales variables del sistema (sustrato y medio acuático), así como de las características moleculares del compuesto que influyen en la concentración disuelta del LAS. Finalmente, el capítulo 6 estudia la toxicidad del LAS en sedimentos usando el anfípodo marino *Corophium volutator*.

Análisis nd-SPME del LAS en distintas matrices acuosas y posibles aplicaciones del coeficiente de reparto fibra-agua

El método de micro extracción en fase sólida, en su variante de “extracción imperceptible”, consiste en fibras ópticas recubiertas de un polímero en el que el compuesto es absorbido, estas fibras permiten extraer una fracción mínima del compuesto disuelto sin afectar el equilibrio del sistema. El uso del método SPME evita muchos de los pasos experimentales previos al análisis cromatográfico de la muestra, como la separación de fases (ej. centrifugación) o purificación. El capítulo 2 muestra que las fibras ópticas revestidas de una capa de 7µm de poliacrilato (PA) pueden ser usadas para el análisis del LAS en agua de mar. Previamente a su uso, estas fibras deben ser tratadas mediante un condicionamiento termal (120°C) en el horno de un cromatógrafo de gases. Con el método SPME optimizado se obtienen coeficientes de reparto fibra-agua (K_{fw}) independientes de la concentración del analito, así como de la presencia de otros compuestos en disolución. El método SPME ha sido utilizado para medir por primera vez la solubilidad de los distintos constituyentes del LAS en agua de mar. Los resultados indican que la solubilidad se puede predecir a partir de la concentración de Ca^{2+} en el medio. En los capítulos 4 y 5, las fibras PA SPME son usadas para analizar la concentración del LAS en experimentos de adsorción con sedimento y ácidos húmicos. La composición del medio acuático afecta en gran medida la adsorción del LAS a la fibra, probablemente debido a la formación de pares iónicos entre LAS y cationes presentes en disolución (especialmente Ca^{2+}).

Aunque los límites de detección del método están restringidos por la contaminación general del compuesto en material y maquinaria del laboratorio, su simplicidad y precisión hacen que pueda considerarse una herramienta útil para estudios donde la extracción de la

fracción disuelta del LAS es dificultosa. Un ejemplo de aplicación es su uso para medir la concentración de agua intersticial en ensayos de adsorción y toxicidad.

Aparte de la aplicación analítica del método SPME, el capítulo 4 explora el uso de los coeficientes K_{fw} para predecir la hidrofobicidad de los distintos compuestos del LAS. En este mismo capítulo se muestra la relación existente entre los coeficientes K_{fw} y los coeficientes de reparto del LAS en sedimentos y ácidos húmicos. Sin embargo, si solo consideramos la relación entre estos dos coeficientes a distintas salinidades, la correlación es menor, debido a que la adsorción del LAS en fibra y sedimento está regida por diferentes mecanismos. Aún así, el método podría ser usado para estimar la adsorción de los distintos constituyentes del LAS, a partir de la obtención experimental del coeficiente de reparto para un compuesto específico y la extrapolación a los demás compuestos a partir de los valores K_{fw} .

Adsorción del LAS: Efecto de las características del sustrato, composición del medio acuático y estructura molecular del compuesto

La adsorción de los constituyentes del LAS en sedimentos ha seguido una isoterma no lineal en todos los sedimentos y salinidades testados. El capítulo 3 estudia la contribución de las distintas propiedades de sedimentos marinos y de estuarios en la adsorción del LAS. Con este objetivo se usaron sedimentos naturales y algunos constituyentes puros (carbono orgánico, arcillas y arena). La relación de las distintas propiedades del sedimento con los coeficientes de reparto, ha mostrado que la adsorción del compuesto modelo C₁₂-2-LAS depende principalmente del contenido en carbono orgánico y de la fracción de arcilla (fracción del sedimento con un tamaño de grano menor a 2 μm de diámetro), y esta dependencia es versátil a la concentración del tensioactivo en el medio. La fracción arcillosa del sedimento es el parámetro mejor relacionado con los coeficientes de adsorción a bajas concentraciones (<10 $\mu\text{g/L}$), mientras que a mayores concentraciones el contenido orgánico es más significativo.

En cuanto a la composición del agua, el capítulo 4 muestra que la adsorción es significativamente distinta en agua dulce y de mar, y que los coeficientes de reparto son mayores en disoluciones con mayor contenido en sales. La adsorción del LAS en sedimentos está relacionada con el contenido en Ca^{2+} del medio, y la variación de los coeficientes de partición es comparable a otros estudios de tensioactivos aniónicos donde solo se usan disoluciones simples de CaCl_2 .

El LAS comercial está formado por más de 20 constituyentes (homólogos e isómeros) que muestran distintos comportamientos de adsorción de acuerdo al número de carbonos de la cadena alquílica y la posición del sulfonato de alquilbenceno. En el capítulo 4, los resultados de los experimentos de adsorción para los distintos componentes del LAS muestran que las diferencias de adsorción están debidas en gran medida a su hidrofobicidad. Los mayores coeficientes de reparto corresponden a los compuestos con las cadenas alquílicas más largas y posiciones fenil más externas.

El capítulo 5 explora el efecto de la presencia de ácidos húmicos en la concentración disuelta del LAS. La adsorción del LAS en los dos tipos de ácidos húmicos usados resultó

ser independiente de la concentración del compuesto. Los coeficientes de reparto obtenidos fueron considerablemente altos ($\text{Log } K_{\text{DOC}} = 4.1-5.6$) y estos valores variaron de acuerdo con las diferencias estructurales de los compuestos. Al contrario de lo obtenido en los ensayos con sedimentos, los coeficientes de reparto K_{DOC} fueron constantes a las diferentes diluciones usadas. En este capítulo se sugiere que la ausencia de efecto de la salinidad puede ser debida a cambios en la conformación de las moléculas de ácido húmico.

Toxicidad del LAS en sedimentos marinos

Uno de los objetivos primordiales de la presente tesis ha sido la validación de la teoría de partición en el equilibrio (EqP) para el LAS en sedimentos. Según esta teoría, la concentración que causa un efecto en los organismos del bentos se debe principalmente a la concentración del contaminante en el agua intersticial. De esta manera, la toxicidad podría predecirse a partir de la exposición del organismo en ensayos solo acuáticos y el análisis o estimación de la concentración del compuesto en el agua intersticial. El capítulo 6 estudia la toxicidad del LAS en sedimentos, usando el anfípodo marino *Corophium volutator*. Este capítulo también investiga en detalle las técnicas para introducir el compuesto en los ensayos de toxicidad y la estabilidad del compuesto durante su exposición en los bioensayos. Los resultados mostraron que las concentraciones que causan un efecto en ensayos acuáticos y en la concentración intersticial coinciden, corroborando así la hipótesis de partida.

CURRICULUM VITAE

Ángeles Rico Rico was born in Huelva, Spain, on 8 May 1979. She attended Diego Guzmán y Quesada high school in the same city, and, after finishing in 1997, she started her academic studies on Marine Sciences at Cadiz University. During her university years, she actively collaborated in the Biological Methods Department with Prof. Alejandro Pérez Hurtado. From October 2001 until June 2002, she participated in several projects of the Aquatic Ecology group at the Biology faculty of the University of Aveiro (Portugal), under the supervision of Dr. Juan Ignacio Gordillo and Prof. Henrique Queiroga. In 2002, Ángeles graduated from the University of Cádiz with a Master's degree in Marine Sciences.

In March 2003, Ángeles acquired a Leonardo Da Vinci Scholarship, which enabled her to start working in setting up toxicological tests with marine algae at the Research Centre Montecatini in Ravenna (Italy).

In October 2003, she started a postgraduate course on Environmental Technology at the Polytechnic University of Catalonia, and she did her research project "Ecotoxicological effects of pesticides in the aquatic environment" under the supervision of Dr. Carlos Barata, for which she was awarded with the note of "excellent".

In November 2004, Ángeles started her PhD at Utrecht University in The Netherlands for the Marie Curie project "Risk Assessment of Surfactants in the Coastal Environment", under the supervision of Dr. Joop Hermens at the Institute for Risk Assessment Sciences. She has visited and collaborated with the department of Ecology and Coastal Management from Cádiz University with prof. Julián Blasco, and the Biological Station of Doñana in Seville, Spain. In 2007, she was one of the main organizers of the PhD annual meeting of the Dutch Society of Toxicology. During her PhD program, Ángeles has presented her research at several national and international congresses.

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- Rico-Rico A., Droge S.T.J, Hermens J.L.M.. Poster: "Analysis of Freely Dissolved Concentration of Linear Alkylbenzene Sulfonates Using Solid-Phase Microextraction Fibers". SETAC Europe 18th Annual Meeting. 25- 29 May 2008, Warsaw, Poland.
- Rico-Rico A., Temara A., Hermens J.L.M. Poster: "Sorption of LAS in Marine and Estuarine Sediments". SETAC Europe 17th Annual Meeting. 20 - 24 May 2007, Porto, Portugal.
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Colleagues from the Environmental Toxicology and Chemistry group (MTX), I am so glad I had the opportunity to meet you all! I specially keep in my memory Montreal and Warsaw meetings, pizza-movie evenings and lunches at the MTX patch of grass. Steven, I

specially want to thank you for all I have learned from you, it was very helpful to meet somebody like you when I started at IRAS, since you were in many cases my “practical-work supervisor”. You made me feel proud of belonging to the surfactant team when sharing our (sometimes) mysterious outcomes. Thomas ter Laak, during the last two years, I had a research encyclopedia next to me, and this was you!, you were always giving clear answers when I asked you any time. I enjoyed a lot discussing with you about anything. I wish you a great time with your new job. Stephan, besides you delight us with secret recipes cakes, you have the capability to make the environment around you friendly and relax. Many thanks for being my paranimf, for helping me with the summary translation, and freaky corrections. I really appreciate the help and the effort you have made. Barry, although our climate preferences differ in a few globe parallels, I think we understood each other very well these years, I wish you a very successful career in one of the poles, good luck! Nynke and Cozmina, I enjoyed a lot our experience during the organization of the AIO dagen. I am happy I can continue a bit longer to be closer to both of you, because of the project (with Nynke) and because of the room we share (with Cozmina), let's enjoy chocolates together! Chiel, it is nice to have a person like you in the group, you keep yourself busy with a lot of “boss staff”, but at the same time you are very close to students in the lab and in discussions. Theo, I could not measure LAS without you, so this is already a big thing for this thesis. Besides, I like the way you react when I go to your office with new experimental set up or results. “I can't almost not believe it” is your sentence to say “I want more information”, with this, you invite students to clarify and order ideas; this is something extra to experimental help, thanks. Frans, thanks to offer me your help late in the evenings, looking for ice, finding lab instruments, or checking the GC-helium flow. Thanks to you, late hours in the lab were easier. I also wish to thank Thomas Hartnik and Heike, who were part of the MTX group when I started my PhD, you both kindly help me to adapt quickly to the new environment.

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I was lucky to meet a very nice group of international and national friends when I arrived in Utrecht. For almost all of us the 2004 and 2005 were our first years in this city, and

since then, I have enjoyed a lot of your warm friendship. I first met Lih, who nicely introduced me to the Cambridgelaan residence's life, and to distinguish Chinese names and food. Lih, thanks for your help correcting the introduction of the thesis during the inspiring "tea" visits. Con Vicky empezó la fiesta, y todavía sigue, gracias por estar siempre, en persona, en skype y dónde sea, gracias Paraninf! And then, suddenly, I don't remember very well how we start to be a group only formed by super nice people: Ale (seguramente te conocí en una cena, o en el portugués, disfrutando de una relajada charla), Andy, Baris, Birka, Carol, Elisabeth, Emilia (I am sure it was in the Dutch course), Joris, Joseph, Julián, Juliana, Lo, Martin, Nuria, Olga (surely dancing), Ryan (listening Pink Floyd), Santi (en el grupo de los primeros, cenas en la cocina de la resi) Sachie, Savvas, Sonia (I miss our lunches!), Takeshi, Tanja, Tina, Wiebke, Willem, Yukina, Yolanda (desde que llegaste, siempre dispuesta a una cena, enhorabuena investigadora!).

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Durante estos cuatro años he tenido la alegría de recibir a muchos de mis amigos de la Universidad. Algunas de las niñas de la Peña Cupulera celebramos la despedida de Carol en Utrecht. Carol e Isaac pasaron de nuevo, haciendo escala para Costa Rica (oh, sorry, para Creta), con Paco tengo más de una experiencia en común (suerte para hoy!), Laia entrenó por aquí y nos vimos. Inma, May y Xavi me hicieron sentir que estaba de puente de la constitución. Mamen, tú hiciste algo muy importante para que me dieran la beca, gracias! A los demás y a vuestros respectivos, os espero, y si no, nos vemos en la próxima reunión!

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Watse, I could write this part in Spanish, because it is the Spanish week, right? Many thanks for your support and comprehension capacity during this time. You have helped me to

be serene when I had to submit a paper or to give an oral presentation, to look the things in other perspectives. You could even follow my “spiking methods” conversations! Arrive home and meet you is everyday a pleasure.

Las experiencias familiares son especiales cuando una vive a algunos kilómetros de ellos. Aún así, mis padres, hermanas y sobrino me han hecho sentir todos los días un ratito en casa. Para mí ha sido muy importante teneros cerca, y lo habéis conseguido en cada momento. Sin saber mucho lo que significaba, os habéis alegrado con mis publicaciones, y hemos hablado de mi trabajo y mi vida en Holanda como si estuviéseis aquí. Mari, Virginia y Nene, vuestra visita fue muy especial para mí, hay que repetirla. A mis padres les tengo que agradecer mucho más que estos cuatro años, gracias a vuestros genes, pero también a vuestro esfuerzo, he escrito este libro. Por esto y por más, es para vosotros.

Ángeles Rico Rico

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