



**Towards a better understanding of the
bioavailability and
partition behaviour of cationic surfactants**

Draft Final Report of the Contribution from IRAS,
Utrecht University

Utrecht, February 2013



Universiteit Utrecht

CEFIC APAG-Sector group

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Preface

This report summarizes the major outcome of the contribution of the Institute for Risk Assessment Sciences (IRAS) to the project “Towards a better understanding of the bioavailability and partition behaviour of cationic surfactants”. This project was funded by the CEFIC Oleochemicals & Surfactants sector group (CEFIC-APAG). Research was performed at Helmholtz Centre for Environmental Research – UFZ, Leipzig for a period of 3 years. The duration of the research at IRAS was four years because it was performed within a PhD study (Yi Chen). Results of the UFZ contribution are reported in 2012. With this report, the contribution of IRAS can be regarded as finished. We regard this as a draft final report because it will be discussed at a meeting in June 2013 of the researchers and representatives of CEFIC-APAG and the discussions may lead to some changes in the document. After the June meeting, a final version will be prepared. Some of the data presented in this report are published or are presented in manuscripts that are submitted for publication or will soon be submitted. Some data are not yet compiled in the form of a manuscript and we present here only the final results. More detailed information about experimental methods will become available in the near future in the form of manuscripts for scientific publication and these manuscripts or publications will be added to this report after the June meeting.

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I Summary and Conclusions

Bioavailability is an important aspect in the ecological risk assessment. Sorption to sediment, but also to dissolved organic matter in an aqueous solution may reduce the uptake and toxicity to organisms. This reduced bioavailability will in particular be visible in a situation where the organism is in equilibrium with its surrounding. This assumption of an equilibrium is often valid if we consider toxicity in relatively small organisms. These concepts also form the rationale for the equilibrium partitioning theory and this theory is well established for neutral organic contaminants [1, 2]. The validity of this theory for ionic compounds is not established yet and one of the main reasons is that experimental data for sorption and bioavailability for ionic compounds are scarce. Moreover, experimental methods to measure bioavailability and sorption of ionic species are not widely available.

In this report, we present the outcome of research within the project “Towards a better understanding of the bioavailability and partition behavior of cationic surfactants”. The focus of the research was on (i) the development of analytical procedures for measurement of total and freely dissolved concentration of cationic surfactants, (ii) to get more understanding of sorption of cationic surfactants to humic matter, minerals and whole sediments, and (iii) to get more insight into toxicity data of cationic surfactants in relation to bioavailability.

Major outcomes of the work for each of these three topics are:

- (i) *Analytical procedures for measuring total and freely dissolved concentrations of cationic surfactants*
 - Oasis WCX SPE columns are useful for clean-up and concentrating aqueous samples that contain cationic surfactants. Recoveries of extractions are close to 100 %, even for solutions where the major surfactant fraction was sorbed to dissolved organic matter.
 - Preconditioning of glass vials is needed to reduce losses of cationic surfactants due to sorption to surfaces. An appropriate pre-conditioning step results in negligible losses.
 - SPME fibers with 7- μm polyacrylate (PA) coating represent excellent samplers for measuring freely dissolved concentrations of hydrophobic cationic surfactants. At low aqueous concentrations, sorption isotherms are linear. At higher concentrations, saturation occurs because the cationic exchange capacity (CEC) is reached.
 - It remains to be tested to what extent 7- μm PA is functional for very hydrophobic cationic surfactants. If affinities to the fiber are too high, they require large sample volumes to fulfil the requirement of negligible depletion. In such cases, e.g. small volumes of *in vitro* tests, we have already shown the applicability of 35- μm PA fiber to be adequate alternatives with a desired lower affinity.
 - The affinity of polar surfactant structures may be too low to 7- μm PA, and C18/SCX provides a suitable alternative with lower detection limits.
 - Sorption of cationic surfactants to ion-exchange based SPME fibers depend on the pH and the presence of inorganic cations (Na^+ , Ca^{2+}) because these cations compete with the surfactant. This is a limitation of the SPME procedure, but it can be solved by performing the SPME calibration at the same conditions as in the laboratory experiments.
 - The SPME procedure is applicable to quaternary cationic surfactants as well as primary amines. Sorption coefficients to the polyacrylate coating increases with the chain length of alkyl chain.
 - A prototype SPME tool with specific strong cation-exchange properties (C18/SCX) was developed to extract moderately hydrophobic cationic surfactants with polar moieties. Lauryl diethanolamine showed a more than 100 fold higher affinity for C18/SCX than for 7- μm PA, allowing for measurements of sorption affinity to humic acids in a low concentration range.
- (ii) *Sorption of cationic surfactants to humic matter, minerals and whole sediments*
 - The SPME method represents a useful tool for measuring sorption of cationic surfactants.

- Sorption of a cationic surfactant (benzyltrimethylammonium chloride or C12-BAC) to organic matter increases with a decrease in pH and an increase in electrolyte concentrations. This change in sorption is related to competition with cationic exchange sites. The observed sorption data (in presence of Na^+) can be modeled with a NICA-Donnan model.
 - Sorption of cationic surfactants increases with the length of the carbon chain.
 - Normalized to cation-exchange capacity, sorption of cationic surfactants to clay minerals can be stronger than to organic matter.
 - Sorption of a cationic surfactant to an artificial sediment can be predicted from the sorption affinities to organic matter and clay as individual components of a sediment.
 - The fiber-water partition coefficient is an excellent predictor for organic matter sorption of cationic surfactants with different structure as well as in different test media.
- (iii) *Toxicity data of cationic surfactants in relation to bioavailability*
- Toxicity studies clearly show that the EqP theory is also applicable to cationic surfactants.
 - Effect concentrations in complex systems (sediment or in medium with a matrix that may bind the compound) are similar as in water only exposures as long as the concentrations are measured as freely dissolved concentrations.
- (iv) *Alignment of APAG project Topic 1 [IRAS] with Topic 2 [UFZ]*
- The dominant environmental sorption sites for all organic cations (small / bulky / polar / hydrophobic / surfactant / primary, secondary, tertiary amines and quaternary ammonium compounds) are cation-exchange sites.
 - Sorption of all organic cations to cation-exchange sites is affected by medium composition, in particular pH and divalent cation concentrations.
 - Medium effects follow empirically straightforward trends, and can be approximated for organic matter by NICA-Donnan modeling.
 - Both organic matter and clay minerals are important cation-exchange sites that should both be accounted for in sorption of any organic cation (IRAS: surfactants, UFZ: smaller bases and QACs) to typical sediment compositions.
 - Sorption differences for various types of clay minerals are reduced when correcting for cation-exchange capacity of the sorbents (IRAS: surfactants, UFZ: smaller bases and QACs).
 - Specific differences have been noticed between the contribution of molecular fragments to sorption affinities of organic cations to clay minerals and organic matter, as well as for sorption of neutral species (by bulk partitioning) and cationic species (to ion-exchange sites).
 - The IRAS-based work demonstrated for cationic surfactants that sorption to an artificial sediment can be accurately predicted by sorption data on individual components. The UFZ-based project demonstrated that sorption data on a single reference organic matter and a single reference clay mineral can be used to adequately assess the sorption affinity of organic cations to natural soil.

II Introduction

As mentioned in the original proposal, the research was focused on two topics..

Topic 1: “Improve the analytical procedure of the freely dissolved concentration of cationic surfactants in environmental samples, including sediment suspension. This work will focus on the use of solid-phase microextraction (SPME) method, and it will build on experience that has been gathered at IRAS in recent years (“Bioavailability of surfactants in marine sediments” funded by ERASM and performed at IRAS, Utrecht University 2004-2007). Once the SPME method is sufficiently validated for several cationic model surfactants, this tool will allow us to carefully examine several urgent questions related to cationics in sediment. Sorption studies will be performed to elucidate the contribution of sorption to clay minerals to the overall sorption capacity of natural sediments. Measuring freely dissolved concentrations in the pore water phase of a sediment toxicity test in combination with a water only exposure will clarify whether the pore water hypothesis is also valid for cationics, i.e., whether adverse effects to biota are solely controlled by the freely dissolved concentration in the pore water”.

Topic 2: “Develop a sound mechanistic model for understanding and predicting sorption of cationic surfactants to minerals and humic matter in soils and sediments. This work should enable us to extrapolate experimental results from a few compounds and soils/sediments that are being used for calibration, to the large number of other cationic surfactants and soils/sediments that cannot possibly all be tested experimentally. To this end we will try to combine a model that predicts partitioning of polar and non-polar organic compounds with an adapted version of a model that predicts sorption of inorganic, ionic compounds in soils and sediments. In a first step such a combined model will be build separately for humic matter and for mineral matter. These separate models will be evaluated with experimental data that have also been measured separately for humic matter and minerals. In a final step, both models –for humic and mineral matter- will then be combined into a model that should work for soils and sediments with variable content of humic and mineral matter”

The results are expected to also provide a good starting point for a better understanding of sorption of cationic surfactants in biota which is relevant for ecotoxicity and bioaccumulation. UFZ was responsible for topic 2 and the research within IRAS was focused on topic 1.

Here, we report on the outcome of the research in topic 1.

Objectives of the proposed work within topic 1:

1. To improve the analytical procedure for measurement of the freely dissolved concentration of cationic surfactants in environmental samples, including sediment suspension.
2. To generate sorption data for cationic surfactants to humic matter, minerals and whole sediments.
3. To generate toxicity data for a benthic organism in sediment as well as in “water only” tests for cationic surfactants.
4. To compare effect concentrations, measured as freely dissolved concentrations in pore water, with effect concentrations in a toxicity tests with “water only” exposure.

Results of research on each of these subtopics 1-4 are briefly described in Chapter III and more detailed information is provided in annex 1-4.

III.1 Analytical procedure for measurement of total and freely dissolved concentration of cationic surfactants

Hexadecyl-trimethylammonium chloride (IV-16) and benzyl-dimethyl-dodecylammonium chloride (C12-BAC) were used as two model cationic surfactants for which the analytical methods were worked out in detail. These quaternary amines are structurally different with an external, charged head group for the monoquat IV-16 and a large polar nonionic secondary side-chain for C12-BAC and both compounds are permanently charged.

A new sampling technique has been tested for these two cationic surfactants based on solid phase micro-extraction (SPME) with a polyacrylate coated fiber. The advantage of the SPME procedure is that it enables to measure freely dissolved concentrations of contaminants in samples from the environment or from toxicity studies [3, 4]. Information about the freely dissolved concentration is relevant for studying the bioavailability of chemicals. In SPME analysis, a proper calibration is needed and this requires the measurement of concentrations in a fiber coating as well as the concentration in the aqueous phase. Detailed information about the analytical procedure as well as the SPME method is given in annex 1 and 2 and are based on two publications [5, 6]. Here we briefly show some of the major outcomes and conclusions.

Optimization of analytical method and solid phase extraction (SPE)

IV-16 and C12-BAC were analyzed on LC-MS/MS, equipped with an electrospray interface in positive ion mode. The condition of electrospray ionization has been optimized to yield the maximum ion intensity. Different eluents have been tested to reduce peak tailing in the chromatogram. Addition of trifluoroacetic acid (TFA) leads to low pH in the eluent, which can minimize the interaction between negatively silicon groups in the HPLC column and cationics, and therefore reduce the tailing. Methanol was preferred as a cosolvent for eluents over acetonitrile because of worldwide shortage of the latter and sufficient yield.

The efficiency of a weak cation-exchange SPE column (Oasis WCX) has been studied, where several mixed solvents were tested as eluents. The mixture of methanol and water (90/10 v/v) containing 0.1% TFA, which has the same composition of HPLC eluent, has resulted in the recoveries of about 100 % (see Table 1). The good recoveries show great potential of measuring total dissolved concentration in water samples, including fractions sorbed to particulate matter.

The positively charged nitrogen makes cationic surfactants susceptible for sorption to surfaces, including glass of the test vials [7-10]. Although actual concentrations were always determined by SPE, it is valuable to have information about sorption to glass ware. The influence of exposure time and concentration of the surfactant on sorption to glass vials were studied and sorption to glass was higher at lower test concentrations (see Table 2)

Different procedures to precondition the 20 mL glass vials were tested, which showed that methanol and water rinses flushed off most of the surfactants that had adsorbed during preconditioning. Instead of these pretreatments, simple discarding of preconditioning solution with the intended concentration without flushing was shown to be sufficient (see Table 1). As standard precondition procedure, vials were equilibrated for >2 h with a solution of similar concentration as intended, then discarded, and new solutions were prepared.

Optimization of SPME fiber extraction for IV-16 and C12-BAC

Disposable SPME fibers with a 7- μ m polyacrylate (PA) coating were selected for the SPME procedure. Initial experiments showed that this coating gave the best results. Exposure times were optimized and calibration curves were established covering a broad concentration range. These calibration curves for the two test chemicals are shown in Figure 1. The sorption isotherm is non-linear reaching a plateau at about 30 mmol/L PA which corresponds with the value for the cation exchange capacity of the 7- μ m polyacrylate (PA) coating determined independently by $\text{Ba}^{2+}/\text{Ca}^{2+}$ exchange. In a range between 0.1 $\mu\text{g/L}$ and 0.1 mg/L , the sorption is linear. Sorption isotherms were fitted with a Freundlich equation and parameters from these fits are presented in Table 3.

One has to carefully assess the effects of the medium composition, especially the divalent cation concentration and pH, as well as the potential influence of the presence of other competing chemicals because sorption is affected by the presence of these inorganic cations (see Figure 2). It is clear that in laboratory based studies, such as sorption and bioaccumulation tests with a single contaminant,

calibration can easily be performed in the same test solution as applied in the sorption. Although the SPME method has some limitations, it is one of the few methods that enable the measurement of freely dissolved concentrations of cationic surfactants and it is more sensitive than other existing procedures.

SPME sorption coefficients for eight cationic surfactants

With the same procedure as presented in [6], SPME sorption isotherms were measured for five other cationic surfactants. The structure of these compounds, that include five quaternary ammonium surfactants and two primary amine surfactant, is given in Table 4. A separate study investigated the SPME method for a tertiary diethanolamine (C12-DEA) surfactant. Results of this specific study are presented in a first draft manuscript [11] that is attached as annex 3. SPME sorption isotherms are presented in Figure 3. The sorption increases with chain length in the order: C14>C12>C10 alkylbenzyl quat; di-C12>di-C10 ammonium quat. and C14>C12 primary amine. This effect of chain length is related to the influence of chain length on the hydrophobicity of these compounds. The protonated form of C12-DEA could be measured with 7- μ m PA, but was shown to be much more efficiently extracted with a coating with a much higher density of strong cation-exchange sites (C18/SCX) as compared to 7- μ m PA.

Conclusions on analytical procedures for measuring total and freely dissolved concentrations of cationic surfactants:

- Oasis WCX SPE columns are useful for clean-up and concentrating aqueous samples that contain cationic surfactants. Recoveries of extractions are close to 100 %, even for solutions where the major surfactant fraction was sorbed to dissolved organic matter.
- Preconditioning of glass vials is needed to reduce losses of cationic surfactants due to sorption to surfaces. An appropriate pre-conditioning step results in negligible losses.
- SPME fibers with 7- μ m polyacrylate (PA) coating represent excellent samplers for measuring freely dissolved concentrations of hydrophobic cationic surfactants. At low aqueous concentrations, sorption isotherms are linear. At higher concentrations, saturation occurs because the cationic exchange capacity (CEC) is reached.
- It remains to be tested to what extent 7- μ m PA is functional for very hydrophobic cationic surfactants. If affinities to the fiber are too high, they require large sample volumes to fulfil the requirement of negligible depletion. In such cases, e.g. small volumes of *in vitro* tests, we have already shown the applicability of 35- μ m PA fiber to be adequate alternatives with a desired lower affinity.
- The affinity of polar surfactant structures may be too low to 7- μ m PA, and C18/SCX provides a suitable alternative with lower detection limits.
- Sorption of cationic surfactants to ion-exchange based SPME fibers depend on the pH and the presence of inorganic cations (Na^+ , Ca^{2+}) because these cations compete with the surfactant. This is a limitation of the SPME procedure, but it can be solved by performing the SPME calibration at the same conditions as in the laboratory experiments.
- The SPME procedure is applicable to quaternary cationic surfactants as well as primary amines. Sorption coefficients to the polyacrylate coating increases with the chain length of alkyl chain.
- A prototype SPME tool with specific strong cation-exchange properties (C18/SCX) was developed to extract moderately hydrophobic cationic surfactants with polar moieties. Lauryl diethanolamine showed a more than 100 fold higher affinity for C18/SCX than for 7- μ m PA, allowing for measurements of sorption affinity to humic acids in a low concentration range.

III.2 Sorption of cationic surfactants to humic matter, minerals and whole sediments

Sorption of C12-BAC to organic matter: effect of organic matter type, pH and salt concentrations

Sorption of benzyl-dimethyl-dodecylammonium chloride (C12-BAC) to natural organic matter was studied at concentrations well below the cation-exchange capacity (CEC). Freely dissolved concentrations were measured with the solid phase micro-extraction techniques as described in [6] (see annex 1). A detailed overview of the experiments, the experimental methods and the results are presented in [12]. A copy of this submitted manuscript is given in annex 4.

Sorption affinities of C12-BAC under constant medium conditions differed within 0.4 log units for two humic acids (Aldrich, Leonardite) and peat samples (Sphagnum, Pahokee), and all sorbents showed a similar slight isotherm nonlinearity (Figure 3). The effect of the composition of the aqueous solution, including pH and various concentrations of Na^+ or Ca^{2+} , on sorption to 10 mg/L Aldrich humic acid (AHA) was systematically studied. Sorption to the polyacrylate coating of the SPME fibers as well as to AHA was reduced at both low pH and high electrolyte concentration, and reduced more strongly by Ca^{2+} compared with Na^+ at similar concentrations (see Figures 4 and 5).

Sorption data obtained for AHA in different Na^+ solutions at pH 6 was modeled successfully by the NICA-Donnan approach. Excluding the non-specific electrostatic attraction caused by NaCl, the intrinsic sorption coefficient to ionic sites in AHA was calculated to be 5.35 log units (at C_{aq} of 1 nM, pH 6, and in the absence of Ca^{2+}). Applying generic input parameters for humic acid, protons and Ca^{2+} , the NICA-Donnan model explained the stronger effect of Ca^{2+} compared to Na^+ by differences in Boltzmann factors, mostly as a result of specific binding of Ca^{2+} whereas Na^+ only “sorbs” indifferently.

Sorption coefficients to organic matter for seven cationic surfactants

Sorption isotherms to Aldrich humic acids were measured for six other cationic surfactants at a pH of 6.0 and with 15 mM Na^+ . The structure of these compounds, that include five quaternary cationics and two primary cationics, is given in Table 4. Sorption isotherms are presented in Figure 7. The sorption increases with chain length in the order: C14>C12>C10 alkylbenzyl quat; di-C12>di-C10 ammonium quat. and C14>C12 primary amine. This effect of chain length is related to the influence of chain length on the hydrophobicity of these compounds. Sorption of C12-DEA to AHA was half a log unit weaker than the C12 primary amine.

Sorption of C12-BAC to organic matter, clay, sand and sediment

Sorption of one surfactant (C12-BAC) was measured to distinct components of sediment: organic matter, clay mineral and sand. Sorption isotherms for C12-BAC with three different type of clay are presented in Figure 8. While the variation in sorption is relatively high if concentrations are expressed on a weight basis, this variation cancels out if sorption data that are normalized for the cation exchange capacity the three clays. Sphagnum peat, kaolinite and quartz sand were selected as representative materials for these three components and the sorption isotherms to these materials as well as whole sediment are presented in Figure 9. OECD artificial sediment was selected for this study.

Based on information of the composition of OECD sediment, sorption to the whole sediment was predicted based on the sorption data for the three components. The plot in Figure 10 of observed versus predicted concentrations in whole sediment, clearly show that the sorption can very well be predicted from the sorption to the individual components. Apparently, sorption is simply an additive process, at least for artificial sediment.

Relation between sorption to organic matter and fiber-water partition coefficients

Sorption data for seven cationic surfactants in a fixed medium composition (pH of 6 and 15 mM Na^+) and sorption data for C12-BAC at several medium compositions (varying concentrations of Na^+ and Ca^{2+}) are plotted against the fiber water partition coefficient measured at the same composition of the aqueous phase in Figure 11 and 12, respectively. These two figures show that the ion-exchange based fiber-water partition coefficient is an excellent predictor for organic matter sorption of cationic surfactants. This similarity in sorption behavior shows that the mechanisms of the sorption process in organic matter and fiber are similar, and are based on cation-exchange.

Conclusions on sorption of cationic surfactants to humic matter, minerals and whole sediments:

- The SPME method represents a useful tool for measuring sorption of cationic surfactants.
- Sorption of a cationic surfactant (benzyltrimethylammonium chloride or C12-BAC) to organic matter increases with a decrease in pH and an increase in electrolyte concentrations. This change in sorption is related to competition with cationic exchange sites. The observed sorption data can be modeled with a NICA-Donnan model.
- Sorption of cationic surfactants increases with the length of the carbon chain.
- Normalized to cation-exchange capacity, sorption of cationic surfactants to clay minerals can be stronger than to organic matter.
- Sorption of a cationic surfactant to an artificial sediment can be predicted from the sorption affinities to organic matter and clay as individual components of a sediment.
- The fiber-water partition coefficient is an excellent predictor for organic matter sorption of cationic surfactants with different structure as well as in different test media.

III.3 Toxicity data of cationic surfactants in relation to bioavailability

An important element of the ecological risk assessment is the equilibrium partitioning (EqP) theory. As pointed out by Di Toro, the effect concentrations in sediment can be predicted from concentrations on a pore water basis and a sediment-water sorption coefficient [13]. Effect data from on water only exposures can then be applied to predict toxicity in sediment. As pointed out by Di Toro [13]: “the equality of the effects concentration on a pore water basis suggests that the route of exposure is via pore water. However, the equality of the effects concentration on a sediment-organic carbon basis, suggests that the ingestion of sediment organic carbon is the primary route of exposure. It is important to realize that if the sediment and pore water are in equilibrium, then the effective exposure concentration is the same regardless of exposure route. Therefore, it is not possible to determine the primary route of exposure from equilibrated experiments”.

This EqP can easily be applied to experiments in water only but in the presence of a matrix that may bind the compound. In those cases, the concentration of the unbound chemical (i.e. the freely dissolved concentration) is the relevant dose parameter. Several studies, both in the field of ecotoxicology, but also in the area of *in vitro* toxicology, have shown that the freely dissolved concentration represents a more intrinsic dose parameter for analyzing toxicity data [14-16]. While this EqP concept is very interesting, the validity of this concept for ionic compounds has hardly been studied. Recently, the concept was applied in a toxicity study with an anionic surfactant [17].

The aims of this part of the project are to verify the EqP for quaternary cationic surfactants in acute toxicity tests with four test organisms that are commonly used in bioassays, and to assess the influence of sorptive processes in such assays by performing detailed mass balance studies. We specifically wanted to determine the difference between effect concentrations based on nominal concentrations (total spiked amount in the medium) and those based on actual measurements of the freely dissolved concentration. Our sorption work showed a strong affinity of QACs to natural humic acids, and we were therefore also interested in the sorptive properties of other types of organic matter that occur in toxicity studies, such as serum protein and algae cells. Bioassays with cells in *in vitro* tests are nearly always performed in medium that contains serum or serum proteins. Bioassays using freshwater instead of synthetic medium may also have humic acids present in the medium. Oligochaete worms exposed to sediment may be exposed to chemicals via ingested sediment, as suggested by [18] for the toxicity of the QAC dimethyldioctadecylammonium chloride to *Lumbriculus variegatus*. Sorbed fractions are not taken up by cell systems such as algae and cultured *in vitro* cells, but filter feeding daphnids that may ingest surfactant sorbed to humic acid and the oligochaete *L. variegatus* can ingest sediment particles, are interesting test cases for validation of the EqP theory.

The effects of benzyl-dimethyl-dodecylammonium chloride (C12-BAC) in four different toxicity assays was determined, including i) mortality of aquatic worm *Lumbriculus variegatus* in presence/absence of a suspension of artificial sediment, (ii) viability of *in-vitro* rainbow trout gill cell line (RT-gill W1) in presence/absence of serum proteins, (iii) immobilization of *Daphnia magna* in presence/absence of dissolved humic acid (HA), and iv) photosynthesis inhibition of green algae *Chorella vulgaris*; iv) The study also determined the loss due to chemical adsorption to the different test vessels applied in these tests. Results of this work are presented here briefly. A manuscript is in preparation with a more detailed outline of these studies [19].

Figure 13 presents dose response curves for the four test systems. Concentrations of C12-BAC are plotted as nominal concentrations, as total concentrations (after SPE) or as freely dissolved concentrations (SPME). It is obvious that the presence of sediment reduces the concentration in the water phase. Based on nominal concentrations, the effect concentration is much higher in the presence of sediment. Effect data based on measured total concentrations without sediment are close to effect data using freely dissolved concentrations. Also in cytotoxicity test with RTgill-W1 cells, measured freely dissolved concentrations in the presence of FBS (fetal bovine serum) are close to concentrations with the same effect in a test without the presence of FBS. The biggest effect of bioavailability on toxicity was observed for the presence of humic acids in tests with *Daphnia magna*. The dose-response curves also show that effects based on measured freely dissolved concentrations correspond very well with data from the toxicity test without humic acids. In the test with algae, dose-response

curves based on total versus freely dissolved concentrations are very similar and this correspond very well with the information about the distribution that show that the amount that is bound to algae is almost negligible in comparison to the amount present as freely dissolved in the medium. This is clearly different in the other studies with humic acids, sediment and proteins (FBS) where the amount that is bound is significantly higher than the amount present as unbound compound in the test medium. Still, even in the test with algae, significant sorption to test vessel walls was observed for C12-BAC to result in lower actual dissolved concentrations compared to nominal concentrations, and stronger effects are to be expected for more hydrophobic cationic surfactants.

Conclusions on toxicity data of cationic surfactants in relation to bioavailability:

- Toxicity studies clearly show that the EqP theory is also applicable to cationic surfactants.
- Effect concentrations in complex systems (sediment or in medium with a matrix that may bind the compound) are similar as in water only exposures as long as the concentrations are measured as freely dissolved concentrations.

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Table 1. Aqueous recoveries for various test concentrations of IV-16 and C12-BAC after pre-conditioning 20mL scintillation vials at intended concentration and only discarding this before preparing new solutions (Data from [6]).

Test conc. (mg/L)	Aqueous recovery (%; Mean \pm SD, n=4)	
	IV-16	C12-BAC
1.0	98 \pm 5	88 \pm 9
0.1	95 \pm 2	89 \pm 2
0.01 ^a	90 \pm 3	88 \pm 9
0.001	105 \pm 20	112 \pm 12
overall recovery of spiked concentrations for 2-d-isotherm in 5 mM CaCl ₂	105 \pm 15	105 \pm 14

^a n=2

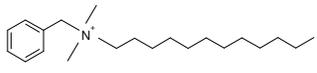
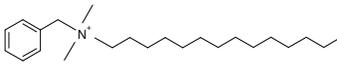
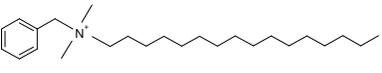
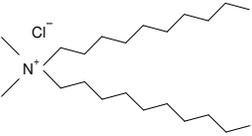
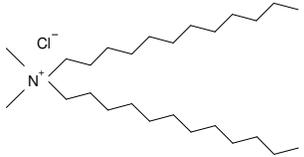
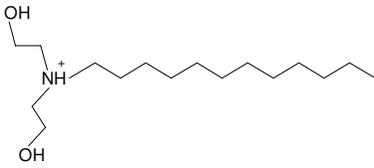
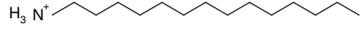
Table 2. Effect of adsorption to glass wall on solutions of cationic surfactants in 20 mL scintillation vials (no pre-conditioning steps), tested for different concentrations (data from [6]).

Test conc. (mg/L)	Aqueous recovery (%; Mean \pm SD, n = 3)	
	IV-16	C12-BAC
1.0	88 \pm 7	91 \pm 7
0.1	51 \pm 1	74 \pm 1
0.01	48 \pm 1	75 \pm 1
0.001	22 \pm 2	63 \pm 7

Table 3. Fiber water sorption coefficients (K_{fw}) and Freundlich sorption coefficients (organic carbon fraction normalized $\log K_F$ in mmol/kg) for C12-BAC on purified AHA at various medium composition given a fixed Freundlich exponent (n_F) of 0.8. $\log K_{fw}$ is obtained from the isotherms fitted with a slope of 1. $\log K_F$ and $\log D_{OC,IE}$ values are derived from the isotherms. Sorption coefficients for 7- μm PA fiber ($\log K_{fw}$ in L/L) measured in the corresponding medium as the sorption test. $\log D_{OC,IE}$ (L/kg_{OC}) are calculated at 1 nM dissolved concentration based on n_F of 0.8. Boltzmann factor (B) are calculated with different electrolytes at pH 6 using ECOSAT.

medium composition		$\log K_{fw} \pm sy.x$ (n) (slope=1) L/L	$\log K_F \pm sy.x$ (n) (fixed $n_F=0.8$) (mmol) ^{0.2} *L ^{0.8} /kg _{OC}	$\log D_{OC,IE}$ ($C_{aq}=1$ nM, $n_F=0.8$) L/ kg _{OC}	Boltzmann factor (B)
CaCl ₂					
pH 6.0	0.5 mM	4.01 ± 0.07 (6)	5.20 ± 0.02 (5)	6.40	17.7
	5 mM	3.76 ± 0.08 (3)	4.93 ± 0.02 (10)	6.13	9.6
	50 mM	3.40 ± 0.01 (4)	4.67 ± 0.02 (10)	5.87	5.0
pH 4.5	5 mM	3.32 ± 0.07 (4)	4.61 ± 0.06 (6)	5.81	
pH 3.0	5 mM	2.75 ± 0.06 (3)	4.21 ± 0.03 (5)	5.64	
NaCl					
pH 6.0	5 mM	4.06 ± 0.05 (4)	5.57 ± 0.04 (8)	6.77	257.4
	50 mM	3.96 ± 0.02 (3)	5.24 ± 0.05 (9)	6.44	92.1
	500 mM	3.72 ± 0.02 (2)	4.94 ± 0.03 (4)	6.14	31.7
pH 3.0	5 mM	2.93 ± 0.1 (3)	4.54 ± 0.03 (11)	5.97	

Table 4. Structures of cationic surfactants used in the sorption studies.

<p>C12-BAC</p> 	<p>C14-BAC</p> 	<p>C16-BAC</p> 
<p>DI-C10-ammonium quat</p> 	<p>DI-C12-ammonium quat</p> 	<p>C12-diethanol amine</p> 
<p>C12 primary amine</p> 	<p>C14 primary amine</p> 	

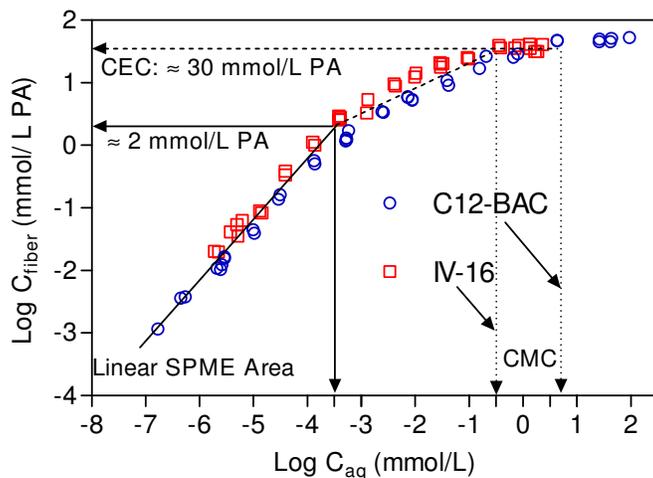


Figure 1. SPME sorption isotherms covering a broad concentration range were measured in 2-d exposure period for IV-16 and C12-BAC in 5 mM CaCl_2 solution at pH 5.5. The unit is expressed as mmol/L for dissolved and fiber sorbed concentrations. Linear SPME calibration can be applied in the aqueous concentration range from nM to μM (solid line arrows). The maximum fiber concentration is approximately 30 mmol/L PA (broken line arrows), which is very close to the measured CEC of 7- μm PA fibers via the cation exchange method. The CMC values of both chemicals in this solution can also be estimated via the isotherms (dotted line arrows). The solid line indicates linearity. Data from [6].

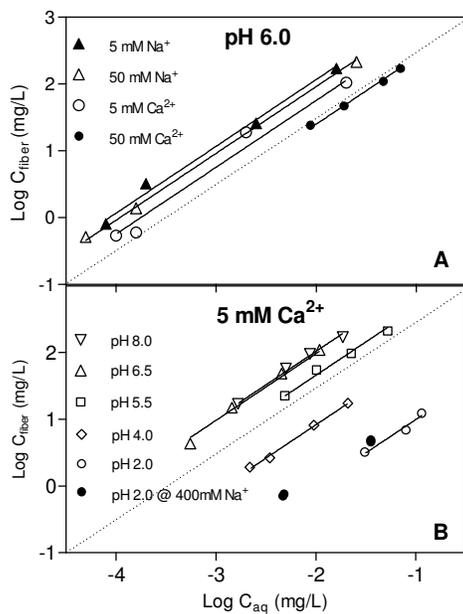


Figure 2. Effect of electrolyte composition (A) and pH (B) on the C12-BAC sorption to 7- μm PA SPME fiber conditioned at 120°C. Fiber-water sorption affinity (K_{fw}) decreases with increasing electrolyte concentration and decreasing pH. Data from [6].

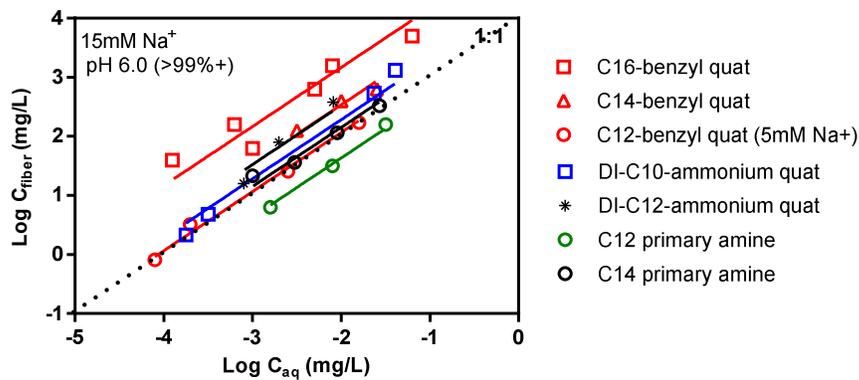


Figure 3. SPME sorption isotherms covering a broad concentration range for seven cationic surfactants, measured at pH of 6.0 and 15 mM Na⁺. Data from [20].

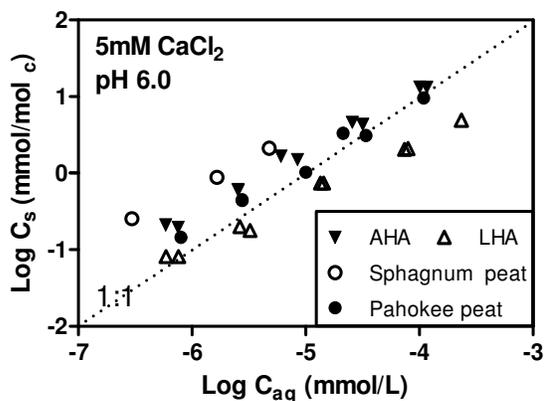


Figure 4. C12-BAC sorption to four types of organic matter normalized with CEC in 5 mM CaCl₂ at pH 6. Dotted line indicates linearity. AHA and LHA indicate purified Aldrich humic acid and Leonardite humic acid, respectively. Data from [12].

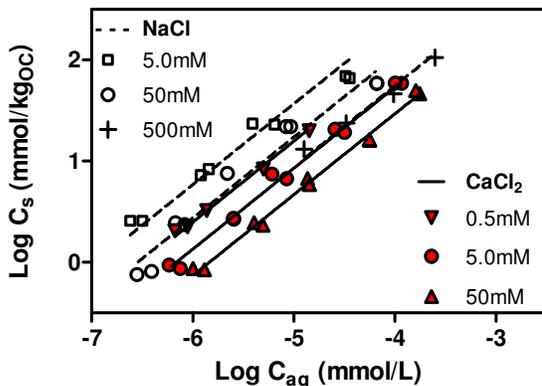


Figure 5. C12-BAC sorption to purified AHA determined at different background electrolytes at pH 6. Isotherms were fitted with Freundlich equation with n_F of 0.8. Dashed and solid lines indicate fitting curve for NaCl and CaCl₂, respectively. Data from [12].

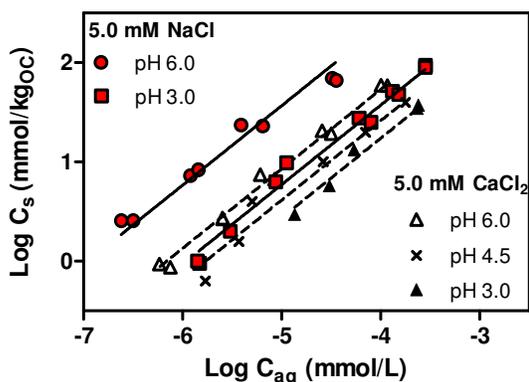


Figure 6. C12-BAC sorption to purified AHA determined at pH 6, pH 4.5 and pH 3 at 5 mM NaCl and CaCl₂ concentration. Isotherms were fitted with Freundlich equation with n_F of 0.8. Data from [12].

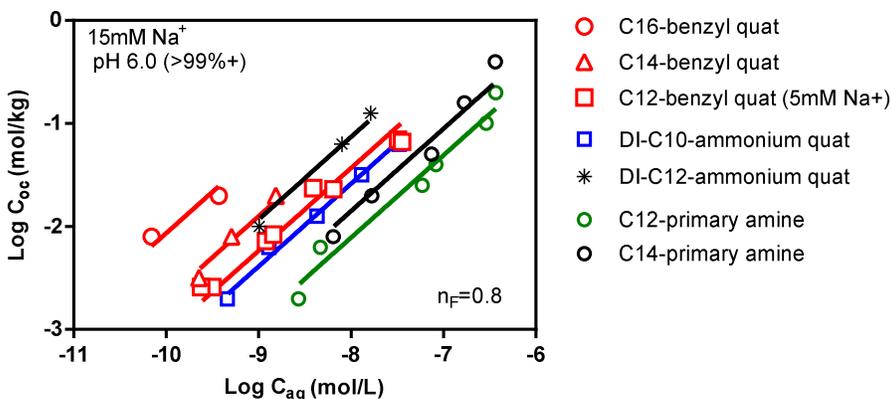


Figure 7. Sorption to purified AHA determined at pH 6 and 15 mM Na⁺ of seven cationic surfactants. Data are fitted with Freundlich equation with n_F of 0.8. Data are from [20].

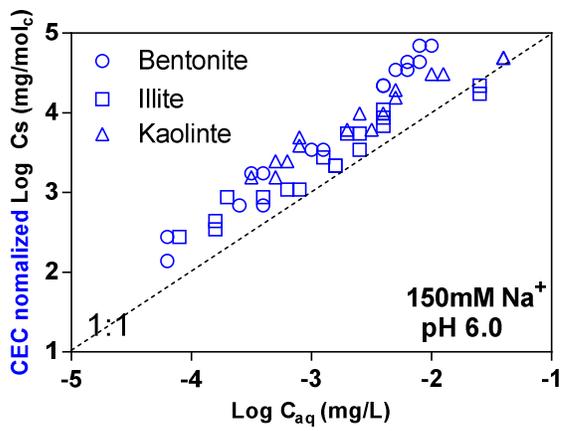
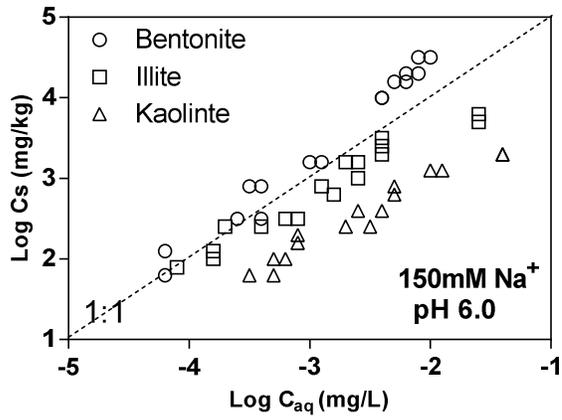


Figure 8. Sorption of C12-BAC to different clay minerals in 150 mM Na⁺ at pH 6.0. Lower panel: concentrations are normalized to cationic exchange capacity (CEC). Data from [20]

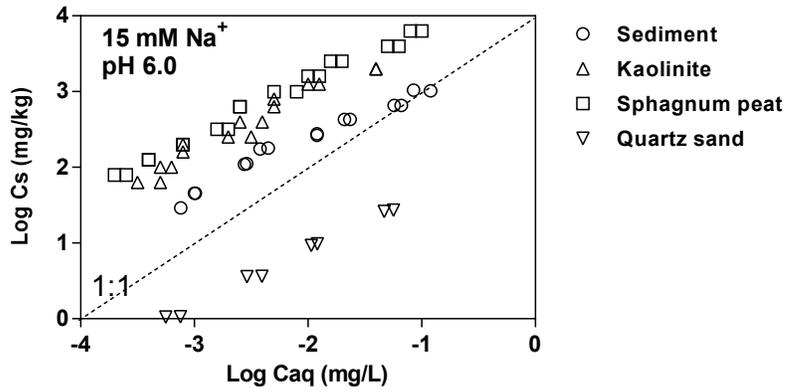


Figure 9. Sorption of C12-BAC to different component of artificial sediment 15 mM Na⁺ at pH 6.0. Data from [20].

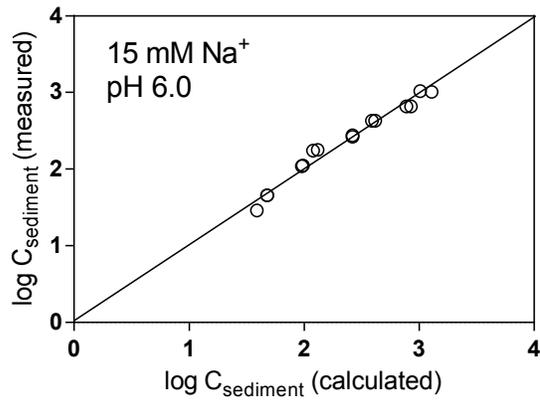


Figure 10. Measured versus predicted concentrations of C12-BAC in sediment based on sorption data for the individual component of the sediment. Data from [20].

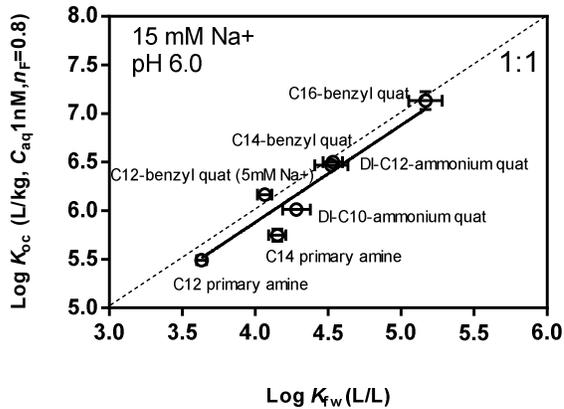


Figure 11. Log $D_{OC,IE}$ (L/kg_{OC}) of seven cationic surfactants C12-BAC to purified Aldrich humic acid calculated at 1 nM dissolved concentration based on n_F of 0.8 as a function of log K_{fw} (L/L). Dotted line indicates linearity. Data from [20].

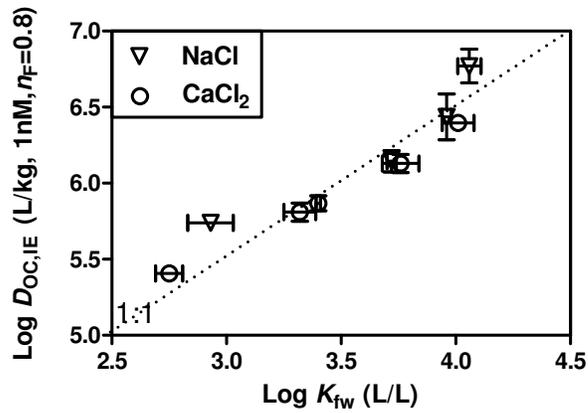


Figure 12. Log $D_{OC,IE}$ (L/kg_{OC}) of C12-BAC to purified Aldrich humic acid calculated at 1 nM dissolved concentration based on n_F of 0.8 as a function of log K_{fw} (L/L) (sorption to PA fiber) measured in different medium compositions. Dotted line indicates linearity. Data from [12].

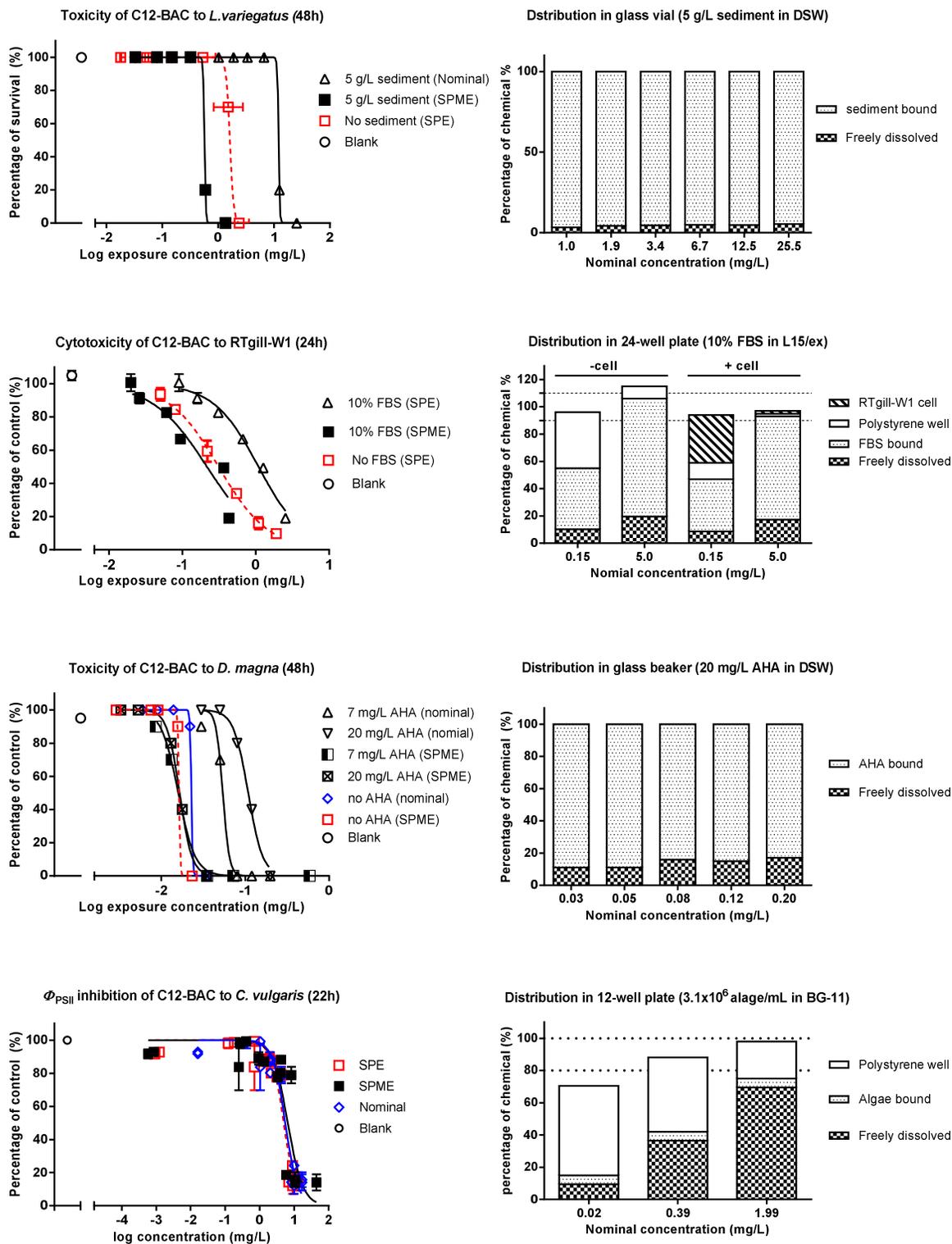


Figure 13. Dose-response curves for the effect of C12-BAC in four different assays.

Annex 1:

Chen, Y.; Droge, S.T.J.; Hermens, J.L.M. Optimization of the chemical analysis of two cationic surfactants; Institute for Risk Assessment Sciences, Utrecht University: Utrecht, Febr. 2013.

Optimization of the chemical analysis of two cationic surfactants

5

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15

Summary

It is well known that cationic surfactants strongly sorb to surfaces. Losses due to sorption will complicate the chemical analysis of aqueous samples. This paper reports the results of a study
20 into the sorption to glassware of two cationic surfactants, hexadecyltrimethylammonium chloride and benzyldimethyldodecyl-ammonium chloride. Moreover, the extraction of the two test compounds with solid phase extraction (SPE) on an OASIS weak cation-exchange (WCX) column was optimized. The results show that sorption to glassware depends both on the exposure time and concentrations. A precondition step clearly reduces the loss due to
25 sorption.

Introduction

A new sampling technique has recently been tested for two cationic surfactants
30 hexadecyltrimethylammonium chloride (IV-16) and benzyldimethyldodecyl-ammonium chloride (C12-BAC), based on solid phase micro-extraction (SPME) with a polyacrylate coated fiber [1]. The advantage of the SPME procedure is that it enables to measure freely dissolved concentrations of contaminants in samples from the environment or from toxicity studies [2, 3]. Information about the freely dissolved concentration is relevant for the studying
35 the bioavailability of chemicals. Sorption of a contaminant to dissolved organic matter in freshwater or to proteins in an *in vitro* toxicity assay may reduce the free concentration and this may affect uptake and toxicity [3]. In SPME analysis, a proper calibration is needed and

this requires the measurement of concentrations in a fiber coating as well as the concentration in the aqueous phase.

40 During the development of this SPME method, two major challenges had to be addressed, including (i) the optimization of an extraction procedure for aqueous samples, and (ii) development of protocols that ensure minimizing the losses of cationic surfactants due to sorption to glass or other equipment. It is well known that it is not simple to keep stable concentrations of cationic surfactants in aqueous solutions, in particular because cationic
45 surfactants have a tendency to sorb to all kinds of material [4, 5].

In the publication that describes the SPME method [1], only the optimal conditions are presented. These conditions were selected from the outcome of a number of detailed experiments. These detailed studies, in particular the studies into the loss of test chemicals in aqueous samples, show a number of interesting complications that may be encountered in
50 experiments with cationic surfactants. In this publication, we describe the outcome of these detailed studies.

Optimization of an extraction procedure for aqueous samples

Calibration of the SPME method for cationic surfactant requires measuring both fiber
55 concentrations and actual aqueous concentrations. Since adsorption to the glass wall was anticipated to cause substantial losses in aqueous samples, accurate analysis of the actual dissolved concentrations is of high importance for SPME calibration. Total dissolved concentrations were determined after solid phase extractions (SPE) using 3cc OASIS weak cation-exchange (WCX) column (Waters, Etten-Leur, The Netherlands). This column is
60 specifically developed to extract cationic compounds [6, 7]. Mechanistically, this mixed-mode SPE sorbent retains analytes by reverse-phase and/or ion-exchange interaction, and is specifically designed for hydrophobic strong bases. At pH 6 and higher, neutral hydrophobic co-solutes can be flushed from the deprotonated WCX with organic solvent, while hydrophobic bases are retained due to ionic interactions. At pH below 3, the carboxylic
65 groups are fully protonated and hydrophobic bases interact only by nonionic interactions. In this state, abundant salt can still be removed by flushing with water and TFA solution if necessary, whereas the hydrophobic bases readily elute with acidified organic solvent. The SPE procedure was optimized for C12-BAC and IV-16 with regard to the composition of the eluent and washing steps.

70

Sorption to glass ware and the development of procedures to minimize the losses

The positively charged nitrogen makes cationic surfactants susceptible for sorption to glass surfaces [8-11]. Although actual concentrations were always determined by SPE, it is valuable to have information about sorption to glass ware. The influence of exposure time and

75 concentration of the surfactant on sorption to glass vials were studied. Moreover, we also
measured losses to cap liners. In order to achieve test conditions where losses were minimal,
several precondition procedures were tested.

Experimental methods

80 *Chemicals and chemical analysis*

Information about the two cationic surfactants that were tested as well as all other compounds
chemicals applied in the sorption tests are given in [1]. Chemical analysis was carried out,
with the internal standard IV-16-d42 in all samples, on a Perkin Elmer (Norwalk, CT) liquid
chromatography system coupled to a triple quadrupole/linear ion trap mass spectrometer.

85 Details are available in [1].

Optimization of an extraction procedure for aqueous samples

The SPE procedure was optimized for permanently charged quaternary surfactants. It was
anticipated that glass wall adsorption and transfer of aqueous samples with plastic pipette tips
90 could include additional losses that affect recovery rates. Therefore, actual SPE recovery was
examined by spiking 1 µg from a surfactant methanol stock solution into 1 mL of water that
was placed on top of a conditioned SPE column and completing the different SPE flushing
and elution steps, in comparison with directly spiking 1 µg in eluents. After setting the
appropriate initial elution steps (flushing out inorganic and organic impurities before eluting
95 the analytes), the best solvent composition for the final elution step was evaluated. Especially
in the sample loading step, the elution rate had to be maintained well below 1 mL/min to
avoid breakthrough of the surfactant (data not shown). Different eluents were examined by
varying the v/v ratio methanol/water, the pH and the addition of NH₄Ac. Based on the
extraction recoveries (see results section) the acidic 90/10 mixture was used as solvent for
100 both SPE extraction and HPLC elution.

The procedure of the SPE extraction and the different steps, including a rationale for each of
the steps, is given in table 1.

To determine the SPE recovery, excluded from losses during transfer from an aqueous
105 sample, clean Millipore water (1 mL) was added on top of the column in Step 3, and this
water was spiked with 1 µg surfactant from a methanol stock solution (mimicking a sample
concentration of 1 mg/L). Following the normal flushing and elution steps, the concentration
in the final eluate was compared with the concentration in a known volume of eluents mixture
spiked with 1 µg.

110

Questions/remarks:

- Did we test in seawater and is that the reason for developing the clean up step for the SPE
- Do we have to include other essential information so that the reader can understand what
115 has been done? (type of water used?).
- Check if we use text from the published article.

Sorption to glass ware and the development of procedures to minimize the losses

A 20 mL scintillation vial (Perkin Elmer, Boston, MA) was used as standard test container
120 for all the sorption experiments. Decrease of relatively low aqueous concentrations (1 µg/L)
due to adsorption to glass vials were checked after exposure for 5 minutes, 2 days and 10
days, and also for different concentrations after 2 days exposure. Pre-conditioning of
glassware for tests with DTDMAC, a more hydrophobic cationic surfactant than our test
compounds, include equilibration of high dissolved concentrations (5 mg/L) followed by
125 flushing with hot tap water, distilled water and methanol [10, 11]. Pilot tests showed that this
resulted in much higher actual concentrations for our cationic surfactants at concentrations
<10 µg/L and therefore different pre-conditioning steps were examined. We tested the effect
on final aqueous recoveries of pre-conditioning with solutions of either intended surfactant
concentrations or ten times higher, and flushing with hot tap water, and/or Milli-Q water
130 and/or methanol.

Losses of the test chemicals and sorption to cap lining were studied in an additional
experiment. Test solutions were prepared by spiking weighed stocks of IV-16 and C12-BAC
in 25 mL and 40 mL vials with Millipore water to attain the concentrations of 1 mg/L and
0.01 mg/L. Vials were completely filled with the solution. Half filled vials were also
135 examined for 1 mg/L, to check if partial filling resulted in higher loss to the glass wall.
Different screw caps lined with polyethylene (PE on 25 mL vials, PerkinElmer, Boston MA)
and Teflon (on 40 mL vials, Supelco, Bellefonte, PA) were tested. Aluminum foils (alu-foil)
were lined on the opening of some vials to prevent the contact between solutions and the
inner layer of the cap. Vials were shaken gently at 20 °C for two days. 1 mL weighed solution
140 was transferred to a 10 mL glass vial and evaporate under nitrogen flow. The test of sorption
to pipette tip was checked separately by flushing the tip with a methanol-water mixture 90/10
(v/v) containing 0.1% TFA (refer to as acidic 90/10 mixture). After the water completely
evaporated, the vials were then extracted by 3 mL acidic 90/10 mixture. Stocks were directly
spiked in the 10 mL glass vial containing 1 mL water to check whether there was compound
145 loss during evaporation procedure. The eluent was analyzed on LC-MS/MS.

Questions/remarks:

- 150 – Do we have to include other essential information so that the reader can understand what has been done?

Results and Discussion

Optimization of an extraction procedure for aqueous samples

An overview of the steps in the SPE extraction is given in Table 1.

155 Small composition difference between water extracts (by SPE) and LC-MS eluent would seriously deform the chromatograms (Figure 1A, C12-BAC measured at a concentration far below the maximum detection limit of LC-MS/MS). A low pH in the HPLC mobile phase could facilitate analysis of cationic drugs [12], by protonating charged sites at silicon groups in the HPLC column (thereby reducing ionic interactions of cationic surfactants in the
160 column), and by ion-pairing of the anions (TFA or formate) with cationic surfactant (thereby improving retention and focusing of cationic surfactants on the HPLC column). TFA has been selected in the HPLC eluent, because serious peak tailing was still observed in the chromatograms when using 0.15% formic acid (pH 3) (figure 1B). Additionally, TFA is a stronger acid and therefore is less needed to lower the pH. Since the addition of acid in
165 mobile phase tends to increase the background noise and deteriorate the HPLC column [13, 14], the concentration of TFA was set to 0.1%, which was also sufficient for SPE extraction. Using acetonitril instead of methanol resulted in similar peak shapes, but was not readily available at the time of the experiments. Figure 1C shows that the presence of 5 mM NH₄Ac in the acidic 90/10 mixture resulted in sharper peak shape, less tailing, but increasing
170 background noise. Still, detection limit and accuracy did not improve, and since the salt also did not improve SPME extraction and SPE elution it was not used in the standard HPLC eluent mixture. Retention time using an isocratic eluents methanol/water mixture of 90/10 (v/v) was 6 minutes shorter than that of 80/20 for both cationic compounds (Figure 1D).

To minimize effects of sample composition (SPE extracts, SPME extracts and external
175 calibration standards injected in the HPLC eluent flow) in the LC-MS/MS detection, we performed an integrated optimization of a single eluent composition that would combine the highest recoveries for SPE and SPME with optimal analytical performance. Although peak shape and tailing may be further improved, LC-MS/MS sensitivity was sufficiently high with isocratic acid 90/10 mixture (pH 1.7) (injection of ~1 pg), SPE recovery (n = 5) 100 ± 4% for
180 IV-16 and 92 ± 3 % for C12-BAC (Table 2). To our knowledge, this is the first generic method for the determination of individual cationic surfactant in aqueous matrices using the SPE WCX column. pH plays an important role in the different steps of the SPE procedure since the carboxyl groups on the SPE sorbent function as an ion-exchange switch for retaining organics (e.g. surfactant) and inorganic cations (e.g. Ca²⁺). The different washing steps isolate

185 the analytes from both organic contaminants and inorganic ions, which facilitate the direct
analysis on LC-MS.

Sorption to glass ware and the development of procedures to minimize the losses

190 Losses due to adsorption to glass wall (Tables 3 and 4) increase with time, rather slowly as
typically observed for silica [15] and are higher with lower concentrations, up to 80% of the
spiked amount for the tested quaternary surfactants. Table 5 shows that about 10% of the total
spiked amount in a 25 mL vial is sorbed to the polypropylene lined cap, whereas teflon lined
caps sorb 50% of the spiked amount in 40 mL vials, but in both cases this is readily
prevented by aluminum foil lining (though care should be taken with NaN_3 , as this corrodes
195 foil after exposure for more than 1 week).

Different procedures to precondition the 20 mL glass vials were tested, which showed that
methanol and water rinses flushed off most of the surfactants that had adsorbed during
preconditioning (Tables 6 and 7). Instead of these pretreatments, simple discarding of
preconditioning solution with the intended concentration without flushing was shown to be
200 sufficient. As standard precondition procedure in all further experiments, vials were
equilibrated for >2 h with a solution of similar concentration as intended, then discarded, and
new solutions were prepared. The polypropylene pipette tips sorbed up to 10% of the total
spiked amount (Table 5), and therefore also had to be flushed with SPE eluent after sample
transfer.

205

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210 European Chemical Industry Council (CEFIC) sector groups.

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260

Tables and Figures

265 Table 1. Steps in the SPE extraction with OASIS weak cation-exchange (WCX) column/

No.	Step	Purpose or rationale
1.	Condition 1: 1mL methanol	moisten the sorbent and remove impurities
2.	Condition 2: 1mL Millipore water	activate the sorbent
3.	Sample load: accurate amount	slow flow rate to enable ion-exchange, preferably without applying vacuum
4.	Wash 1: 1 mL 5% NH ₃ solution	keep the sorbent in ionic mode and remove most inorganic cations such as Ca ²⁺
5.	Wash 2: 1 mL MeOH	remove impurities which bound to the sorbent with reversed-phase interaction
6.	Wash 3: 2 mL Millipore water	remove methanol
7.	Wash 4: 2 mL 0.5% TFA in Millipore, take to dryness to remove water?	make the sorbent fully protonated and remove ionic contaminants, e.g. Ca ²⁺
8.	Elute: with 3 mL mixture of 0.1% TFA in methanol/water (90:10, v:v). Rinse inside of the transfer pipette tip with the first mL of elution mixture. Final elution volume is checked by weighing.	Obtain solute in eluent of same composition as LC mobile phase

270 Table 2. Recoveries of SPE extraction for individual IV-16 and C12-BAC at an aqueous concentration of 1 mg/L tested with five eluent compositions.

SPE eluent composition			SPE Recovery (%; mean \pm SD; n = 5)	
MeOH/H ₂ O (v:v)	acid (pH)	5 mM NH ₄ Ac	IV-16	C12-BAC
90/10	0.1% TFA (1.7)	no	100 \pm 4	92 \pm 3
90/10	0.1%% TFA (1.9)	yes	100 \pm 10	97 \pm 7
90/10	0.02% TFA (3.4)	yes	105 \pm 6	87 \pm 10
90/10	0.15% FA (3.0)	yes	88 \pm 7	99 \pm 6
80/20	0.1% TFA (1.9)	yes	78 \pm 2	78 \pm 6

TFA = trifluoric acid, FA = formic acid, NH₄Ac = ammonium acetate.

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Table 3. Effect of adsorption to the glass wall in time on low concentrations of cationic surfactants in 20 mL scintillation vials (no pre-conditioning steps), followed over 10 days. The initial test concentration was 1 μ g/L.

contact time	Aqueous recovery (%; Mean \pm SD, n=3)	
	IV-16	C12-BAC
5 minutes	47 \pm 2	64 \pm 2
2 days	22 \pm 2	63 \pm 7
10 days	26 \pm 5	46 \pm 10

280

285 Table 4. Effect of adsorption to glass wall on solutions of cationic surfactants in 20 mL scintillation vials (no pre-conditioning steps), tested for different concentrations.

Test conc. (mg/L)	Aqueous recovery (%; Mean \pm SD, n = 3)	
	IV-16	C12-BAC
1.0	88 \pm 7	91 \pm 7
0.1	51 \pm 1	74 \pm 1
0.01	48 \pm 1	75 \pm 1
0.001	22 \pm 2	63 \pm 7

290

Table 5. Losses to cap lining and aluminum foil, and the effect of partial filling of vials.

IV-16 Test concentration: 1.0 mg/L	Test vial: 25 mL scintillation vial, n = 3					
	PE lined cap	alum-foil covered	half filled, alum-foil covered	sorption to pipet tip	evaporation control	
Average	76%	88%	93%	4%	105%	
SD	2%	7%	1%	3%	1%	
IV-16 Test concentration: 0.010 mg/L	Test vial: 25 mL scintillation vial, n = 3					
	PE lined cap	alum-foil covered	half filled, alum-foil covered	sorption to pipet tip	evaporation control	
	Average	38%	48%	17%	95%	
SD	9%	1%	9%	11%		
Test vial: 40 mL scintillation vial, n = 1						
	Teflon lined cap	alum-foil covered				
	13%	62%				
C12-BAC Test concentration: 1.0 mg/L	Test vial: 25 mL scintillation vial, n = 3					
	PE lined cap	alum-foil covered	half filled, alum-foil covered	sorption to pipet tip	evaporation control	
Average	88%	91%	89%	3%	102%	
SD	1%	1%	2%	0%	2%	
C12-BAC Test concentration: 0.010 mg/L	Test vial: 25 mL scintillation vial, n = 3					
	PE lined cap	alum-foil covered	half filled, alum-foil covered	sorption to pipet tip	evaporation control	
	Average	67%	75%	13%	103%	
SD	2%	6%		5%		
Test vial: 40 mL scintillation vial, n = 2						
	Teflon lined cap	alum-foil covered				
Average	27%	74%				

Table 6. Aqueous recoveries for various test concentrations of IV-16 and C12-BAC after different pre-conditioning^a steps in 20 mL scintillation vials.

test concentration (mg/L)		Aqueous recovery (%; Mean \pm SD, n = 3)		
		pre-conditioned concentration (mg/L)		
0.001		0.001	0.01	0.01 + MeOH
	IV-16	38 \pm 2	137 \pm 47	47 \pm 2
	C12-BAC	68 \pm 1	79 \pm 9	79 \pm 9
		pre-conditioned concentration (mg/L)		
0.01		0.01	0.1	0.1 + MeOH
	IV-16	63 \pm 1	129 \pm 11	54 \pm 1
	C12-BAC	75 \pm 3	76 \pm 2	68 \pm 1
		pre-conditioned concentration (mg/L)		
0.1		0.1	5.0	
	IV-16	51 \pm 1	92 \pm 9	
	C12-BAC	74 \pm 2	99 \pm 1	

^aPreconditioning solutions were left overnight, then discarded, rinsed with once hot tap water and twice with demi water and left to dry. In some instances, a single methanol flush (5 mL) was used (steps with “+ MeOH”). The spiked test solutions were analyzed by SPE after 2 days on a mild shaking device.

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Table 7. Aqueous recoveries for various test concentrations of IV-16 and C12-BAC after pre-conditioning 20mL scintillation vials at intended concentration and only discarding this before preparing new solutions.

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Test conc. (mg/L)	Aqueous recovery (%; Mean \pm SD, n=4)	
	IV-16	C12-BAC
1.0	98 \pm 5	88 \pm 9
0.1	95 \pm 2	89 \pm 2
0.01 ^a	90 \pm 3	88 \pm 9
0.001	105 \pm 20	112 \pm 12
overall recovery of spiked concentrations for 2-d-isotherm in 5 mM CaCl ₂	105 \pm 15	105 \pm 14

^a n=2

315

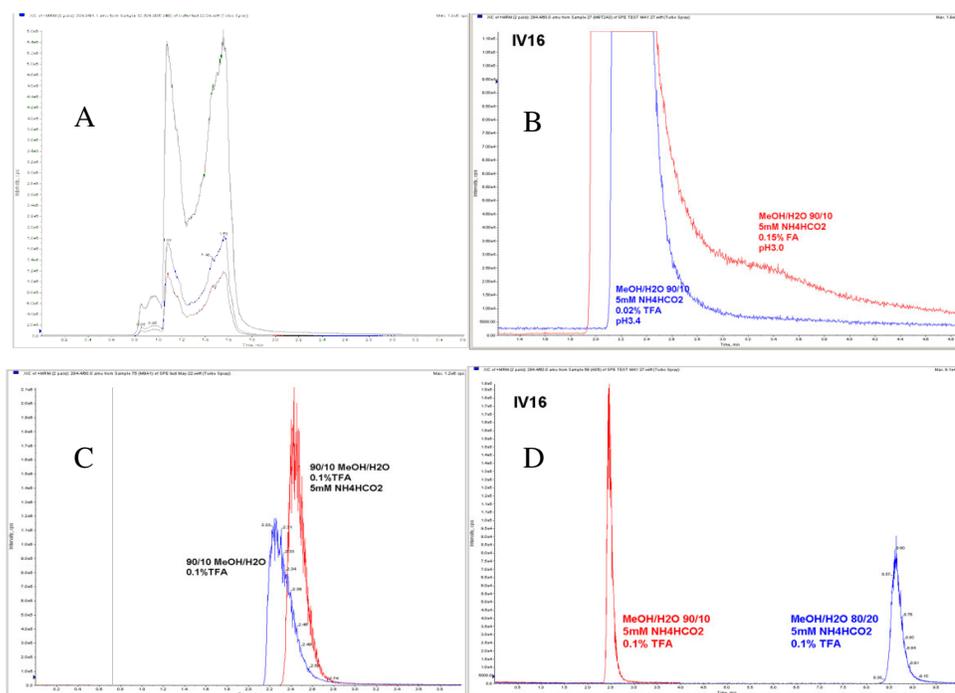


Figure 1. Optimization of LC-MS eluents composition: effect of solvent, acidifier and ammonium acetate.

Annex 2.

Chen, Y.; Droge, S.T.J.; Hermens, J.L.M. Analyzing freely dissolved concentrations of cationic surfactant utilizing ion-exchange capability of polyacrylate coated solid-phase microextraction fibers. *J. Chromatogr. A* 2012, 1252, 15-22.



Analyzing freely dissolved concentrations of cationic surfactant utilizing ion-exchange capability of polyacrylate coated solid-phase microextraction fibers

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ABSTRACT

A 7- μm polyacrylate (PA) coated fiber was successfully employed to determine freely dissolved concentrations of cationic surfactants by solid-phase microextraction (SPME) and utilizing the capability of the PA-coating to sorb organic cations via ion-exchange at carboxylic groups. Measured fiber–water partitioning coefficients (K_{fw}) were constant below a fiber loading of 2 mmol per liter polyacrylate, allowing for simple and accurate analysis in a concentration range that is relevant from a risk assessment point of view. Ion-exchange was confirmed to be the main sorption mechanism because of a decreasing K_{fw} with either higher CaCl_2 concentrations or lower pH, and maximum fiber uptake at the polyacrylate cation-exchange capacity (CEC, at 30 mmol/L PA). Fiber–water sorption isotherms were established in various aqueous media in toxicological relevant concentrations. The developed SPME method has a high potential for application in ecotoxicological studies, as demonstrated in sorption studies with humic acid in different electrolyte solutions at aqueous concentrations down to the sub nM range. Cationic surfactant sorption affinities for humic acid also depend on medium composition but are orders of magnitude higher than to the PA fiber on a sorbent weight basis.

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1. Introduction

The ecological risk assessment process needs input data for both environmental fate and toxic potential of chemicals. For hydrophobic organic contaminants, both fate and toxicity in aqueous systems are strongly influenced by sorption processes [1]. Due to strong sorption to colloidal phases, only a small fraction of the total amount of hydrophobic contaminants in aqueous environments is freely dissolved. It is the freely dissolved concentration that drives partitioning to other biotic and abiotic phases, thereby determining toxic potential, overall dispersion rates and removal efficiencies. Information about the freely dissolved concentration is therefore essential for risk assessment of strongly sorbing chemicals [2–5]. Tools to accurately determine the freely dissolved concentration of classical hydrophobic contaminants, such as PAHs and PCBs, are typically based on equilibration with an extractable polymer phase. In this study, we examine if such extraction tools can also be optimized to determine the freely dissolved concentration of cationic surfactants, which are not very hydrophobic, but which do

have a strong affinity to sorb to negatively charged environmental substrates such as humic acids and clay minerals [6–8].

Cationic surfactants have numerous applications in industrial and household applications, such as in fabric softeners, detergents, biocides and personal care products [9]. Quaternary ammonium compounds (QACs), one of the most extensively used type of cationic surfactants, have been detected in wastewater treatment sludge [10,11], freshwater and marine sediments [11–14], surface water [10,15–17], and in drinking water [18,19]. These monitoring studies have mostly focused on the total amount of cationics either sorbed in sediments or present in the aqueous phase. A significant fraction of cationic surfactants in the aqueous phase in such tests may be bound to non-separable matter, such as dissolved organic matter, resulting in underestimation of sorption coefficients [20] or overestimation of the concentration that is available for uptake [21,22]. Conventional techniques to separate the binding phase from an aqueous solution are not suitable for analyzing freely dissolved cationic surfactants: centrifugation or filtration fails to completely separate dissolved organic matter, whereas equilibrium dialysis may easily lead to losses of surfactants as they tend to accumulate at interfaces [9]. To avoid phase separations, a surfactant electrode potentiometric titration method has been developed to study the sorption of cationics to humic acids [8,23,24]. Although successfully applied in several studies, this method is difficult to

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implement and the sensitivity is insufficient at aqueous concentration level around mM [8,24]. Those techniques that measure freely dissolved concentrations would greatly enhance our insight into the bioavailability and potential risk of cationic surfactants in the environment. The application of a technique to measure freely dissolved concentrations of cationic surfactants would also circumvent artifacts in sorption studies and toxicity tests.

The solid-phase microextraction (SPME) method is a widely used passive sampling technique to determine freely dissolved concentrations of polar and non-polar chemicals in diverse matrices [25–27]. Once equilibrated, the concentration of neutral chemicals in absorptive polymer fiber coatings, such as PDMS and polyacrylate (PA), is proportional to freely dissolved concentration by a compound specific fiber–water sorption coefficient (K_{fw}) [28,29]. Freely dissolved concentrations can thus be determined by measuring the concentrations in the fiber polymer and applying the K_{fw} . SPME has been recently applied to hydrophilic and ionizable chemicals in pharmacokinetics studies [30,31]. Furthermore, the SPME method has been shown to be also applicable for amphiphilic compounds, such as anionic surfactants (linear alkylbenzene sulfonates, LAS) as well as nonionic surfactants (alcohol ethoxylates, AEs) [32–34], and was successfully applied in sorption and sediment toxicity studies [33,35–37]. The successful application of the PA fibers with anionic and nonionic surfactants was the main starting point for the current work with cationic surfactants.

The main objective of the present work was to examine whether the SPME method could be optimized for controlled laboratory studies with permanently charged quaternary surfactants, in an operational concentration range covering toxic levels and several orders of magnitude lower. Various SPME coatings were tested, after which kinetic uptake studies were performed and sorption isotherms over broad concentration ranges at several equilibration times were established. Losses caused by adsorption of cationic surfactant to (negatively charged) test apparatus surfaces were anticipated [12,14], and test conditions were optimized in order to minimize these losses. A second objective in the current study was to explore the sorption mechanism (adsorption, absorption or ion-exchange) by which cationic surfactants sorb to SPME coating, in order to better define the variables that affect SPME applicability. The effect of differences in salinity and pH, the possibility for competition effects in surfactant mixtures on the fiber uptake, and the role of polymer characteristics were assessed. Thirdly, analysis of freely dissolved cationic surfactant concentrations by SPME was applied in a study with dissolved purified humic acids, establishing sorption isotherms between water and standardized humic acid material in different electrolyte solutions.

2. Experimental

2.1. Chemicals

Hexadecyltrimethylammonium chloride (IV-16, $\geq 98.0\%$), benzyldimethyldodecyl-ammonium chloride (C12-BAC, $\geq 99.0\%$) and benzyldimethylhexadecylammonium chloride (C16-BAC, $\geq 97.0\%$) were purchased from Sigma–Aldrich (Zwijndrecht, The Netherlands). Hexadecyltrimethylammonium-d42 chloride (CDNisotope, Quebec, Canada) was used as internal standard (IV-16-d42) for analysis on LC–MS/MS. Standard surfactant stocks were prepared with analytical methanol (Lab-scan, Gliwice, Poland) and stored at +4 °C. Solutions of sodium chloride, calcium chloride and barium chloride (NaCl, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ and $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$, respectively, all from Merck, Darmstadt, Germany) were prepared in Milli-Q water ($>18 \text{ M}\Omega \text{ cm}$, Millipore Waters, Amsterdam, The Netherlands). Artificial seawater was made according to EPA protocol [38] with all salts from Merck except KBr (Sigma–Aldrich). Formaldehyde solution (37%, Merck) was added

to all test mediums to prevent biodegradation. Potassium chloride (Riedel de Haen, Seelze, Germany), ammonium acetate (NH_4Ac) (Fluka, Zwijndrecht, The Netherlands), ammonium hydroxide (28.1% NH_3 , Sigma–Aldrich), formic acid (Fluka), trifluoroacetic acid (TFA) (Sigma–Aldrich), hydrochloric acid (Merck) and sodium hydroxide (Merck) were all in analytical grade. Humic acid was acquired from Aldrich, and purified as described in the literature [39].

2.2. Selection of eluent composition and recovery for solid phase extraction (SPE)

We intended to use a single eluent mixture that allowed for effective SPE elution, as well as full desorption of surfactant from the SPME fiber and efficient HPLC mobile phase. After some initial experiments, an eluent consisting of 0.1% TFA (pH ~ 1.7) added to a 90/10 (v/v) methanol–water mixture was selected.

Total dissolved surfactant concentrations were determined by solid phase extraction (SPE), using 3 cc/60 mg OASIS weak cation-exchange (WCX) column (Waters, Etten-Leur, The Netherlands). The SPE procedure was optimized and consisted of the following steps: (1) condition 1: 1 mL methanol; (2) condition 2: 1 mL Milli-Q water; (3) sample load (slow flow rate to enable ion-exchange, preferably without applying vacuum); (4) wash 1: 1 mL 5% NH_3 solution; (5) wash 2: 1 mL MeOH; (6) wash 3: 2 mL Milli-Q water; (7) wash 4: 2 mL 0.5% TFA in Milli-Q; (8) elute: with 3 mL mixture of 0.1% TFA in methanol/water (90:10, v/v).

It was anticipated that glass wall adsorption and transfer of aqueous samples could include additional losses that affect SPE recovery rates. Therefore, SPE recovery was tested with 1 μg surfactant spiked from a methanol stock solution into 1 mL of water added on top of a column, in comparison with directly spiking 1 μg surfactant in 3 mL eluent solution.

After optimizing the SPE procedure with solutions spiked on column, SPE for measuring actual total dissolved concentrations was always determined by extracting 1 mL aqueous samples. To minimize losses during sample transfer, the inside of 1-mL tips (Greiner Bio-one GmbH, Austria) was always flushed with SPE eluent during the final elution step.

To determine the efficiency of SPME fiber desorption, four SPME fibers (7- μm polyacrylate) were simultaneously exposed to 1 mg/L solutions for both quaternary ammonium compounds. After 2 h exposure the fibers were taken out, and extracted analytes were desorbed in 1 mL of the 90/10 eluent with 0.1% TFA in duplicate. After 1 h desorption, fibers were transferred to new desorption solutions to determine the efficiency of a single extraction step.

2.3. Losses of cationic surfactant to test equipment and preconditioning of glassware

Because it is well known that cationic surfactants can adsorb to glass or other surfaces, we investigated the effects of a number of preconditioning procedures on losses due to sorption. The optimal pretreatment of the test vials consisted of a pre-conditioning step of the test vials with the intended concentration for more than 1 h before preparing new solutions, without additional flushing steps in between. Aqueous recoveries of test solutions in Milli-Q water were close to 90% or higher and in 5 mM CaCl_2 recoveries were 100% for the tested compounds (see Table S1).

2.4. Selection of SPME fiber coating type

Various types of polymer coated glass fibers were purchased from Polymicro Technologies (Phoenix, AZ), which could be cut into disposable SPME fibers of any desired length. All have glass

cores of 110 μm diameter, and either polyacrylate (PA) or polydimethylsiloxane (PDMS) coating with different thickness. Four types of SPME fibers (abbreviated as 7- μm PA, 35- μm PA, 7- μm PDMS, 30- μm PDMS), were tested to select the most suitable fiber coating and thickness for sampling freely dissolved cationic surfactants.

Fibers were cut to four different lengths to differentiate between the fiber types when exposed together in one vial. Following the protocol for the SPME method for the anionic surfactant LAS [34], fibers were conditioned at 120 °C at GC oven with a nitrogen flow rate of 30 mL/min for 16 h, and immersed in Milli-Q water for at least 24 h before use.

Cation-exchange capacity (CEC) was determined in triplicate for 7- μm PA (conditioned to 120 °C overnight for 2 h) by exposing 5 m fiber in 20 mL of 0.1 M BaCl_2 , after which the fiber was blotted dry, and Ba^{2+} exchanged in 20 mL of 0.1 M CaCl_2 . The concentration of Ba^{2+} and K^+ were determined on ICP-OES (Spectro Ciros). CEC values and some other characteristics of the different fiber coatings are given in Table S2.

Sorption isotherms with the different fibers were established for IV-16. 20-mL vials containing four types of fibers in duplicate, were spiked with IV-16 at a concentration ranging from 2 $\mu\text{g/L}$ to 4 mg/L in 5 mM CaCl_2 solution with 0.2% formaldehyde (pH \sim 6). Next, vials were kept on a gentle shaking device (Stuart Roller Mixer SRT9) in a climate room (20 ± 1 °C) for 2 d. Afterwards, SPME fibers were blotted dry on a tissue, cut into small pieces and extracted in HPLC vials (Grace, Deerfield, IL), which contained 1 mL eluent. Samples were subsequently stored at -20 °C until analysis. All the aqueous samples were analyzed by SPE extraction as described above.

2.5. SPME sorption isotherms with the 7- μm PA fiber

The 7- μm PA fiber was considered the most efficient SPME fiber type in terms of uptake kinetics, yield and linearity (discussed in Section 3), and used in all further tests. The effects of shaking on the 2-d SPME sorption isotherm were studied with surfactant solutions (in Milli-Q) left standing still (0 rpm), or shaken at two different speeds (5 rpm and 60 rpm on Stuart Roller Mixer SRT9), with duplicate SPME fibers (50 mm of 7 μm -PA). Although no effect of shaking was noted on the 2-d sorption isotherm (Table S3), shaking at 60 rpm at 20 °C was used in all experiments.

An experiment to determine the SPME uptake kinetics was performed over several weeks and was run in parallel with experiments to establish sorption isotherms after a short term (2 d) and a long term (35/48 d) exposure for both IV-16 and C12-BAC individually in 5 mM CaCl_2 solutions (pH 5.5). Duplicate 7- μm PA SPME fibers were used in each vial for each time point and test concentration. For the uptake study, fibers were exposed at 0.6 mg/L of the test chemical. Sorption isotherms were acquired covering the concentrations from slightly above the limit of detection to above the CMC. For the 2-d sorption isotherm, three out of sixteen different concentrations were tested in quadruplicate vials (each containing two fibers). Based on the findings of these tests, a 2-d exposure period was considered to be most appropriate and used throughout the rest of the study.

The effect of the ionic composition of the medium on PA-SPME sorption isotherms was tested at 2-d exposure for C12-BAC in different concentrations of NaCl and CaCl_2 solutions at pH 5.5 (all with 0.2% formaldehyde). To investigate the effect of H^+ on the surfactant sorption behavior to PA fiber, sorption isotherms were obtained in 5 mM CaCl_2 solutions where the pH was adjusted to 2.0, 4.0, 5.5, 6.5 and 8.0 by addition of HCl or NaOH. For pH 2.0, also sorption in 400 mM NaCl was tested.

To examine whether two surfactants would compete for sorption sites in or on the polymer phase, duplicate 7- μm PA fibers were exposed in solutions with either an individual compound or

a binary mixture of C12-BAC and C16-BAC. Triplicate tests were performed at two aqueous concentrations, resulting in total fiber-sorbed surfactant concentrations that were either below or above 2 mmol/L PA (for reasons explained in Section 3).

2.6. Sorption of quaternary ammonium surfactant to humic acid using ion-exchange SPME

The optimized SPME analysis was applied in a sorption study with Purified Aldrich Humic Acid (PAHA). Purification served to maintain Na^+ as main initial counter ion, which, upon full release in PAHA solutions, would account for \sim 0.05 mM in the experimental set-up used, i.e. negligible compared to the tested electrolyte compositions. A concentrated PAHA stock (3 g/L) was stored at 4 °C until use. Total organic carbon (TOC) content of PAHA was 58.6% (Shimadzu TOC-5050 analyzer). Sorption tests were performed at field-relevant solids concentration of 9.8 mg/L with 0.2% formaldehyde to prevent biodegradation. Aluminum foil was used to prevent losses onto the screw cap lining. Sorption of C12-BAC to PAHA was studied in four different electrolyte solutions (5 and 50 mM NaCl, 5 and 50 mM CaCl_2) in the linear SPME range (<0.1 mg/L). Solutions were prepared in unconditioned 20-mL glass vials, adjusted to pH 6.0, and allowed to equilibrate with spiked compound for 1 d before introducing duplicate 7- μm PA SPME fibers (120 °C conditioned). From four different surfactant concentrations in solutions without PAHA, SPME calibration curves were obtained for each electrolyte solution, with aqueous concentrations determined by SPE. After 2 d, SPME fibers were taken out from the solutions, wiped along a wet tissue (to remove any attached PAHA), and desorbed and analyzed as described above. Average freely dissolved concentrations in each test vial were derived only when duplicate fibers differed less than 10%. To verify the 100% mass balance assumption by which PAHA sorbed concentration are derived, nine aqueous samples with PAHA were extracted for the determination of total dissolved surfactant concentration (freely dissolved and HA bound) by a slightly adapted SPE procedure (5% NaOH in wash step 1 instead of 5% NH_3 solution).

2.7. Chemical analysis

Sample analysis was carried out on a Perkin Elmer (Norwalk, CT) liquid chromatography system, coupled to a triple quadrupole/linear ion trap mass spectrometer (MDS Sciex API 3000 LC-MS/MS System, Applied Biosystems, Foster City, CA). The interface was a Turbo Ion spray source set in the positive ion mode at 1700 V and operated at 400 °C. Separation was achieved by a reverse phase Luna C18(2) column (5 μm , 100 mm \times 3.0 mm, Phenomenex, Torrance, CA) maintained at 20 °C. An isocratic flow rate of 400 $\mu\text{L/min}$ was applied with the acidic 90/10 eluent. The injection volume was 20 μL . Prior to analysis, the internal standard was added at a concentration of 20 ng/mL in each vial to account for MS ionization variability. Acquisition was operated with the software Analyst 1.4.2 (MDS Sciex Applied Biosystems) in multiple reaction monitoring mode leading to the fragmentation of molecular ion at m/z 304/212, m/z 360/268, m/z 284/60 and m/z 326/70 for C12-BAC, C16-BAC, IV-16 and the internal standard (IV-16-d42), respectively. Quantification was executed by integrating the peak area of the target compounds normalized with those of the internal standard using the same software.

2.8. Data analysis

Experimental data were analyzed using Graphpad Prism Version 4.00.

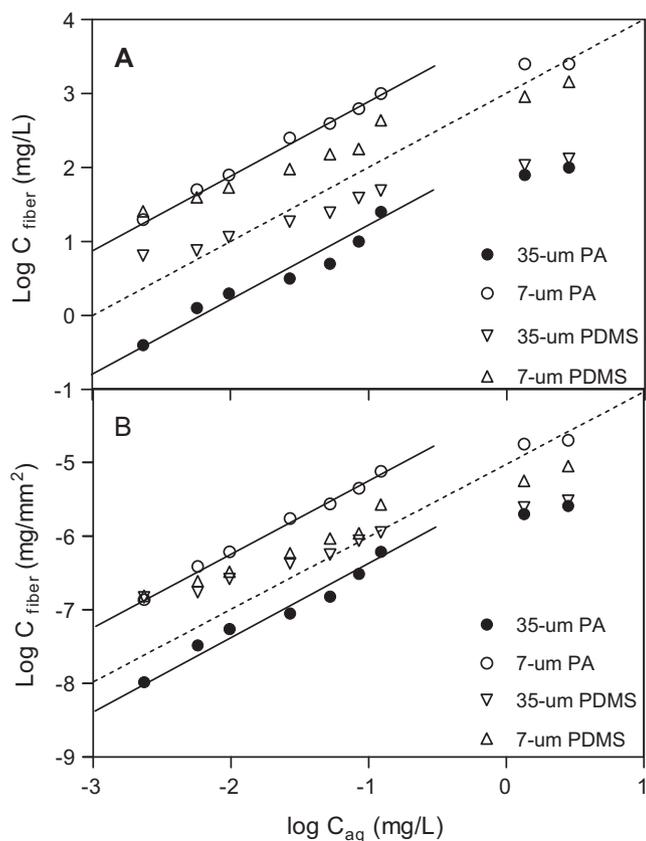


Fig. 1. Sorption isotherms for IV-16 on four types of SPME fiber in 5 mM CaCl_2 solution at pH 5.5 for 2 d. The sorbed amounts of IV-16 are normalized with both fiber volume (A) and fiber surface area (B). The dotted line indicates linearity.

3. Results and discussion

3.1. Optimization extractions and selection of SPME fiber

The analytical test conditions resulted in a sensitive LC–MS/MS method with a LOQ injected amount of ~ 1 pg. SPE recovery of aqueous samples is nearly complete for both IV-16 ($100.3 \pm 3.8\%$) and C12-BAC ($91.7 \pm 3.2\%$). The SPME fiber desorption is complete after a first desorption step with the acidic 90/10 methanol–water mixture because a second SPME fiber desorption step showed no detectable signal.

For PDMS fibers, as shown in Fig. 1, IV-16 sorption isotherms are nonlinear, whereas for PA fibers isotherms are linear in the lower concentration range (< 0.1 mg/L), and thereby provide a constant fiber–water distribution coefficient ($\log D_{\text{fw}}$). Upon surface area normalization of the sorption isotherms, the nonlinear sorption affinity of IV-16 is similar for both PDMS fibers. In contrast, the affinity of both surfactants was higher for the 7- μm PA compared to the 35- μm PA based on volume as well as on surface area. All further tests were therefore performed with this 7- μm PA SPME. Shaking speed during SPME equilibration does not seem to affect the D_{fw} after 2 d, but solutions in all further tests were shaken at 60 rpm (see Table S3).

3.2. Sorption to 7- μm PA SPME: kinetics and sorption isotherms

The effect of exposure time on $\log D_{\text{fw}}$ values are shown in Fig. S1. The concentrations in the fiber reach steady state within 2 d, which was chosen as the standard equilibration time for establishing SPME sorption isotherms for both surfactants. Although initial uptake is very fast ($\sim 45\%$ of steady state within 20 min), this is

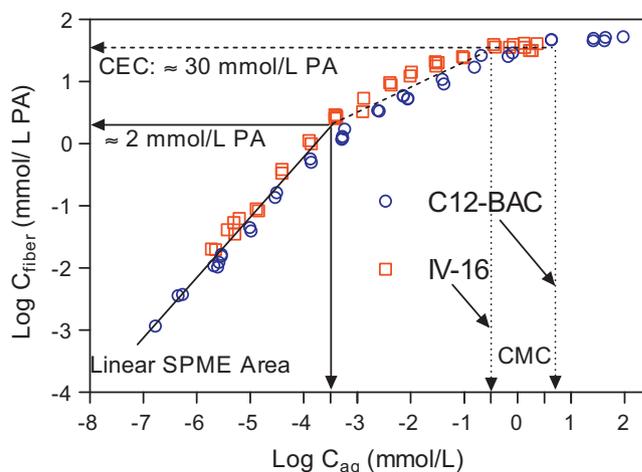


Fig. 2. SPME sorption isotherms covering a broad concentration range were measured in 2-d exposure period for IV-16 and C12-BAC in 5 mM CaCl_2 solution at pH 5.5. The unit is expressed as mmol/L for dissolved and fiber sorbed concentrations. Linear SPME calibration can be applied in the aqueous concentration range from nM to μM (solid line arrows). The maximum fiber concentration is approximately 30 mmol/L PA (broken line arrows), which is very close to the measured CEC of 7- μm PA fibers via the cation exchange method. The CMC values of both chemicals in this solution can also be estimated via the isotherms (dotted line arrows). The solid line indicates linearity.

not much faster than a neutral compound with comparable K_{fw} [26]. The D_{fw} for IV-16 remain relatively constant until the end of the long exposure period (~ 40 d), whereas the uptake for C12-BAC slightly declined after 15 d. The reason for this small decline is unknown. SPME sorption isotherms from 2-d exposure (short term) for C12-BAC and IV-16 are presented in Fig. 2. The tested concentration range covers more than 7 orders of magnitude, from the LC–MS/MS detection limit for SPE samples to above the CMC, and extends to a more than 100 times lower concentration range than the surfactant electrode method [8]. The results of the short and long term (~ 40 d) sorption isotherms (see Fig. S2) agree well with the kinetic data, since the sorption data obtained from long exposure tests are either equal (IV-16) or slightly lower (C12-BAC) than those acquired after 2 d. Fiber measurements vary within 10% and all the duplicate and quadruplicate vials show reproducible results (SD for $\log K_{\text{fw}} < 0.1$) in the 2-d sorption isotherm.

As shown in Fig. 2, the 2-d SPME sorption isotherms for both surfactants can be divided into three distinct regions. In the first region, at aqueous concentrations below $\log C_{\text{aq}} \sim -3.5$ mmol/L (~ 0.1 mg/L), the sorption isotherms are linear for both compounds (slopes in the logarithmic plot are very close to 1). In the linear range of the isotherms, the equilibrated fiber–water sorption coefficients ($\log K_{\text{fw}}$, expressed as liter water per liter PA) in 5 mM CaCl_2 solution (pH 5.5) are 3.92 ± 0.10 ($n = 18$) and 3.65 ± 0.08 ($n = 18$) for IV-16 and C12-BAC, respectively (see Table 1). In the second region, the slope in the logarithmic plot of Fig. 2 is lower than 0.5, indicating that the K_{fw} decreases considerably with increasing aqueous concentrations. This occurs for both compounds above a fiber concentration of about 2 mmol/L PA. Possible explanations for the observed nonlinearity are discussed further on. The nonlinearity implies that the SPME technique is less applicable above the aqueous concentration of 0.1 mg/L for these two surfactants, because minor changes in fiber concentration could lead to relatively large errors in the calculation of the corresponding free aqueous concentration. In the third region, the sorption isotherms level off. The aqueous concentration for IV-16 at which the isotherm starts to level off horizontally (~ 0.33 mM) in 5 mM CaCl_2 matches well with the reported critical micelle concentration (CMC) of 0.3 mM in 10 mM CaCl_2 [40] (3–4 times lower than those in pure water

Table 1

C12-BAC fiber–water sorption coefficients (K_{fw} in liter water per liter PA) for 7- μm PA in the linear range <0.1 mg/L were measured in solutions of different ionic composition and pH. Data are based on single test series exposed for 2 d except the values from 5 mM CaCl_2 pH 5.5 ($n = 18$ for 2 d, and $n = 18$ for 40 d).

Composition	$\log K_{fw}^a \pm \text{SE} (n^b)$	$n_F^c \pm \text{SE}$
5 mM NaCl pH 6.0	$4.06 \pm 0.05 (4)$	0.97 ± 0.06
50 mM NaCl pH 6.0	$3.96 \pm 0.02 (3)$	1.00 ± 0.12
5 mM CaCl_2 pH 6.0	$3.76 \pm 0.08 (4)$	1.06 ± 0.10
50 mM CaCl_2 pH 6.0	$3.40 \pm 0.01 (4)$	0.94 ± 0.03
5 mM CaCl_2 pH 8.0	$4.02 \pm 0.02 (4)$	0.96 ± 0.06
5 mM CaCl_2 pH 6.5	$3.99 \pm 0.03 (4)$	1.08 ± 0.05
5 mM CaCl_2 pH 5.5	$3.65 \pm 0.08 (18^e)$	0.95 ± 0.04
5 mM CaCl_2 pH 4.0	$2.92 \pm 0.01 (4)$	1.01 ± 0.04
5 mM CaCl_2 pH 2.0	$2.00 \pm 0.03 (3)$	0.98 ± 0.17
400 mM NaCl pH 2.0	$2.11 \pm 0.05 (4)$	1.06 ± 0.15
Artificial seawater ^d	$3.76 \pm 0.02 (3)$	1.00 ± 0.06

^a $\log K_{fw}$ derived from $\log K_{fw} = \log(C_f/C_{aq})$, assuming the isotherm is linear.

^b Number of samples determining $\log K_{fw}$.

^c n_F is the fitted slope of the logarithmic Freundlich isotherm, $\log C_f = \log K_f - n_F \cdot \log C_{aq}$ (both C_f and C_{aq} are in mg/L).

^d The composition of artificial seawater is shown in the protocol [38].

^e Data from the completed isotherm in Fig. 1.

[41,42]). As has already been shown for nonionic surfactants [32], SPME fibers extract only freely dissolved surfactant monomers, and the CMC can therefore be derived from the plateau of the sorption isotherm.

3.3. SPME sorption isotherms: effect of solution chemistry

Aqueous solution chemistry is expected to play an important role in the cationic surfactant sorption in general [6,43] and therefore also in the sorption to the PA SPME fiber. Only the linear part of the SPME sorption curves (below a fiber loading of 2 mmol/L PA) is of interest for future application of the SPME method in controlled environmental fate studies. Therefore, the effects of electrolyte concentrations and pH were tested in this concentration range. Sorption isotherms for all electrolyte solutions were linear, and the fitted K_{fw} values of C12-BAC for different solutions are listed in Table 1. Fig. 3A shows that higher electrolyte concentration decreases C12-BAC sorption affinity to 7- μm PA fibers in the pH 6.0 solution. In 50 mM CaCl_2 the K_{fw} is lower (~ 0.36 log units) than in a 5 mM CaCl_2 solution, while the difference of $\log K_{fw}$ measured between 5 mM and 50 mM NaCl is less than 0.1 log units. Furthermore, it was observed that the divalent Ca^{2+} is more competitive than the monovalent Na^+ when sorbed on the SPME PA fiber, since $\log K_{fw}$ values at 5 mM CaCl_2 are 0.2 units lower than those in 50 mM NaCl. Fig. 3B shows that pH can strongly affect the SPME sorption isotherms. The isotherms obtained in 5 mM CaCl_2 solution at pH 6.5 and 8.0 overlap. In contrast, while maintaining the same electrolyte solution, the K_{fw} measured at pH 5.5, 4.0, and 2.0 decreases about 0.4, 1.1 and 2.0 log units, respectively, compared to pH 6.5 or 8.0.

The relatively small effect of Na^+ compared to Ca^{2+} is also observed for the sorption isotherm in artificial seawater (pH 8.2, total concentration of divalent Ca^{2+} and Mg^{2+} is about 50 mM), for which the K_{fw} is about 0.26 log units lower than the 5 mM CaCl_2 solution (pH 8.0), which is comparable with the difference of K_{fw} (0.36 log unit) between 50 mM and 5 mM CaCl_2 measured at pH 6.0 (Table 1). The high Na^+ concentration in seawater (420 mM) apparently has little effect on the K_{fw} . Comparing SPME sorption isotherms at pH 2.0 for 400 mM NaCl and 5 mM CaCl_2 showed that a very high Cl^- concentration also has negligible effects on the affinity of cationic surfactants for the 7- μm PA coating.

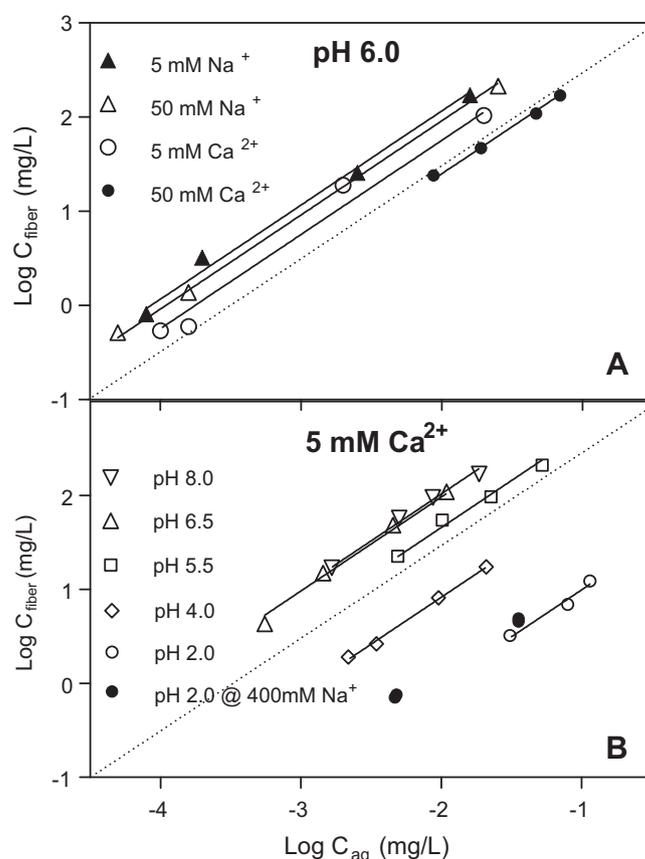


Fig. 3. Effect of electrolyte composition (A) and pH (B) on the C12-BAC sorption to 7- μm PA SPME fiber conditioned at 120 °C. Fiber–water sorption affinity (K_{fw}) decreases with increasing electrolyte concentration and decreasing pH.

3.4. Sorption mechanism for quaternary surfactants on SPME fibers

From the SPME sorption isotherms that cover a broad concentration range and that were established at different salinity and pH, we can derive several conclusions on the sorption mechanism of quaternary ammonium compounds on the SPME coatings. We would like to emphasize that the PA coating in SPME fibers is generally considered a neutral polymer phase in other SPME studies. Also worth mentioning, is that the PA coating is a (patented) complex, mostly esterified acrylic co-polymer, with the intended use only to protect the glassy data-communication fiber from breaking. This PA coating differs substantially from the strongly swelling PA gels, e.g. used in diapers, that only contain ionizable carboxylic acids. All our results suggest that ion-exchange on such carboxylic groups is the driving sorption process for the quaternary ammonium compounds on PA SPME, not partitioning into the polymer. Firstly, at higher CaCl_2 background concentrations, the K_{fw} values of cationic surfactants decrease. Sorption studies with protonated bases and cationic surfactants on humic acids demonstrate that inorganic cations compete with organic cations in a similar way for binding at cation-exchange groups, such as carboxylic acids in humic acid structures [8,44]. As shown for octanol–water systems [43,45], partitioning or absorption mainly occurs as a neutral ion-pair complex, and the K_{fw} should then increase with higher electrolyte concentrations as more anions are available for ion-pairing. Secondly, below pH 6 the K_{fw} values of cationic surfactants decrease. Estimating a pK_a of 4.72 for 2-methylpropanoic acid (SPARC V4.5 [46]), 0.2%, 16% and 95% ionization at pH 2.0, 4.0, and 6.0, is expected, respectively, which convincingly explains the decreasing K_{fw} with lower

pH (Fig. 3A and Table 1). Accordingly, no increase in K_{fw} is expected above pH 6.5. The relatively low affinity at pH 2.0 could be related to the remaining absorption of neutral ion-pair complexes (since most ion-exchange groups on PA should be protonated). However, the $\log K_{fw}$ measured in 400 mM NaCl at pH 2.0 does not differentiate from the $\log K_{fw}$ value measured in 5 mM CaCl_2 solution at pH 2.0 (2.1 vs. 2.0, Table 1). As suggested above, partitioning via ion-pairing with chloride anions does not substantially contribute to the overall SPME affinity of these quaternary compounds. Thirdly, the CEC measurements confirm the presence of negatively charged groups on the 7- μm PA fibers (see Table S2 for CEC values). Moreover, the CEC obtained from either $\text{Ba}^{2+}/\text{Ca}^{2+}$ and K^+/Na^+ exchange methods (both ~ 30 mmol/L PA), corresponds well to the maximum fiber uptake for both cationic surfactants (Fig. 2). In the absence of ion-exchange sites for PDMS coated SPME fibers (measured CEC was 3.4 mmol/L which was much lower than PA and also within the error margin obtained for PA fibers), sorption to PDMS is surprisingly strong compared to PA (Fig. 1). Presumably this is due to the more nonpolar character of PDMS compared to PA, which favors adsorption of the aliphatic tails of surfactants at the PDMS surface. This surface process would also explain overlapping data for the two PDMS fibers when normalized on surface area. Still, due to the undesirable overall nonlinearity of PDMS isotherms, these fibers were not further tested.

The three distinct regions in the full sorption isotherm for 7- μm PA SPME in Fig. 2, suggest the following ideas about the surface processes. At low sorbed concentrations (<2 mmol/L PA, Fig. 2) the isotherm linearity indicates that there is no distinct heterogeneity in sorption ion-exchange sites. At higher sorbed concentrations on the PA fibers (>2 mmol/L PA) resulted in ongoing decrease in affinity. The negative charge arising from the dissociated groups at the PA “surface” attract a higher cation concentrations near the surface compared to the bulk solution, but at sorbed surfactant levels within 10% of the CEC this attraction is being increasingly reduced, thus lowering the K_{fw} . The increase from 5 to 50 mM CaCl_2 may have also resulted in a significantly decreased negative charge on the fiber surface, thereby explaining the lower K_{fw} at e.g. 50 mM compared to 5 mM Ca^{2+} . The leveling-off of the isotherms at ~ 30 mmol surfactant/L PA indicates the maximum exchange capacity of PA, at a considerably lower level than >1000 mmol/kg for humic acids [8]. The SPME sorption isotherm did not show the typical S-curve that is often observed for sorption isotherms on clay minerals [47], fully ionizable poly(acrylic acid) [48] and humic acids [8], where at relatively high sorbed concentrations the packing density is so high that sorbate–sorbate interactions cause a sudden strong increase of the sorption affinity until CMC is reached. We hypothesize that a relatively sparse distribution of charged sites on PA compared to humic acids may be the reason that sorbate–sorbate interactions are limited, and therefore no S-curve is observed. In addition, the much lower CEC of PA compared to HA would also partially explain why the ion-exchange affinity for 7- μm PA SPME fibers is orders of magnitude lower in comparison with the sorption affinity of quaternary surfactants to humic acids, as discussed also below [8,24].

3.5. Competitive sorption of cationic surfactants in mixtures to polyacrylate SPME fibers

Because sorption isotherms are linear at concentrations in PA coating below 2 mmol/L, no interaction between sorbed cationic surfactants is expected due to “decreased surface charge” at total surfactant concentrations below this level of 2 mmol/L PA. We tested whether competition occurs between C12-BAC and the more hydrophobic C16-BAC below and slightly above this concentration of 2 mmol/L PA. The total cationic surfactant concentration of C12-BAC and C16-BAC together in the low concentration mixtures is 0.12 mmol/L PA (Fig. 4) and indeed no effect on the K_{fw} of C12-BAC

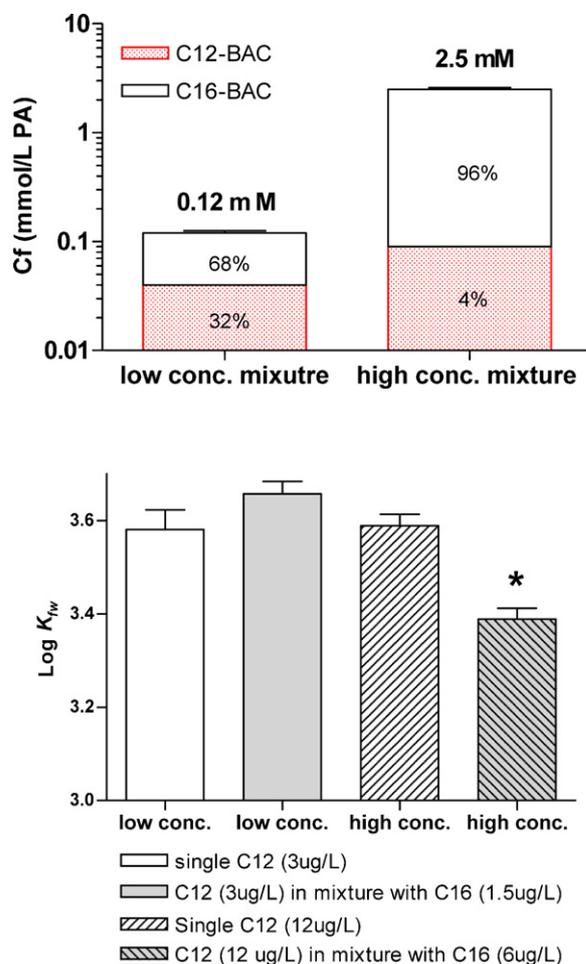


Fig. 4. The total concentrations of C12-BAC and C16-BAC (measured in triplicate). The percentage represents the proportion of individual concentration in the fiber.

was observed. As shown in Fig. 4, only in the mixture with high concentrations the K_{fw} of C12-BAC is significantly reduced (ANOVA, $p=0.0003$) because the total sorbed concentration was 2.5 mmol/L PA (the C12-BAC fiber concentration was still only 0.1 mmol/L PA). Apparently, there is indeed no competition between comparable cationic compounds for ion-exchange sites on PA SPME fiber in the linear SPME range. This suggests that the SPME method can be applied in mixtures of cationic surfactants as long as the total concentration of surfactant mixture in fiber does not exceed 2 mmol/L PA.

3.6. Sorption of quaternary ammonium surfactants to humic acid using ion-exchange SPME

SPE recovery for total extraction of surfactant solutions with Purified Aldrich Humic Acid (PAHA) reaches $96.4 \pm 7.6\%$ ($n=9$), demonstrating negligible degradation and complete mass balance. More than 70% of surfactant in the test systems with 10 mg/L PAHA was sorbed. SPME sorption isotherms obtained in the different electrolyte solutions without PAHA were all linear, as presented in Fig. 3A. Sorption isotherms of C12-BAC to PAHA, however, were slightly non-linear, with Freundlich exponent n_F of 0.79 ± 0.06 ($n=4$) (Fig. 5). As a result, sorption coefficients (K_{oc} , in L/kg organic carbon) increase at lower surfactant concentrations. At the lowest tested concentrations, the sorption experiments demonstrate that $>90\%$ of the compounds in solution were sorbed to the humic acid, which is of high interest for risk assessment issues because 10 mg dissolved organic carbon per liter are field relevant solids

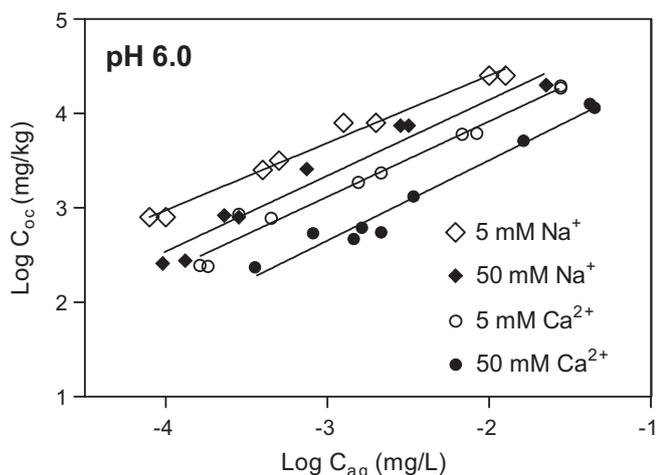


Fig. 5. Sorption isotherms of C12-BAC to PAHA at pH 6.0 solution, measured by SPME, in different NaCl and CaCl₂ concentrations (5 and 50 mM). Sorption isotherms were fitted with Freundlich equation.

Table 2

Sorption coefficients (K_{oc} in L/kg organic carbon) of C12-BAC to purified humic acid and 7- μ m PA fiber (K_{fw}) measured in different electrolyte solutions at pH 6.0. $\log K_{oc}$ were calculated at aqueous concentration of 1 μ g/L.

Electrolyte solution	$\log K_{fw}^a \pm SE (n^b)$	$\log K_{oc}^a \pm sy.x^c (n)$
5 mM NaCl	4.06 \pm 0.05 (4)	6.70 \pm 0.11 (8)
50 mM NaCl	3.96 \pm 0.02 (3)	6.34 \pm 0.15 (9)
5 mM CaCl ₂	3.76 \pm 0.08 (4)	6.12 \pm 0.11 (10)
50 mM CaCl ₂	3.40 \pm 0.01 (4)	5.67 \pm 0.11 (9)

^a $\log K_{fw}$ derived from $\log K_{fw} = \log(C_f/C_{aq})$, assuming the isotherm is linear.

^b Number of samples (plotted in Fig. 3) determining $\log K_{fw}$.

^c Standard deviation of the residuals.

concentrations. $\log K_{oc}$, calculated at 1 μ g/L and at different electrolyte solutions, with the corresponding $\log K_{fw}$, are presented in Table 2. The high sorption coefficient for C12-BAC in 5 mM NaCl (calculated $\log K_{oc} \approx 6.8$ at 1 nM) are in the same range as sorption coefficients determined for a comparable quaternary ammonium surfactant such as hexadecylpyridinium ($\log K_{oc} \approx 7.0$ at 1 nM) at similar solid concentrations of PAHA and in 5 mM NaCl [8]. Similar as the effects of salts on the affinity of cationic surfactants to PA in the SPME fiber sorption tests, sorption of C12-BAC to PAHA also decrease with increasing salt concentrations, as was observed also in sorption studies with other cationic surfactants and ionized bases [8]. The relatively stronger effect of Ca²⁺ on the sorption of cationic surfactant suggests that Ca²⁺ has a higher specific affinity for ion-exchange sites on PAHA compared to Na⁺ [49,50].

4. Conclusions

The 7- μ m PA SPME method can be a sensitive and suitable tool for measuring freely dissolved quaternary ammonium surfactants, for IV-16 and C12-BAC in a range between 0.1 μ g/L and 0.1 mg/L. This range covers the highest concentrations measured in environmental monitoring (total concentration of 9.9 μ g/L and 1.9 μ g/L in surface waters in Austria for IV-16 and C12-BAC, respectively [16]) as well as concentrations that show toxicity to aquatic organisms (For IV-16 *Daphnia magna* 48-h EC50 is 10 μ g/L [51]).

As shown in this study, if one is to use 7- μ m PA SPME to determine freely dissolved concentrations of cationic surfactants, one has to carefully assess the effects of the medium composition, especially the divalent cation concentration, as well as the potential influence of the presence of other competing chemicals. In sorption studies with a defined medium, the SPME calibration and the

sorption test itself should be performed in the same medium. In application to field samples (e.g. waste water effluent or soil pore water) this is of course more complex. Calibration with labeled test compounds could possibly be an option. It is clear that in laboratory based studies, such as sorption and bioaccumulation tests with a single contaminant, calibration can easily be performed in the same test solution as applied in the sorption.

Although the SPME method has some limitations, it is one of the few methods that enable the measurement of freely dissolved concentrations of cationic surfactants and it is more sensitive than the existing procedure based on a potentiometric titration method.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.chroma.2012.06.080>.

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Annex 3

Wang, F.; Chen, Y.; Hermens, J.L.M.; Droge, S.T.J. Evaluation of both neutral and ion-exchange SPME fiber coatings to extract the basic surfactant lauryl diethanolamine: measurements of pKa and Koc. draft manuscript.

**Evaluation of both neutral and ion-exchange SPME
fiber coatings to extract the basic surfactant
lauryl diethanolamine: measurements of pK_a and K_{OC} .**

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(4100 words in total, 5 figures, 1 table, 2 figures for supporting information?)

Abstract

25 A passive sampler tool (SPME) was optimized to measure freely dissolved concentrations of lauryl diethanolamine (C12-DEA). C12-DEA can be protonated and act as a cationic surfactant in the environment after waste water disposal. Measurements with the neutral polymer SPME coating polyacrylate, which only takes up neutral C12-DEA, in solutions of different pH suggest a pKa around 8.5, much higher than previously recorded pKa of 6.24.
30 Sorption isotherms with neutral polymers (PDMS, polyacrylate) showed a strongly increased affinity of C12-DEA between pH 8 and 10. As a result of the even lower affinity at pH6, it was difficult to determine the sorption affinity of C12-DEA to dissolved organic matter at that pH. A prototype SPME phase with a silica based coating of both hydrophobic (C18) and strong cation exchange (propylsulfonic acid, SCX) properties showed thousand-fold stronger
35 affinity of protonated C12-DEA, and a tenfold stronger affinity of neutral C12-DEA. This extraction was linear in a concentration range of 1nM – 1 μM, well below the CMC. Using

the C18/SCX fiber, sorption to aldrich humic acid was observed to be linear over a broad concentration range with a sorption coefficient of 5.0 log units.

1. Introduction

The aim of this study is to develop a solid phase microextraction method to measure freely dissolved concentrations of the cationic surfactant lauryl diethanolamine (*N*-(*n*-dodecyl)diethanol-amine, or C12-DEA). The freely dissolved concentration ($C_{w,free}$) of a compound controls many processes important for technical applications and environmental processes, such as evaporation, reactivity, precipitation, sorption, biodegradation, bioconcentration and toxicity. Therefore, $C_{w,free}$ is a relevant parameter in environmental chemistry, pharmacology and toxicology. The general purposes of *N*-alkyldiethanolamines are as antistatic agent and cosmetic ingredient (hair conditioning), and fractions of the total consumed tonnage may enter environmental systems via waste water effluents. C12-DEA has also been used as a bacteriostatic compound in tests with *Escherichia coli*^{1,2} One study reported a pK_a of 6.4 determined by titration (at 25°C), and critical micelle concentration (CMC) of 1-1.5 mM in 0.1 M buffers of pH 4.6 and 7.0 (both 25°C), respectively. The presence of the polar ethanol moieties and the relatively low pK_a of C12-DEA may require specific sampler material for the analysis of a broad range of $C_{w,free}$ at a pH either above or below the pK_a .

The solid phase microextraction (SPME) method has been developed in the early 1990s as a passive sampling tool to facilitate the determination of $C_{w,free}$ for volatile and semi-volatile nonionic organics in diverse matrices³. Since the early development, SPME fibers were fused silica cores with coatings of different polarity and porosity. The polymers commonly used for SPME are either homogenous phases, such as the nonpolar polydimethylsiloxane (PDMS) and the polar polyacrylate (PA), or porous crystalline adsorbent phases embedded in homogenous phases, such as PDMS-DVB, carbowax-DVB, PDMS-Carboxen⁴. Dissolved organic structures that are mostly ionized at the tested pH, or which contain permanently charged moieties, are difficult to extract by common SPME coatings, because the affinity of the ionic species to neutral polymers is orders of magnitude lower than the neutral species. Modifying the pH of the solution or adding derivatization agents may increase the neutral fraction and thereby enhance extraction efficiency,^{5,6} but this may not be the desired procedure in many test systems if it disturbs the actual distribution of the chemical. Polyacrylate SPME fibers have recently been used to measure the $C_{w,free}$ of the basic drug chlorpromazine at pH 7.4, where the neutral fraction is less than 1%, because the affinity of the neutral species is sufficiently high to yield low detection limits⁷. Permanently charged quaternary ammonium surfactants can also be extracted with PA SPME fibers, but only with disposable 7- μ m PA fibers, because these appear to have a sufficiently high density of cation-exchange sites in/on the polymer to sorb these hydrophobic cations⁸. This may also be the

reason why disposable 7- μm PA fibers worked well in calcium rich solutions for the analysis of anionic surfactants⁹. Still, these PA fibers are not sufficiently sensitive for the direct analysis of small and polar organic ions.

80 Recently, a new way of creating SPME fibers has been developed, with (biocompatible) stainless steel wires and a wide variety of solid phase extraction (SPE) materials, as coatings of porous silica based particles bound with a polymeric binder^{10,11}. Including ion-exchange based SPE material on SPME samplers provides clear opportunities to facilitate extraction of $C_{w,\text{free}}$ of ionogenic compounds directly in test medium. Using a prototype SPME phase based on SPE material with a mixed-mode coating of hydrophobic C_{18} and propylsulfonic acid
85 (C18/SCX) yielded much higher extractions for the more than 99% protonated drug amphetamine in physiological pH than PA-fibers¹². Figure 1 shows a graphical interpretation of the SPME types and their most outstandingly different properties used in this study for the basic compound C12-DEA.

The first objective of the present study was to apply the SPME method with polyacrylate
90 coating to verify the pK_a , in order to decide which pH range to test to examine the functionality of different fiber coatings. Disposable 35- μm PA coated fibers were selected, because it was expected that this polymer specifically sorbed neutral species so that a clear relation would be obtained between affinity and ionisation, and PA was expected to be more efficient than the non-polar PDMS. The second objective was to determine the most efficient
95 SPME fiber for use with C12-DEA in environmentally relevant research conditions, e.g. sorption studies, and biodegradation and toxicity tests. Uptake kinetics at three pH with different fractions of ionisation were then studied for PA, PDMS and C18/SCX fibers, in order to establish equilibration times and measure sorption isotherms over broad concentration ranges in fully equilibrated fibers. The effect of differences ionic strength on
100 sorption affinity to PA and C18/SCX fibers were studied at the pH where >99% of C12-DEA was ionized, to elucidate the differences in sorption processes. The final goal was to use the optimized SPME tool to determine the sorption affinity of C12-DEA to dissolved organic matter, by analysis of $C_{w,\text{free}}$ and a mass balance approach, in order to compare the sorptive properties with other cationic surfactants we worked on other studies⁸. (Add new refs Yi
105 Chen)

2. Materials and methods

2.1. Chemicals and materials

110 SPME fibers with a 110 μm diameter fused silica core coated with 30- μm poly-(dimethylsiloxane) (PDMS) coating (volume 13.2 $\mu\text{L}/\text{m}$), 35- μm polyacrylate (PA) coating (15.4 $\mu\text{L}/\text{m}$), and a 7- μm PA coating (2.6 $\mu\text{L}/\text{m}$) were purchased from Polymicro

Technologies (Phoenix, AZ, USA). From these fiber-batches of originally >200 m length, we used a guillotine paper-cutter to cut batches of ~50 pieces to the desired length (generally 30-50 mm, depending on the expected partitioning coefficient) and further used as received as disposable fibers. C18/SCX mixed-mode fibers were kindly provided by Supelco, Sigma Aldrich (Bellefonte, PA, USA). These fibers consisted of 3 cm pieces of Nitinol wire with a 1.5 cm coating length at an average thickness of 45 μm (2.44 $\mu\text{L}/\text{m}$)¹². The coating was prepared with HPLC column grade silica based material bound on the wire with a biocompatible polymeric binder.

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120 N-dodecyl-N,N-diethanol amine (C12-DEA, $\geq 98.0\%$, CAS No. 1541-67-9, Mw 273.45) was provided by Akzo Nobel N.V. (Arnhem, the Netherland). The salts for buffer solutions ($\text{H}_3\text{PO}_4/\text{NaH}_2\text{PO}_4$ for pH 2, acetic acid(HAc)/NaAc for pH 4, $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ for pH 6/7/8, $\text{NaHCO}_3/\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$ for pH 9/10, and $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}/\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$ for pH 11/12) were all from Merck, Darmstadt, Germany. The 10 mM buffers were prepared in

125 highly pure deionized water with $R \geq 18 \text{ M}\Omega\text{cm}$ (Millipore Waters, Amsterdam, The Netherlands). Sodium azide (Merck) was added at 5 mM to all test media to prevent biodegradation, resulting in a total Na^+ concentration of 15 mM in most test solutions. Standard surfactant stocks were prepared with analytical methanol (BioSolve, Valkenswaard, The Netherlands) and stored at $-20 \text{ }^\circ\text{C}$. Aldrich Humic acid (AHA, Cat. No. H1675-2) was

130 acquired from Sigma-Aldrich (Zwijndrecht, The Netherlands), and purified in the similar batch as used in an earlier study⁸. All tests were performed at room temperature (20°C).

2.2. *pKa determination with PA-SPME*

Single vials with nominal concentrations of 0.2, 2 and 25 mg/L C12-DEA were prepared in

135 24 mL vials for each different buffer solution in the range of pH 2-11.

A single PA fiber (50 mm for pH2-8, 30 mm for pH8-11) was exposed in each solution for 3 d, after which the fiber was taken out with tweezers, blotted dry on a tissue, and desorbed in a 60/40 methanol/water mixture with 0.2% acetic acid (0.1 mL for pH2-8, 1 mL for pH9-11). Samples were stored at $-20 \text{ }^\circ\text{C}$ until analysis.

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2.3. *SPME fiber extraction sensitivity optimization*

The fiber-uptake kinetics were determined for 35- μm PA and 30- μm PDMS for 0.46 mg/L (1.7 μM) C12-DEA solutions in buffers of pH 6, 8, and 10. The kinetics for the C18/SCX fiber was determined for pH 6. Since pilot samples showed full recovery for water samples

145 (data not shown), aqueous concentrations were not measured. Single PA and PDMS fibers were exposed individually in 20-mL solutions and taken out after 0.25, 0.5, 1, 2, 4, 10, 24, 48, and 72 h, and further handled and desorbed as described above. C18/SCX fibers were exposed individually in 290 mL solutions of 0.43 $\mu\text{g}/\text{L}$ (1.6 nM), and extracted in 120 μL of

90/10 methanol/water, with 0.1% NH₄ in the Millipore water (pH 11), in a 200 µL glass insert.
150 After 4 h, this was acidified to pH 3 with 60 µL of 0.1 M HCl solution, and the HPLC-vial
was vortexed upside down to improve mixing. Desorbed C18/SCX fibers were considered
(and verified) clean and re-used several times, stored in Millipore water in between
experiments.

Sorption isotherms to the different fibers were determined at pH 6 and pH 10, at which C12-
155 DEA should be >95% ionized or >95% neutral, according to the pK_a determination test with
PA-fibers described above. Initial SPME calibration isotherms for the 35-µm PA and 30-µm
PDMS at both pH 6 and 10 (in 20 mL solutions) ranged over several orders of magnitude at
aqueous concentration well below the expected CMC of 1 mM¹. Since the uptake curves
already indicated a slightly higher affinity for PA than for PDMS, additional measurements
160 were made only for 35-µm PA down to detection limits of the fiber extracts. Higher sorption
affinities at pH 6 were expected for the 7-µm PA fibers and C18/SCX fibers due to the
additional sorption of cationic species at anionic sorption sites, which would allow lower
detection limits. Tested concentration ranges at pH 6 and pH 10 for 7-µm PA and C18/SCX
were 10⁻⁵-10⁻¹ mM (in 24 mL solutions) and 10⁻⁷-10⁻⁴ mM (in 290 mL solutions), respectively.
165 The influence of ionic strength on sorption to SPME fibers at pH 6, with most of C12-DEA as
cationic species, was tested with 35-µm PA as neutral 'reference' polymer and C18/SCX as
ion-exchange polymer. Single measurements were made for several concentrations, in a
similar range for the fiber types as above, in 5 mM sodium buffer with addition of either 10
mM or 100 mM NaCl. Samples were always 24 mL for 35-µm PA, and 250 and 100 mL for
170 C18/SCX in NaCl solutions of 10 mM and 100 mM, respectively.

2.4. Sorption to humic acid

Initial sorption experiments with C12-DEA to Aldrich humic acid (AHA) were performed
with 35-µm PA fibers at pH 6 and pH 10 (10 mM buffers). A stock solution of 1 g/L AHA
175 was used to prepare 15 mg/L AHA solutions for tests with 35-µm PA. Measurements of the
free concentrations were performed to the lowest detection limits of SPME extracts. The PA
fiber extraction was not further optimized in terms of higher volume of PA extraction phase
or decreased desorption volumes, because this was not expected to increase sensitivity by
more than a factor of three. Regarding the much higher affinity of C12-DEA for C18/SCX,
180 additional series of experiments were then performed with C18/SCX SPME fibers to
determine the sorption behavior to AHA at even lower concentrations. Using sample volumes
of 110 mL, 90 mg/L AHA solutions were spiked and C_{free,w} measured with C18/SCX as
described above. The amount of methanol spiked from stock solutions was always less than
0.5% of the total sample volume. Concentrations of C12-DEA sorbed to AHA were
185 determined assuming 100% mass balance, using the measured C_f and calculated C_{w,free}.

Mass balance calculations were only considered if the AHA sorbed fraction was more than 30%.

2.5. Analysis

190 All samples were analyzed by LC-MS/MS, using a MDS Sciex API 3000 (Applied Biosystems, Foster City, CA) with Turbo Ion spray set in positive ion mode at 400 °C, scanning for m/z 274.1/88.0. C12-DEA was separated from the solvent on a RP-Luna C18(2) column (Phenomenex, Torrance, CA) with isocratic eluent of 60% methanol and 40% water with 0.2% acetic acid. Repeated injection of external calibration samples controlled response
195 drift of the MS from calibration curves within 5%.

All data was analyzed using Graph Pad Prism 6. First-order kinetic curves were fitted to the uptake curves. Generic exponential sorption isotherms (Freundlich equation) were plotted to all sorption isotherms with log-transformed data. By plotting sorption coefficients to PA ($K_{fw,PA35}$) on a normal scale Y-axis against pH, a sigmoidal curve was plotted and the pK_a of
200 C12-DEA was determined as this is the pH at which 50% of the maximum $K_{fw,PA35}$ (at high pH) is reached.

Results and discussion

3.1. pK_a determination of C12-DEA with 35- μ m PA-SPME

205 Figure S1 in the Supporting Information (SI) plots the measured C12-DEA concentration on the PA-SPME fiber, which were exposed for 3 d, at each pH at three different concentrations. All tested concentrations were at least a factor of 10 below the reported CMC for both protonated and neutral species, according to ref ¹. Sorption to PA increased for all pH at higher aqueous concentrations. Sorption did not increase linearly for the three tested
210 concentrations at each pH, and as a result the sorption coefficients ($K_{fw,PA35}$) appeared to be higher at lower tested concentrations. This may partially be due to the limited amount of data. Extended sorption isotherm data for 35- μ m PA at pH6, 8, and 10, indicated with smaller symbols in Figure S2, showed that sorption isotherms should be close to linearity, at least for the lower two test concentrations. Overall, it is evident that sorption to PA is fairly constant
215 from pH 9 and higher, and gradually decreases with lower pH to orders of magnitude lower. The specific affinity of cationic species for the PA phase cannot be determined, since $K_{fw,PA30}$ plotted on a logarithmic scale in Fig.1 does not reach a plateau at low pH as would be expected at a pH sufficiently below the pK_a .

The plot with $K_{fw,PA35}$ on a normal scale indicates an apparent pK_a (50% of the maximum
220 $K_{fw,PA35}$) for C12-DEA of about pH 8.5. This is considerably higher than the pK_a of 6.4 determined by titration and detection by complexation with dyes, as reported by Lambert and Smith in 1977¹. It is not clear what caused this factor 100 difference in proton association

constant. It appears that the reported pK_a was also measured at room temperature (25°C), and the higher buffer of 0.1M compared to our 5 mM is not likely to have caused this shift. The
225 1977 study did report only a notably small difference in measured octanol-water distribution
coefficients ($\log D_{ow}$) of 1.8 log units between pH4 and 8, perhaps an indication that even
higher ($\log D_{ow}$) would have been obtained at higher pH values than 8. On the other hand, the
testing of $\log D_{ow}$ with cationic surfactants by a shaking method is commonly regarded as
insupportable,¹³ because of emulsifying properties and tendencies of surfactants to
230 accumulate at interfaces between water and solvent. Defining the pK_a with good accuracy was
considered essential for additional SPME testing with C12-DEA. Peltenburg et al. recently
performed the same 35- μ m PA SPME method to determine the pK_a for amphetamine and
found a value of 9.5, against reported 9.9,¹² suggesting the SPME- pK_a method to be an
appropriate indicator for C12-DEA, too.

235 Additional evidence for our finding of a pK_a higher than 8 for C12-DEA was observed in the
fiber uptake curves from a constant dissolved concentration (0.1mM) at pH 6, 8, and 10, as
shown in SI-Figure S2. For both PA and PDMS, the fiber concentrations over the whole
uptake curve are considerably higher at pH 10 than at pH 8, indicating that the fraction of
neutral species is not equal in these two solutions. The uptake curves further show that the
240 fibers are equilibrated within 24-48 h, so the 3 d exposure of fibers in the broad pH range
should have allowed for sufficient equilibration.

Several tools exist to estimate the pK_a of ionizable compounds from the molecular structure.
The online tool SPARC, co-developed by the US-Environmental Protection Agency and
calibrated by a vast amount of data, predicts a pK_a of 6.24 for C12-DEA, supporting the
245 reported pK_a . However, smaller N-alkyldiethanolamine structures with smaller alkyl chains
are predicted higher pK_a values, e.g. 8.04 for n-octyldiethanolamine, and 8.77 for n-
butyldiethanolamine (and pK_a drops to 5.7 for the C₁₆-compound). The factor ~100 difference
for a C₈- and a C₁₂-alkylated structure does not make sense mechanistically, and we suspect
an artifact in the calibration set, perhaps due to the value reported for C12-DEA in 1977.

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3.2 SPME fiber extraction sensitivity optimization

Figure 3 plots sorption data of C12-DEA to the four different fibers at pH 6 and pH 10, with
sorbed concentrations based on volume of the used SPME coatings. This may not represent
the volume of the actual sorption phases, as shown in Figure 1. In addition to neutral
255 partitioning, (ionized) surfactants may also accumulate on surfaces of the homogenous
polymers, cationic species may favorably sorb to ion-exchange sites on 7- μ m PA, and the
C18/SCX extraction phase is actually a coated layer on porous silica particles. The fitted
Freundlich parameters are presented in Table 1. There is little doubt that C12-DEA exists
only as neutral species at pH 10. At this pH, the volume-normalized sorption affinities to the

260 two PA thicknesses are similar, and only slightly higher than for PDMS. Apparently, the
expected polarity of the two ethanol groups and the neutral nitrogen moiety makes not much
difference in the general affinity to polar PA or non-polar PDMS. The affinity of neutral C12-
DEA to C18/SCX is even higher than to a comparable volume of PA, even though the actual
C18/SCX phase must be considerably smaller. This was also observed for neutral
265 amphetamine¹². At pH 6, the sorption affinity to 35- μ m PA and PDMS decreases 2-2.5
orders of magnitude compared to pH 10. In contrast, the sorption affinity to 7- μ m PA
decreases only 1 order of magnitude between pH 10-6, once more demonstrating the presence
of additional ion-exchange sites on the surface of this fiber material,^{8,12} which allows for
limited extraction efficiency of the cationic C12-DEA species. Still, the gained sensitivity due
270 to this increased affinity compared to 35- μ m PA is limited, because the (carboxylic) ion-
exchange sites become protonated below pH 6, and the sorption affinity is still too low for
measurements at pH 6 at concentrations below 20 μ g/L. The prototype C18/SCX-SPME
fibers (Supelco) are very efficient in extracting the cationic C12-DEA, even slightly better
than the neutral species. The advantage is a lower detection limit in measurements of $C_{w,free}$ of
275 a factor of 1000 at pH6 compared to the common homogeneous polar polymer that only
extracts neutral species. A first disadvantage of C18/SCX is that considerably larger sample
volumes should be deployed if one wants to ensure negligible depletion of dissolved C12-
DEA upon introduction of this SPME fiber into a sample. Secondly, although the current data
show linear isotherms for C12-DEA (Freundlich exponent \sim 1.0), our previous work with
280 amphetamine extraction by C18/SCX showed increased nonlinearity higher concentration
range,¹² although this does not necessarily limit the C18/SCX-SPME applicability in the
range between 1 μ M and the CMC at 1 mM. A third disadvantage of an ion-exchange based
extraction tool is that the sorption affinity may be influenced by other dissolved cations, either
organic or inorganic. We tested only the difference of a ten times increased sodium content,
285 as shown in Figure 4. As expected, no effect was observed for 35- μ m PA, because it extracts
only the neutral species and strong salting out effects are not expected at 100 mM salt.
Surprisingly, we also noted hardly any effect for C12-DEA ($K_{fw,C18/SCX}$ of 5.00 and 5.06)
whereas the C18/SCX sorption affinity of protonated amphetamine was decreased by a factor
of 3. The study on amphetamine did not find specific effects of Ca^{2+} compared to equal ionic
290 strength solutions with Na^+ , although K^+ showed a two-fold stronger reduction of
amphetamine sorption affinity compared to Na^+ ¹². Apparently, inorganic medium
electrolytes, including H^+ , are not expected to affect the extraction efficiency of C12-DEA by
C18/SCX more than 2-5 fold. The small influence of electrolytes does require one needs to
calibrate the SPME isotherm in every test medium. The current study used the same 15 mM
295 buffers for pH 6 and pH 10 to determine the sorption of C12-DEA to humic acid, which

cannot be readily filtered or centrifuged out of solution to obtain measurements of $C_{w,free}$, and the optimized SPME method for C12-DEA was tested for its applicability in this matrix.

3.4 C12-DEA sorption onto Aldrich humic acid

300 Figure 5 shows the sorption isotherms of C12-DEA to Aldrich humic acid (AHA) measured by 35- μ m PA in a pilot experiment and C18/SCX-SPME fiber in an extended measurement series. The 35- μ m PA could only be used for freely dissolved C12-DEA concentrations above 0.1 μ M (0.3 mg/L), corresponding to AHA sorbed concentrations of 10 mmol/kg dry weight (2.7 g/kg). The sorption affinity of C12-DEA at pH 6 to AHA in this concentration range was
305 already so high that in the suspensions of 15 mg AHA/L the sorbed fraction ranged 35-80%. In this relatively high concentration range at pH 6, the limited amount of pilot data suggested AHA sorption isotherm was strongly nonlinear, with a Freundlich exponent (n_F) of 0.43 (see Table 1).

For risk assessment purposes, it is important to know whether obtained sorption data can be
310 extrapolated to lower concentration ranges. It was therefore important to confirm sorption affinities in lower concentrations range, and therefore the C18/SCX-SPME was of value. Using C18/SCX measurements of the free concentration in the range of 1-200 nM C12-DEA showed a nearly linear sorption isotherm ($n_F=0.98 \pm 0.04$), comparable to other cationic surfactants to humic acid,⁸ and the C18/SCX-SPME data unites with the PA data for high
315 concentrations. Fitting the C18/SCX-SPME data with a linear sorption isotherm shows a logarithmic AHA sorption coefficient of 5.12 (95% c.i. 5.03-5.22). Although the dominant sorption sites in AHA for C12-DEA at pH 6 are most likely cation-exchange sites, such as carboxylic acids, sorption coefficients based on organic carbon content (K_{OC}) facilitate comparisons of sorption affinities with neutral contaminants. Applying a organic carbon
320 content of 0.59 for AHA,⁸ gives a log K_{OC} of 5.34. This is considerably lower than the log K_{OC} of 6.8 determined on the same AHA for C12-benzalkonium, a quaternary ammonium compound with a dodecyl-chain, in 5 mM NaCl solution at pH 6⁸. The value for C12-DEA is closer to the log K_{OC} of 5.8 determined *in our lab* for the primary amine n-dodecylamine (unpublished data).

325 Using the PA fibers, some pilot measurements were also made for AHA solutions at pH 10 (Figure 5). Whereas it is difficult to examine the sorption to AHA for fully neutral n-dodecylamine, because the pK_a for that compound is 10.6, our findings for C12-DEA suggest a comparable Log K_{OC} for the neutral amine species compared to the protonated amine species (at high concentrations). Similar findings for neutral and protonated bases were shown
330 recently for a series of smaller amines,¹⁴ suggesting this may be a rule of thumb for an initial estimate of the sorption affinity of cationic surfactants to humic organic matter. However, that

study also showed that the clear differences increments of sorption coefficients due to specific molecular moieties, such as -CH₂- and -OH, when neutral partitioning of the free base compared to the ion-exchange affinity of the protonated base. In the case of protonated C12-DEA, it seems that the lower increment of -CH₂- is balanced by a much smaller effect of the OH groups compared to neutral partitioning. The study of Droge and Goss¹⁴ indeed showed that protonated 2,2'-(benzylimino)-diethanol (“T28”), the small analogue of C12-DEA, sorbed as strong as protonated N-benzyl dimethylamine (“T20”), a comparable tertiary amine structure without OH moieties (at pH4.5 a log *K*_{OC} of 2.18 and 2.20, respectively).

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350 **Appendix A. Supplementary data** Supplementary data associated with this article can be found, in the online version, at ...

Table 1. Fitted Freundlich parameters (K_F and n_F ^a) for C12-DEA sorption onto different fibers and Aldrich humic acid.

	pH	LogK_F (1 μM)	95% C.i.	n_F	Sy.x	test range C_w in μM	N^b
C18/SCX ^a	pH 6	4.98	4.77- 5.19	1.01	0.09	0.0003- 0.15	6
	pH 10	4.85	4.75- 4.94	1.03	0.04	0.0003- 0.15	6
PA 7 μ m	pH 6	2.55	2.17- 2.93	0.99	0.12	0.03-100	5
	pH 10	3.79	3.54- 4.05	0.99	0.01	0.03-3.5	3
PA 35 μ m	pH 6	1.07	0.9-1.24	0.99	0.14	0.5-8000	8
	pH 10	3.63	3.57-3.7	0.76	0.07	0.03-100	9
PDMS 30 μ m	pH 6	0.28	0.17-0.4	0.67	0.02	1-100	5
	pH 10	2.50	2.28- 2.71	0.73	0.08	0.03-100	9
Aldrich Humic Acid (AHA) ^c	pH 6	5.16	5.08- 5.26	0.89	0.08	0.0001- 0.10	8

^a Using the logarithmic Freundlich equation $\text{Log}C_f = n_F \cdot \text{log}C_w + \text{log}K_F$; ^b Number of data points; ^c 95% c.i. and Sy.x for the sorbed concentration of 1 mmol/kg dw.

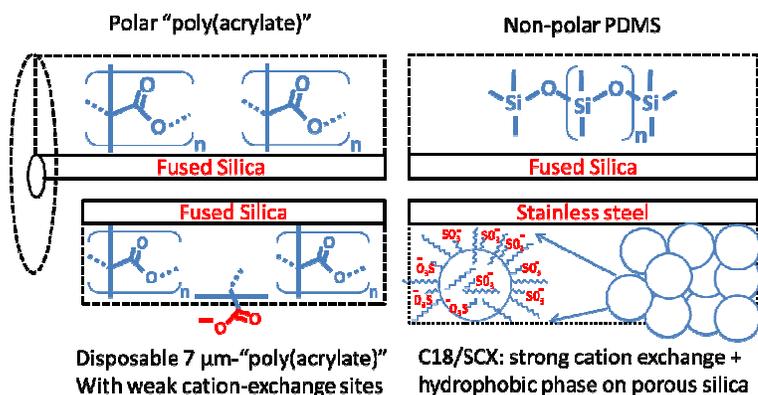


Figure 1. Schematic overview of the different SPME coatings used in this study. Polyacrylate is a poorly defined acrylate co-polymer with a moderate degree of cross-linking⁴. The cation-exchange site density on 7- μm PA was measured to be 30 mmol/L PA,⁸ but it has not been verified that this comes from carboxylic acids, nor that they only occur on the surface. It is also not clear to what extent the polymeric binder is present as an extraction phase in the C18/SCX coating.

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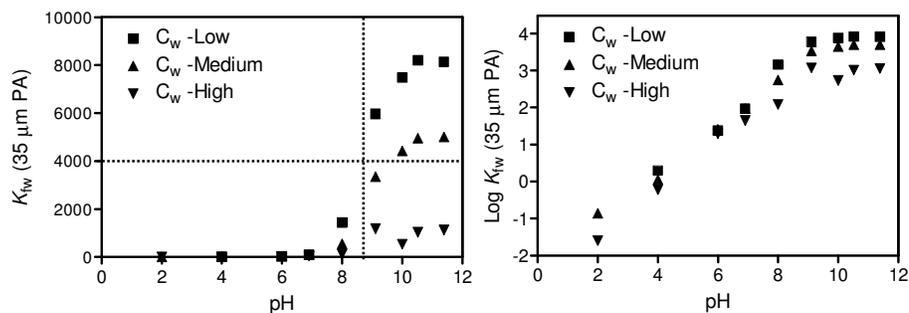


Figure 2. Sorption coefficients of C12-DEA to 35- μm PA SPME fiber at different pH, measured for different concentrations (0.2, 2, and 25 mg/L), plotted on a normal scale (left) and logarithmic scale (right). Dotted lines indicate the level of 50% $K_{fw,max}$ for low concentrations, and the corresponding apparent $\text{p}K_a$.

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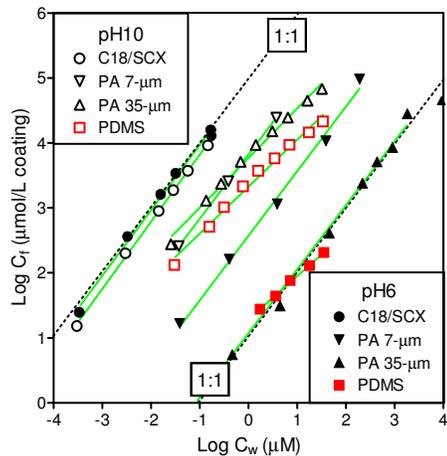
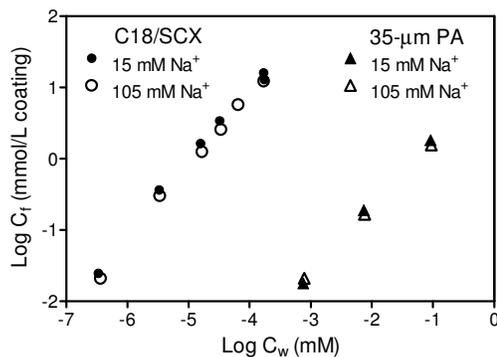
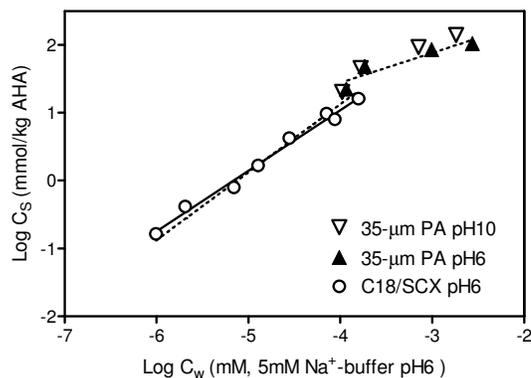


Figure 3. C12-DEA sorption onto different fibers at pH 6 and pH 10



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Figure 4. C12-DEA sorption to 35- μ m PA and C18/SCX-SPME fiber at different ionic strength.



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Figure 5. C12-DEA sorption onto Aldrich humic acid (AHA), with freely dissolved concentrations measured by 35- μ m PA and C18/SCX-SPME and sorbed concentrations calculated using a mass balance approach. The broken line is the Freundlich curve for the

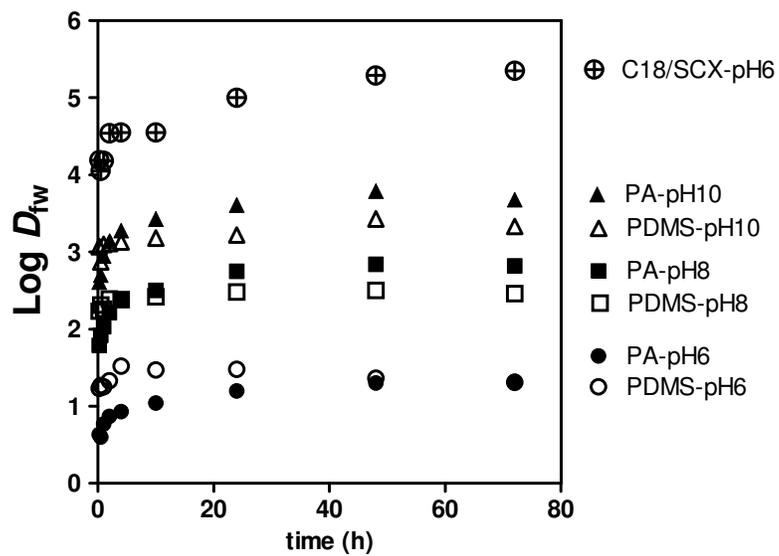
high concentration range measured by PA, the other lines are linear and Freundlich curves for the C18/SCX-SPME data.

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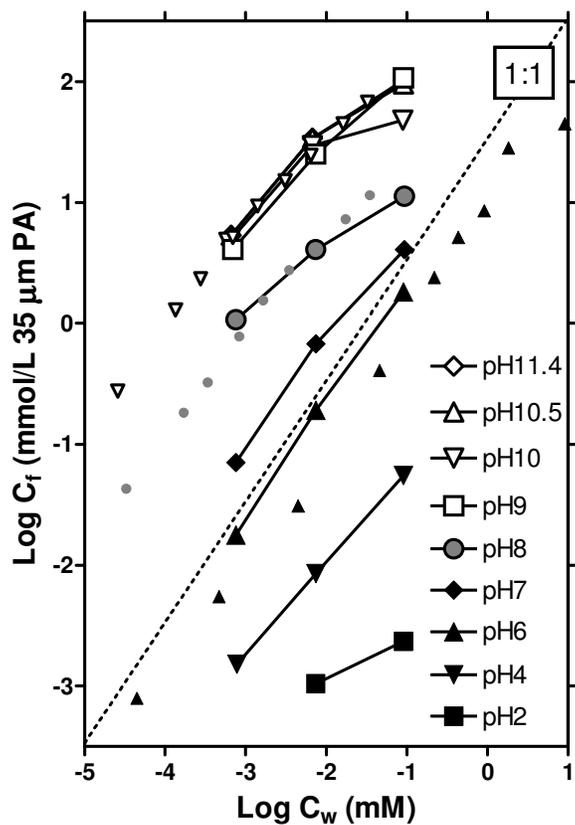
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Supporting Information



405 **Figure S1.** Kinetics of C12-DEA sorption onto different fibers at different pH



410 **Figure S2.** C12-DEA sorption data to 35- $\mu\text{m PA}$ at different pH. Small symbols represent extended measurement series for pH 10, 8 and 6. The slope of the dotted line indicates linearity.

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Annex 4

Chen, Y.; Hermens, J.L.M.; Droge, S.T.J. Influence of organic matter type and medium composition on the sorption affinity of C12-benzalkonium. Submitted for publication.

Influence of organic matter type and medium composition on the sorption affinity of C12-benzalkonium.

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Abstract (258 words)

10 We used 7- μm polyacrylate coated silica fibers as an efficient ion-exchange SPME tool to investigate the sorption behaviour of C12-benzalkonium (C12-BAC, benzyldimethyldodecylammonium chloride) to natural organic matter at concentrations well below the cation-exchange capacity (CEC). SPME measurements at lowest test concentrations overlap with the upper limits of environmental monitoring concentrations. Sorption affinities of C12-BAC
15 under constant medium conditions differed within 0.4 log units for two humic acids (Aldrich, leonardite) and peat samples (Sphagnum, Pahokee), and all sorbents showed a similar slight isotherm nonlinearity. The effect of the composition of the aqueous solution, including pH and various concentrations of Na^+ or Ca^{2+} , on sorption to 10 mg/L Aldrich humic acid (AHA) was systematically studied. Sorption to the polyacrylate coating of the SPME fibers as well as
20 to AHA was reduced at both low pH and high electrolyte concentration, and reduced more strongly by Ca^{2+} compared with Na^+ at similar concentrations. Sorption data obtained for AHA in different Na^+ solutions at pH 6 was modeled successfully by the NICA-Donnan approach. Excluding the non-specific electrostatic attraction caused by NaCl, the intrinsic sorption coefficient to ionic sites in AHA was calculated to be 5.35 log units (at C_{aq} of 1 nM,
25 pH6, and in the absence of Ca^{2+}). Applying generic input parameters for humic acid, protons and Ca^{2+} , the NICA-Donnan model explained the stronger effect of Ca^{2+} compared to Na^+ by differences in Boltzmann factors, mostly as a result of specific binding of Ca^{2+} whereas Na^+ only “sorbs” indifferently. This study provides relevant insight for environmental risk assessment of quaternary ammonium compounds, and possibly for a wide variety of organic
30 cations.

1. Introduction

Quaternary ammonium compounds (QACs) are widely used, permanently charged, organic cations, for example as biocides and as anti-electrostatics ingredient (e.g. hair conditioner and fabric softener) (Cross and Singer, 1994). The usage of QACs leads to their release into the environment through various ways, such as waste water effluent (Ding and Tsai, 2003), leaching from QACs-contained products (Van De Voorde et al., 2012), land-applied wastewater biosolids (Ismail et al., 2010), etc. Concentrations of these QACs are reported in a number of monitoring studies (Ding and Tsai, 2003; Ferrer and Furlong, 2001; Li and Brownawell, 2009, 2010; Martínez-Carballo et al., 2007a; Martínez-Carballo et al., 2007b). Sorption is one of the key processes when evaluating the fate and occurrence of organic pollutants in environment. Comprising both a positively charged head group and a hydrophobic alkyl chain, QACs generally adsorb strongly to negatively charged environmental surfaces such as organic matter, but also clay minerals (Xu and Boyd, 1995; Ying, 2006). A problem in the risk assessment is that sorption processes for organic ions are still poorly understood (Tolls, 2001).

In our previous study on QACs (Chen et al., 2012), we demonstrated that ion-exchange based solid-phase microextraction (SPME) can be used to determine freely dissolved organic cations in batch sorption studies, at concentrations well below both the critical micelle concentration (CMC) of the surfactant and the cation-exchange capacity (CEC) of humic acid (HA). This SPME method allows for measurements in a dissolved concentration range ranging above and well below observed toxic effects. As had also been observed in other studies (Droge and Goss, 2012; Fábrega et al., 2001; Ishiguro et al., 2007; van Wijk et al., 2009; Yee et al., 2006; Yee et al., 2009), our previous study demonstrated a high affinity of QACs for HA, suggesting that environmentally relevant concentrations of dissolved organic matter (DOC) can affect the bioavailability of QAC (Chen et al., 2012). Furthermore, our work and related studies have shown that the solution chemistry can influence the sorption affinity of organic cations to HA (Chen et al., 2012; Droge and Goss, 2012; Fábrega et al., 2001; Ishiguro et al., 2007). In this study, we aimed to extend the use of SPME to study sorption of QACs to organic matter in a concentration range well below the CEC.

Firstly, we have examined the difference in QAC sorption affinity for four different types of organic matter tested at the same solution chemistry conditions. Secondly, we aimed to investigate the effect of solution parameters on sorption to one type of HA more systematically, including pH and various concentrations of Na^+ or Ca^{2+} in an environmentally relevant range. The final objective was to use this data to explore the use of the NICA-Donnan sorption model, which is developed and widely used as a sorption model for multivalent metals, as a possible model that can aid predictive sorption models for QACs.

2. Materials and methods

2.1 Chemicals.

70 Benzyltrimethylammonium chloride (C12-BAC, $\geq 99.0\%$) was purchased from Sigma-Aldrich (Zwijndrecht, The Netherlands). Hexadecyltrimethylammonium-d42 chloride (CDNisotope, Quebec, Canada) served as internal standard for analysis on LC-MS/MS. Aldrich humic sodium salt (Cat. No. H1675-2) was from Sigma-Aldrich, Leonardite humic acid (Cat. No. 1S104H-5) and Pahokee peat (bulk solid Cat. No. 2BS103P) from the
75 International Humic Substances Society (IHSS, Golden, CO). Sphagnum peat was from garden centre Intratuin, The Netherlands. Solutions of sodium chloride and calcium chloride (both from Merck, Darmstadt, Germany) were prepared in Milli-Q water ($>18\text{M}\Omega\cdot\text{cm}$, Millipore Waters, Amsterdam, The Netherlands). NaOH (Merck), HCl (Merck), methanol (Lab-scan, Gliwice, Poland) and trifluoroacetic acid (TFA) (Sigma-Aldrich) were all
80 analytical grade. To prevent biodegradation of the C12-BAC, 0.2% formaldehyde solution (37%, Merck) was added to test medium.

2.2 Ion-exchange SPME.

Disposable 7- μm polyacrylate (PA) coated SPME fiber (Polymicro Technologies, Phoenix, AZ) were used throughout the sorption experiments. Fibers were cut to a suitable length (30
85 or 50 mm, so 0.07 or 0.11 μL PA) to maintain a negligible ($<5\%$) depletion of the dissolved concentration upon introduction of the SPME-fiber. Our previous study showed that PA fiber-water sorption coefficients (K_{fw}) depend on the medium composition (Chen et al., 2012) and we thus determined K_{fw} individually in each specific medium identical as those for HS sorption tests. Fibers were equilibrated in test systems for 48 h. After exposure, fibers were
90 quickly cleaned with a wet tissue and desorbed in 1.5 mL HPLC vial (Grace, Deerfield, IL) containing 1 mL of LC-MS/MS eluent. Our previous work showed that the QACs have a constant K_{fw} as long as fiber sorbed concentration were below 2 mmol/L PA (Chen et al., 2012). Thus, experiments were designed to operate at that concentration range and as low as detection limits allowed.

95 2.3 Sorption experiments.

Batch experiments were conducted in 20 mL scintillation vial with polyethylene-lined screw caps (Perkin Elmer, Waltham, MA). Aluminum foil was employed to prevent losses due to sorption onto the screw cap lining. Due to the high sorption affinity of C12-BAC, we could apply a field relevant organic matter concentration of 10 mg/L (dry weight based) for four
100 different organic matter throughout the tests. Sorption experiments with purified Aldrich

humic acid (AHA) were performed in the same way as in our previous study (Chen et al., 2012). C12-BAC stock in methanol was spiked in the test medium, where the amount of solvent was below 0.5% of the total volume of the system. The sample vials were placed on a gentle shaking device (Stuart Roller Mixer SRT9) in a climate room ($20 \pm 1^\circ\text{C}$) for 2 d, allowing for equilibration between natural organic matter and the chemical. Dissolved AHA was used as the model sorbent for tests with different medium compositions. Leonardite humic acid (LHA) stock (2.0 g/L) was prepared by first dissolving LHA in NaOH at pH 10 overnight, and then readjusting to pH 7. To prepare homogeneous solution with the bulk peat materials, a small amount of Pahokee and Sphagnum peat (10 mg/L) was first swirled in an Erlenmeyer with some drops of methanol, to prevent aggregation on the aqueous surface after mixing with electrolyte medium (final methanol content <1%), and then constantly stirred in the required aqueous medium. The 20 mL test vials were filled with the “peat medium” from the stirred solution. Two duplicate samples from the stirred solution showed the same peat concentration in each sample vial after taking to dry weight, indicating that a homogeneous system could be attained. To avoid calcium phosphate precipitation and to minimize the effect of buffer ions, phosphate buffer was not used in the test medium.

The effect of pH on QAC sorption affinity was tested with AHA at pH 3, pH 4.5 and pH 6 in 5 mM CaCl_2 solution. Electrolyte effects on QAC sorption to AHA were conducted at a fixed pH 6, because the pH of the AHA medium was already close to 6, and fine tuning the pH only introduced trace amount of Na^+ . The desired pH of the medium containing peat or HA was obtained by adding HCl or NaOH solution. The prepared AHA solutions contained either NaCl (5-50-500 mM, concentrations representing freshwater to seawater conditions) or CaCl_2 (0.5-5-50 mM, which covers (hard) Dutch Standard Water (1.2mM Na^+ , 1.4 mM Ca^{2+})(NEN, 1980), seawater (420mM Na^+ , 9 mM Ca^{2+})(EPA, 2002), and most soils (5-20 mM Ca^{2+})(Bradford et al., 1971). AHA solutions were always stored in the dark for 24 h to check whether the pH was stabilized before use. Although H^+/QAC^+ exchange was not expected to initiate substantial pH change in the tested concentration range well below the CEC, sample pH were always measured before and after the sorption experiment. Data were excluded when the pH changed more than 0.1 units. To compare the effects of organic matter type or medium composition on sorption, each comparison has included at least one duplicated testing series. To stress that ion-exchange (IE) is probably the dominant sorption process, and to avoid confusion with bulk partition coefficient K_{OC} , the OC-normalized sorption coefficients are denoted $D_{\text{OC,IE}}$, as was also done recently for other organic cations (Droge and Goss, 2013). Since ion-exchange sorption is related to the cation-exchange capacity (CEC, in total mol charged sites per kg, $\text{mol}_\text{C}/\text{kg dw}$), we also plot data as sorbed concentration (mmol/kg) normalized to CEC ($\text{mol}_\text{C}/\text{kg}$), resulting in units of mmol surfactant per mol_C .

2.3 Mass balance checks.

140 Solid phase extraction (SPE) with Oasis-WCX columns was applied to determine dissolved concentrations in the fiber calibration samples, but also, in a slightly adapted form, in determining the total concentration of C12-BAC in humic acid solutions (so, including the >70% C12-BAC bound to DOC). Both detailed SPE procedures are provided in previous work (Chen et al., 2012). The SPE measurements are used to check for the validity of the assumption of a complete mass balance and to check whether sorption to glass-ware is
145 negligible. For “peat medium” samples, both peat and aqueous medium were analyzed separately. After centrifugation (Hermle centrifuge Z400, Germany) at 3000 rpm for 20 min, the supernatant of the sample was extracted with SPE. Once the aqueous medium was carefully removed, the peat was extracted with 90/10 (v/v) methanol-water mixture containing 0.1% TFA (pH ~1.7) for 3 h in a sonication bath (Elma Transsonic T460,
150 Germany). At least two samples were analyzed for each test series, no evident degradation or loss were found as the recovery was $95.8 \pm 8.8\%$.

2.4 Analytical details.

155 Total organic carbon (TOC) content of each HS was determined on the Shimadzu TOC-5050 analyzer, CEC values were obtained from literature (Appendix Table S1). The samples were analyzed by LC-MS/MS with acidified 90/10 (MeOH/water) isocratic eluent and a Phenomenex Luna C18(2) 100x3 mm column, with detailed settings documented elsewhere (Chen et al., 2012).

160 3. Results and discussion

3.1 Sorption data obtained by SPME.

165 PA fibers were calibrated in the same medium as applied in the sorption experiments. Calibration was performed in the concentration range where sorption to the PA fiber is linear (Figure S1). The corresponding K_{fw} values are summarized in Table 1. As observed in our previous study with QACs (Chen et al., 2012), K_{fw} values decreased at higher electrolyte concentrations and at lower pH. K_{fw} at 5 mM Ca^{2+} were 0.3 log units lower compared to 5 mM Na^+ , and almost equal to K_{fw} at 500 mM Na^+ . All data was obtained at a pH with an error margin of less than ± 0.1 units. In all reported humic substance (HS) sorption data, at least 70% of C12-BAC was sorbed on organic matter.

170

3.2 Sorption of C12-BAC to various types of organic matter.

To facilitate the comparison of sorption affinities for organic cations to those for neutral compounds, we normalize sorbed concentrations to the OC-content as done by others (Chen et al., 2012; Droge and Goss, 2012). However, since earlier sorption work have all indicated that sorption of QACs is most likely an ion-exchange based sorption process, the sorption data can also be normalized to the CEC of the sorbents (van Wijk et al., 2009). Figure 1 shows the CEC-normalized sorption isotherms of C12-BAC to four types of HS in 5 mM CaCl₂. Figure S2 shows data normalized to OC-content and dry weight. Whereas sorption to the SPME fibers was linear in the tested concentration range (Figure S1), sorption isotherms are equally nonlinear for all four HS ($n_F \sim 0.8$, Table 1). The tested sorbed concentrations of 0.2-80 mmol/kg (~0.070-28 g/kg) are well below the CEC of HS (>1000 mmol/kg) and nonlinearity is therefore probably not due to saturation effects, but rather to HS sorption site heterogeneity. Table 1, therefore, lists not only the re-fitted Freundlich constant (K_F), but also compares $D_{OC,IE}$ values at single aqueous concentration (C_{aq}) of 1 nM

Purified AHA (Na⁺ saturated) showed no difference in sorption affinity with unpurified AHA (data not shown). Generally, C12-BAC sorb strongly to all four HS at C12-BAC concentrations around nM to μM, with the OC-normalized log $D_{OC,IE}$ ranging 5.71-6.13 at $C_{aq} = 1$ nM (see Table S1). Apparently, the log $D_{OC,IE}$ values for these types of organic matter are very comparable, as they vary within 0.4 log units. Although AHA and LHA readily dissolve, while the two peat samples are more or less suspended particles, the four tested HS differ only slightly in physiochemical properties (Table S1). The OC content varies between 47-59% and the CEC range from approximately 0.4 to 4 mmol/kg. The H/C ratios for the tested substances vary too little to really appoint differences in sorption to such parameters, as done earlier (Ishiguro et al., 2007). It also seems that the hydrophobicity or aromaticity of internal HS domains (indicated by the H/C ratio) is not very decisive for QACs sorption, as sorption of QACs probably occurs more on charged groups on the HS surfaces. The similarity in QAC sorption affinity for these HS facilitates comparison among different studies that considered different aspects of risk assessment of such organic cations. The artificially created and purified AHA has been widely used to study the interaction with various cations, including protons (Avena et al., 1999; Vermeer et al., 1998), inorganic cations (Christl et al., 2001; Milne et al., 2003) and organic cations (Ishiguro et al., 2007; Koopal et al., 2004; Matsuda et al., 2009). LHA is a natural and rather hydrophobic HA (Niederer et al., 2006) and used in another study demonstrating strong cation-binding (Richter et al., 2009). Pahokee peat was used in related sorption work with a wide variety of organic cations (Droge and Goss, 2012; Droge and Goss, 2013) and Spaghnum peat is commonly used to prepare artificial OECD soil in toxicity tests, for example with a dialkyl quat (Thomas et al., 2009).

This study suggests that the average HS sorption coefficient presented in this study is representative for C12-BAC to other HA and soil organic matter materials within limited uncertainty margins. The HS sorption coefficient uncertainty margin is even smaller than for
210 a neutral hydrophobic compound like pyrene, where sorption to sphagnum peat and AHA vary one log unit (Perminova et al., 1999). Earlier sorption studies with QACs displayed hundred times lower sorption affinities for fulvic acids compared to HA (Ishiguro et al., 2007; Yee et al., 2006), which could hamper extrapolation to natural DOC. A sorption study with another QAC, n-dodecyl-pyridinium (Ishiguro et al., 2007), observed a ten times higher
215 sorption affinity of cations to AHA compared to natural HA. Generalisations of our findings to other QACs and organic matter types should therefore be done with care (Ishiguro et al., 2007). Besides the characteristics of sorbent (e.g. hydrophobicity and CEC) and sorbates (e.g. alkyl chain length of QAC) (Li and Gallus, 2007; Wang et al., 1999; Yee et al., 2006), the sorption affinity of cationic surfactants to HS also depend on aqueous phase chemistry,
220 including pH, electrolyte type or concentration (Droge and Goss, 2012; Ishiguro and Koopal, 2011).

3.3 Medium Composition Effects.

Our previous study demonstrated the effect of inorganic cations on C12-BAC sorption to both the polyacrylate coated SPME fibers and AHA (Chen et al., 2012). In the current study, the
225 medium composition effects were studied for a wider range of conditions with the same method. Repeated tests conducted in 5 and 50 mM CaCl_2 at pH 6 show good consistency with our previous study (Figure S3). Figure 2 shows the sorption data of C12-BAC to AHA at different NaCl and CaCl_2 concentrations. The fitted n_F ranges from 0.68 to 0.82 ($\text{sy.x} < 0.06$), but we noted that the lowest n_F values were affected by decreased sorption affinity at highest
230 test concentrations. To facilitate comparison of $\log D_{\text{OC,IE}}$, all isotherms were fitted with a fixed n_F of 0.8, because this was the average nonlinearity shown for the other HS discussed above. These isotherms are drawn in Figure 2 (sy.x of < 0.1 for all isotherms). The fitted Freundlich $\log K_F$ and calculated $\log D_{\text{OC,IE}}$ are listed in Table 1, together with the corresponding SPME $\log K_{\text{fw}}$ at each specific electrolyte composition.

235 As shown in Figure 2, C12-BAC sorption to HA is affected by the medium composition. We divide the electrolyte effect on sorption into two aspects. First, the effect of electrolyte concentration, at pH 6, is evident because the $\log D_{\text{OC,IE}}$ is approximately 0.3 units decreased for a 10-fold increase in both Na^+ (5–50–500 mM) and Ca^{2+} (0.5–5–50 mM) concentration (Table 1). Second, at the same electrolyte concentration, Ca^{2+} can more effectively reduce the
240 sorption than Na^+ , for example 0.64 and 0.57 log units difference at 5 and 50 mM inorganic cations, respectively. Consequently, we observed a rather comparable sorption affinity for 5 mM Ca^{2+} and 500 mM Na^+ despite huge difference in ionic strength (Figure 2). The sorption

of a variety of smaller organic cations (with much lower sorption affinities) to Pahokee peat also showed that 5 mM Ca^{2+} reduced sorption only slightly stronger than 420 mM Na^+ (Droge and Goss, 2012). The decreasing sorption at higher concentrations of Na^+ and Ca^{2+} and differences between inorganic cations suggests that C12-BAC sorption occurs via cation exchange, as was also concluded for other positively charged organic compounds (e.g. pharmaceuticals, antibiotics) in sorption to various HS (Droge and Goss, 2012; Pils and Laird, 2007).

The plot of $\log D_{\text{OC,IE}}$ against $\log [\text{Na}^+]$ or $\log [\text{Ca}^{2+}]$ shows that both slopes (ion selectivity coefficient) are about -0.3, indicating no ideally proportional replacement between organic and mono/divalent cation on AHA (Figure S4). Different selectivity coefficients have been reported for organic cation sorption to Pahokee peat in presence of Na^+ or Ca^{2+} (-0.8 and -0.5, respectively) (Droge and Goss, 2012). A proportional exchange (selectivity coefficient of -1) between Na^+ and organic cation on Elliott humic acid (Sibley and Pedersen, 2008) and negatively charged polymer (Ishiguro and Koopal, 2009) have also been reported. Detailed sorption studies with metals showed that the ion-exchange ratios are overall dependent on sorption site heterogeneity, can differ amongst various HS, and may in general be higher for peat material compared to humic acids (Cooke et al., 2008; Kinniburgh et al., 1999; Tipping, 2002). Ideally, with a fixed negative charged cation exchanger, a selectivity coefficient of 1.0 is expected when applying mass action laws if all cation binding is monodentate, and of 0.5 for divalent cations assuming bidentate binding. Ca^{2+} does not seem to be able to form bidentate binding to most HA (Droge and Goss, 2012; Kinniburgh et al., 1999; Matsuda et al., 2009). Instead of this basic ion-exchange model, the NICA-Donnan model considers the sorption of cations in the presence of Na^+ or Ca^{2+} as a complex set of processes, including electrostatic effects, ion specific binding, non-ideality, competition effects and sorption site heterogeneity, as will be discussed further on, which could substantiate the discussions on stoichiometry and ion-selectivity for our findings better.

Figure 3 shows that C12-BAC sorption to AHA decreases from pH 6 to 3 at the same electrolyte concentration. This makes sense because many carboxylic acids will be protonated at pH 3 and unavailable as ion-exchange sorption sites. It depends on the pK_a of the carboxyl groups to what extent the amount of negative charge is decreased at pH 3, but ion-exchange still does seem to occur, because at pH 3 sorption in 5 mM Na^+ is still about two times stronger than that in 5 mM Ca^{2+} . Sorption was reduced by 0.8 and 0.5 log units (at $C_{\text{aq}} = 1$ nM), for Na^+ and Ca^{2+} , respectively. The reason for the slightly smaller shift with Ca^{2+} is not clear, but corresponds to findings for Pahokee peat for NaCl and CaCl_2 solutions tested between pH 4.5 and 7. Similar as at pH 6, small difference in ionic strength at pH 3 is not responsible for such an effect. The ionic strength is also not significantly influenced by the

280 additional HCl concentration at pH 3, when compared to background electrolyte concentrations. As will be discussed further on, the NICA-Donnan considers protons as cations that can influence the sorption of QACs by specific competition.

3.4 Sorption to polyacrylate SPME fiber versus sorption to organic matter.

285 The sorption of cationic surfactants to the 7 μ m-PA fiber coating occurs by ion-exchange at deprotonated carboxyl groups at the polymer surface. Therefore, it is interesting to compare the fiber sorption coefficients with the obtained sorption data for AHA. Of course there are obvious differences between the fiber and AHA, such as linear sorption to fiber and nonlinear sorption to AHA, a CEC of 0.03 mol/L PA compared to 2 mol/kg AHA, and a relatively dense polymer compared to a ‘spongy’ humic acid. Still, a strong correlation is observed
290 when plotting $\log D_{OC,IE}$ calculated for all medium compositions tested in this work against $\log K_{fw}$ measured in the same medium as applied in the sorption experiments with AHA, despite including different electrolyte types and data at different pH (Figure 4). Overall, the constant in the forced linear regression ($\log D_{OC,IE} = \log K_{fw} + 2.53(\pm 0.05)$, $R^2 = 0.86$), closely relates to the approximately 100 fold difference in CEC. The $\log K_{fw}$ may function as
295 a screening parameter to estimate the sorption coefficient to natural organic matter under various medium conditions and at concentrations well below the CEC.

3.5 Modeling Approach.

300 Simple mass action law equations for ion-exchange are not sufficient to fully explain the effect of medium composition on sorption coefficients of organic cations (Droge and Goss, 2012). The NICA-Donnan model has been developed to describe metal or proton binding to HS (Kinniburgh et al., 1999; Koopal et al., 2005), and has recently been adopted for modeling organic cation binding to HS (Iglesias et al., 2009; Richter et al., 2009), including a slightly altered model for cationic surfactant (Ishiguro and Koopal, 2011). Thorough reviews of the
305 model are available in literature,(Kinniburgh et al., 1999; Koopal et al., 2005; Milne et al., 2001; Milne et al., 2003; Tipping, 2002) and equations and assumptions are provided in SI-Section 2. Briefly, the NICA-Donnan model treats the overall sorption of cations to HS as the product of two distinct processes, non-specific electrostatic attraction and specific sorption to charged sites. The contribution of non-specific attraction is accounted for by the Donnan submodel, and assumes that the negatively charged groups in the HS attract cations into the
310 aqueous phase encompassing the HS (Donnan phase) to maintain electroneutrality (Benedetti et al., 1996; Kinniburgh et al., 1996). This creates a higher cation concentration in the aqueous Donnan phase ($C_{i,D}$) compared to bulk phase ($C_{i,B}$). Note, however, that this apparent

315 accumulation is not related to sorption to specific ion-exchange sites. The electrostatic attraction depends on the charge density on the HS, the Donnan phase volume (V_D), and the ionic strength of the medium. The NICA submodel accounts for specific sorption to the charged groups, specific properties of the HS, and functions as a competition model using the concentrations of ions in the Donnan phase.

320 The basic assumption of the NICA-Donnan approach is that monovalent inorganic cations (e.g., Na^+) only influence the sorption process via the non-specific electrostatic attraction, whereas protons and multivalent ions (e.g., Ca^{2+}) also contribute via specific sorption. Sorption of monovalent organic cations operates by both specific sorption and non-specific attraction. Both processes need to be solved together iteratively, because (i) the charge density that controls the Donnan attraction depends on the specific sorption of H^+ and multivalent 325 ions, whereas (ii) the specific sorption depends on the Donnan phase concentrations. We used the program ECOSAT (Keizer and Van Riemsdijk, 1998) to evaluate to what extent NICA-Donnan can explain the observed effect of medium composition on sorption affinity of quaternary compounds. The NICA-Donnan input parameters (e.g., dependency of V_D on ionic strength, sorption site heterogeneity, charge density of carboxylic acids and sorption affinity 330 of Ca^{2+}) are specific for each type of organic matter. The parameters used by ECOSAT are presented in Table S2.

As a first evaluation of the NICA-Donnan approach, we tried to distinguish between the intrinsic sorption affinity of the surfactant and the additional contribution of (non-specific) electrostatic attraction. This is relatively straightforward from the data obtained at pH6 with 335 different NaCl concentrations, because only the Donnan submodel is required (Na^+ does not sorb specifically, and influence of ionic strength on specific H^+ sorption is assumed negligible for simplicity). The plot of the sorbed surfactant concentration on HS against the dissolved surfactant concentration in the Donnan phase ($C_{i,D}$) should be independent of the electrostatic attraction. At a constant HS charge density, only the Donnan parameter b (indicator for the dependency of V_D on ionic strength, Appendix SI-Section 2) influences the relationship 340 between electrostatic attraction and ionic strength. Following the Donnan approach, isotherms for C12-BAC on AHA measured in 5, 50, 500 mM NaCl can be successfully merged into one master-curve (MC) (Figure 5), by setting the b to 0.59. The b value is typically around 0.6 based on proton binding studies for different types of HA (Milne et al., 2001), and further 345 agrees well with the value (0.63) obtained in a metal binding study that used the same purified AHA (Ishiguro and Koopal, 2011). Lower b values would result in stronger effects of NaCl concentrations on organic cation sorption, as may be applicable to sorption data for the soil organic matter in (Droge and Goss, 2012). The master-curve suggests an intrinsic sorption coefficient ($\log D_{\text{OC,IE,int}}$) for C12-BAC to AHA of 5.35 ± 0.05 L/kg ($C_{\text{aq}}=1$ nM). Again, we

350 want to stress that the effect of Na^+ on sorption of C12-BAC is only the result of variable electrostatic attraction.

As a second evaluation of the NICA-Donnan approach, we compared model predictions for NaCl solutions with CaCl_2 solutions. For the CaCl_2 solutions the model should account for the effect of specific sorption of Ca^{2+} . According to the NICA-Donnan approach, Ca^{2+} influences
355 the electrostatic attraction not only via the ionic strength, but also because sorption of Ca^{2+} reduces the charge density Q , which also reduces the electrostatic attraction. The contribution of electrostatic attraction is directly quantified by the Boltzmann factor (B) (Appendix equation S1), which is the ratio for cations between ($C_{i,D}$) and ($C_{i,B}$). ECOSAT was used to calculate Boltzmann factors for all tested medium compositions at pH 6 (Table 1). At lower
360 pH, NICA-Donnan predictions are sensitive to specific parameters for the pKa distribution parameter (p) of carboxylic acids in AHA, as well as specific H^+ and Ca^{2+} sorption parameters. Since these are not all available, we only focus on the NICA-Donnan predictions for pH 6 in this paper. Figure 6 plots the observed sorption coefficients at NaCl and CaCl_2 solutions against the calculated Boltzmann factors for each system, using generic HA input parameters.
365 The Boltzmann factors for the three tested CaCl_2 solutions are much lower than for the NaCl solutions with comparable concentrations, because of the specific Ca^{2+} sorption to AHA. Figure 6 shows that the Ca^{2+} data fall almost in one line with the Na^+ data, and that all observed effects of electrolyte composition at pH6 can be explained by the Boltzmann factor, using mostly generic HA input parameters.

370

4. Environmental relevance

The NICA-Donnan modeling explorations showed that observations in $D_{\text{OC,IE}}$ effects by different medium compositions can be approached reasonably well, and potentially the model can also be used in the future to compare different types of organic matter and pH effects if
375 specific NICA-Donnan parameters are available. The ion-exchange based SPME measurements at low sorbed concentrations provide detailed insight for environmental risk assessment of QAC, and may it be possible to apply this method to a wide variety of cationic surfactants.

Depending on the medium electrolyte composition, the sorption affinity of C12-BAC to
380 humic substances ranges from $7.4 \cdot 10^5$ to $5.8 \cdot 10^6$ L/kg_{OC} ($C_{\text{aq}}=1$ nM, pH 6). Sorption to dissolved organic matter may affect the bioavailability of cationic surfactants in the environment. Realistic estimates of concentrations of humic acids for riverine samples are in the range of 10 mg/L at pH 6. From the presented sorption data, we can estimate that at 10 mg/L humic acids (5.8 mg/L OC), the fraction freely dissolved of highest monitored C12-

385 BAC concentrations in rivers, about 10 nM (Ding and Tsai, 2003; Martínez-Carballo et al.,
2007b), is between 19 % and 2.9 %. If aqueous toxicity of QACs is related only to freely
dissolved concentration, as is generally accepted for neutral compounds, this study shows that
information on freely dissolved concentrations is relevant for the interpretation of potential
effects of this cationic surfactant and related compounds in the environment.

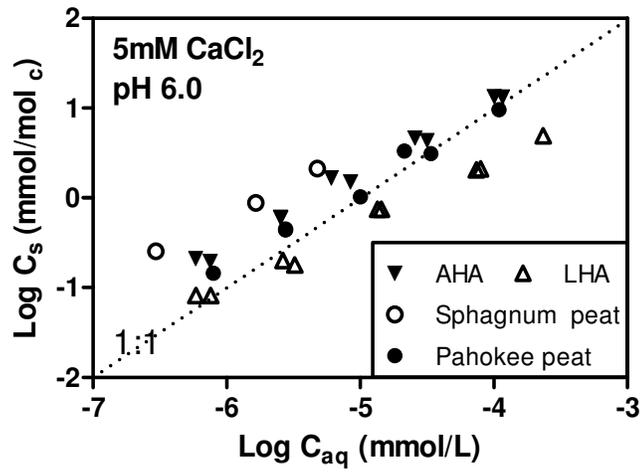
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Table 1. Fiber water sorption coefficients (K_{fw}) and Freundlich sorption coefficients (organic carbon fraction normalized $\log K_F$ in mmol/kg) for C12-BAC on purified AHA at various medium composition given a fixed Freundlich exponent (n_F) of 0.8. $\log K_{fw}$ is obtained from the isotherms fitted with a slope of 1 (see Figure S1 in Appendix). $\log K_F$ and $\log D_{OC,IE}$ values are derived from the isotherms shown in Figure 2 and 3. Sorption coefficients for 7- μm PA fiber ($\log K_{fw}$ in L/L) measured in the corresponding medium as the sorption test. $\log D_{OC,IE}$ (L/kg_{OC}) are calculated at 1 nM dissolved concentration based on n_F of 0.8. Boltzmann factor (B) are calculated with different electrolytes at pH 6 using ECOSAT (SI-Section 2).

medium composition	$\log K_{fw} \pm sy.x$ (n) (slope=1) L/L	$\log K_F \pm sy.x$ (n) (fixed $n_F=0.8$) (mmol) ^{0.2} *L ^{0.8} /kg _{OC}	$\log D_{OC,IE}$ ($C_{aq}= 1$ nM, $n_F=0.8$) L/ kg _{OC}	Boltzman n factor (B)
	CaCl₂			
	0.5 mM	4.01 ± 0.07 (6)	5.20 ± 0.02 (5)	6.40
pH 6.0	5 mM	3.76 ± 0.08 (3)	4.93 ± 0.02 (10)	6.13
	50 mM	3.40 ± 0.01 (4)	4.67 ± 0.02 (10)	5.87
pH 4.5	5 mM	3.32 ± 0.07 (4)	4.61 ± 0.06 (6)	5.81
pH 3.0	5 mM	2.75 ± 0.06 (3)	4.21 ± 0.03 (5)	5.64
	NaCl			
	5 mM	4.06 ± 0.05 (4)	5.57 ± 0.04 (8)	6.77
pH 6.0	50 mM	3.96 ± 0.02 (3)	5.24 ± 0.05 (9)	6.44
	500 mM	3.72 ± 0.02 (2)	4.94 ± 0.03 (4)	6.14
pH 3.0	5 mM	2.93 ± 0.1 (3)	4.54 ± 0.03 (11)	5.97

Figure 1. C12-BAC sorption to four types of organic matter normalized with CEC in 5 mM CaCl₂ at pH 6. Dotted line indicates linearity. AHA and LHA indicate purified Aldrich humic acid and Leonardite humic acid, respectively.

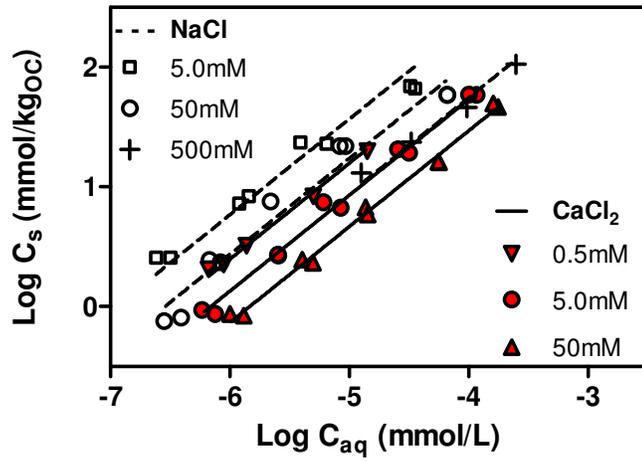
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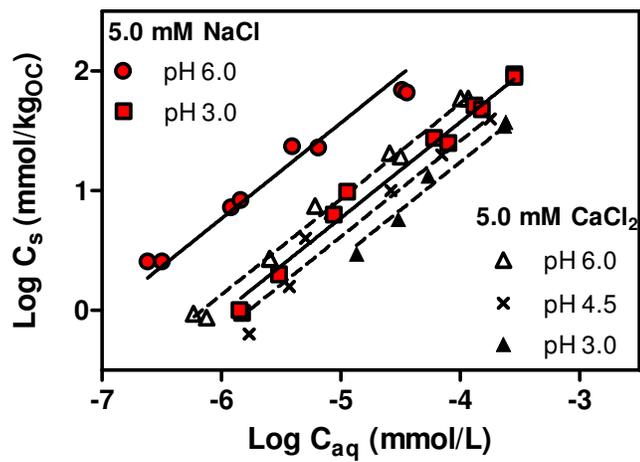
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Figure 2. C12-BAC sorption to purified AHA determined at different background electrolytes at pH 6. Isotherms were fitted with Freundlich equation with n_F of 0.8. Dashed and solid lines indicate fitting curve for NaCl and CaCl₂, respectively.

415



420 **Figure 3.** C12-BAC sorption to purified AHA determined at pH 6, pH 4.5 and pH 3 at 5 mM NaCl and CaCl₂ concentration. Isotherms were fitted with Freundlich equation with n_F of 0.8.

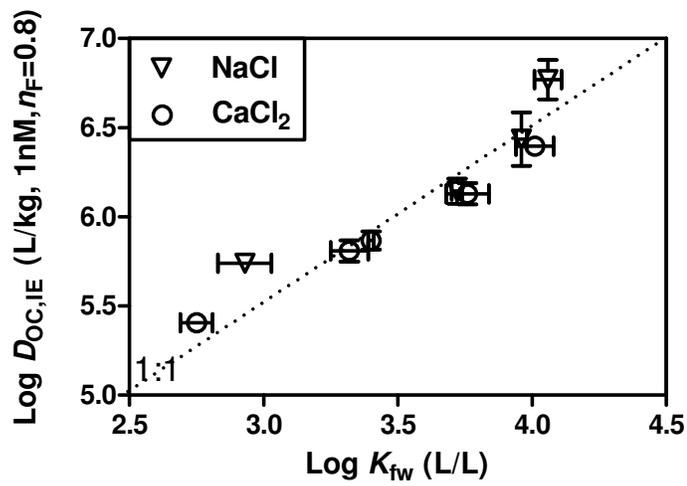


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Figure 4. Log $D_{OC,IE}$ (L/kg_{OC}) of C12-BAC to purified Aldrich humic acid calculated at 1 nM dissolved concentration based on n_F of 0.8 as a function of log K_{fw} (L/L) (sorption to PA fiber) measured in different medium compositions. Dotted line indicates linearity.

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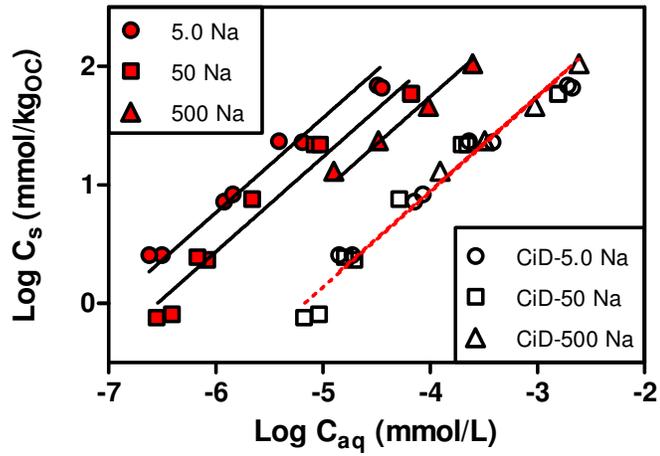
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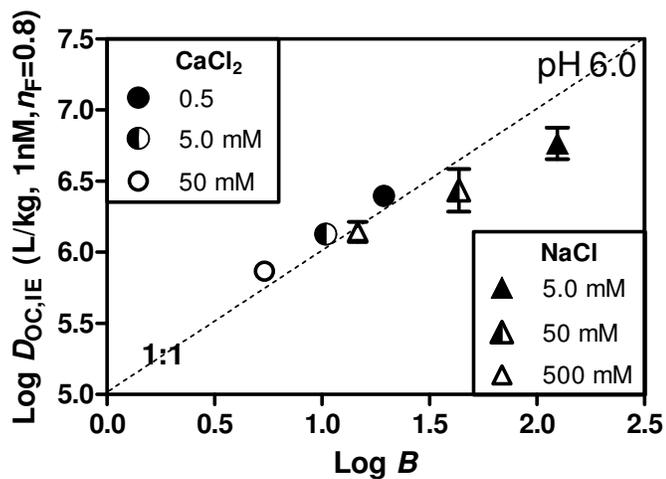
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Figure 5. Isotherms of C12-BAC to purified Aldrich humic acid measured at pH 6 for different Na⁺ solution merge into a master curve (MC) expressed by red dotted lines using NICA model with *b* of 0.59. Dashed lines represent shifted MC for Ca²⁺ using the same *b*.

445



450 **Figure 6.** The observed $\log D_{OC,IE}$ of C12-BAC at NaCl and CaCl₂ solutions against the calculated Boltzmann factors (B) for each system at pH 6, using generic HA input parameters from (Milne et al., 2001).



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