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Quantification of solute–solute interactions using negligible-depletion solid phase microextraction: Measuring the affinity of estradiol to bulk organic matter

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Abstract

The interaction of trace organic contaminants with bulk organic matter has implications for the transport and behaviour of organic trace contaminants within the aquatic environment as well as water and wastewater treatment processes. Partition coefficients (K_{OM}) of the steroidal trace organic contaminant estradiol were quantified for environmentally relevant concentrations of bulk organic matter (12.5 mgC/L) using a full mass balance form of solid phase microextraction (SPME). The results indicated that the method is successful and can be used at environmental concentrations. Estradiol had the greatest affinity for bulk organic matter that contained phenolic and benzoic acid ester groups, namely tannic acid, compared to organics containing predominately carboxylic functional groups. The solution chemistry (pH) was found to influence the interaction, as estradiol had a lower affinity for negatively charged and hydrophilic bulk organic matter. The partition coefficients determined using SPME were consistent with partition coefficients derived using solubility enhancement and fluorescence quenching measurements, confirming that SPME is a powerful technique to quantify the affinity of estradiol for low concentrations of bulk organic matter and trace contaminants. Further, this novel method can be applied to a range of trace contaminants.

Introduction

The behaviour and transport of trace organic contaminants within aquatic systems is affected by their affinity for bulk organic matter (1-3). This interaction can be described as a ‘solute-solute interaction’, and has implications for aqueous solubility (4), biological uptake and contaminant degradation (5) as well as removal of trace organic contaminants in water and wastewater treatment systems (6, 7). However, measuring such interactions at environmentally relevant (generally low) concentrations has, to date, been difficult.

Many trace organic contaminants have adverse effects on environmental and human health. Of particular concern are endocrine disrupting chemicals due to their potential to interfere with the endocrine system leading to effects on reproduction of vertebrates (8). Endocrine disrupting chemicals include natural and synthetic hormones, certain industrial chemicals and pesticides and pharmaceutically active products (9). The trace organic contaminant estradiol (E2) was selected as it is a steroidal hormone which is produced naturally by humans and some animals, and it is considered one of the most potent endocrine disrupting chemicals (10). The aquatic environment has a greater susceptibility to estradiol and other trace organic contaminants compared to other environments, due to the constant introduction of wastewater effluent (11). Effluent from conventional biological treatment plants can have estradiol concentrations ranging from low nanograms per litre to micrograms per litre (8) and researchers have demonstrated that concentrations as low as 5 ng/L of estradiol can have significant implications for aquatic life (12).

The bioavailability of estradiol within an aquatic system can be influenced by the type of bulk organic matter present (13). Bulk organic matter can be used to describe particulate, colloidal and dissolved phases of organic matter of varying origins and characteristics. In water and wastewater bulk organic matter can include natural organic matter (NOM) surrogates, polysaccharides, polyphenols and surfactants. The charge, polarity, aromaticity, hydrophobicity and molecular weight of bulk organic matter can all affect the interaction with trace organic contaminants (14, 15). Therefore, as the different types of bulk organic matter have different properties, it is expected that estradiol will show a greater affinity for certain types of bulk organic matter compared to others and hence different transport and treatment properties depending on water composition. Therefore, the purpose of this research is to quantify the interaction of estradiol with a wide range of bulk organic matter at environmentally relevant concentrations. In addition, the interaction will be studied at different pH values (4-9). Estradiol is a weak acid with an acid dissociation constant (pKa) of 10.23, hence it will be mainly undissociated under the studied conditions (6% dissociated at pH 9) (16). Most bulk organics have ionisable groups, and therefore partitioning will be influenced by pH (17).

Previously, the interaction of estradiol with various types of bulk organic matter has been studied using techniques such as fluorescence quenching (FQ) (18, 19) and solubility enhancement (SE) (18). However, these techniques have limitations which can restrict measurement of partition coefficients, particularly at low concentrations. For example, SE requires a high concentration of bulk organic matter, often in excess of 100 mg/L (approximately 52 mg carbon per litre (mgC/L) for humic acid) (18), while in FQ the presence of molecular oxygen can quench trace organic contaminants, which overestimates partitioning (20). Researchers have previously identified the need for further investigation of this interaction using techniques such as negligible-depletion solid phase microextraction (nd-SPME) (18). nd-SPME is based on the principle that if less than 5% of freely dissolved trace organic contaminants are extracted, the extraction does not significantly disturb the equilibrium with bulk organic matter (21). nd-SPME has been previously used to quantify the interaction between chlorobenzenes and dissolved organic carbon (22), estradiol and protein (bovine serum albumin) (21) and DDT and humic acid (23).

Materials and Methods

Chemicals. All chemicals were of analytical grade. NaOH and HCl (1 M) were used for pH adjustments and 1 mM NaHCO3: 20 mM NaCl was the background electrolyte (Sigma Aldrich UK). Radiolabelled [2,4,6-3H]Estradiol (3.15 TBq/mmol, 37 MBq/mL) was purchased from GE Healthcare UK, while non-radiolabelled [17β-Estradiol (>98% purity) was purchased from Sigma Aldrich UK. Estradiol is a moderately hydrophobic steroidal hormone (log K_{OM} 3.94–4.01) with a solubility of 13 mg/L (in water at 20°C) (24). The estradiol concentrations ranged from 100 ng/L to 100 µg/L. This concentration range was selected as it represents realistic concentrations of estrogens in conventional wastewater effluent (10).

Bulk Organic Matter. Several different types of organic matter were studied including NOM surrogates, polysaccharides, polyphenols and surfactants. These were selected as representatives of organic matter found in water and wastewater. Suwannee River standard IHSS Humic Acid II (HA)
Environmental Science & Technology, 42(8); 2886-2892.


\[
\text{C}_{\text{tot}} = C_f + C_W + C_{\text{OM}}
\]

**Determination of Organic Matter Partition Coefficient (KOM):** The affinity of estradiol for bulk organic matter was measured as a function of bulk organic matter type (11 organics) and pH (4-9). The sorption isotherms, plotted as the freely dissolved estradiol concentration (ng/L) versus bound estradiol concentration (ng/L), were all similar, except for SDS and tannic acid, and ranged from log KOM values listed in Table 2. Previously, studies have suggested that an increase in bulk organic concentration can reduce estradiol partitioning (31). Therefore, the partition coefficients may be different at higher bulk organic concentrations.

Effect of Bulk Organic Matter (Neutral pH): Properties of the bulk organic matter, such as size and structure, can influence partitioning (3). However the interaction of estradiol with bulk organic matter is primarily related to the functional groups present and the molecular interactions between the solute and sorbent. The functional group contents (13C NMR) for NOM surrogates are shown in Table 1, however functional group contents were not available for the other organics used. Based on the characteristics of estradiol and bulk organic matter the mechanisms of interaction are expected to be hydrogen bonding and van der Waals interactions. Hydrogen bonding is a specific dipole-dipole interaction that occurs between hydrogen donors and acceptors. Functional groups that act as both hydrogen donors and acceptors are termed bipolar and include phenol, carboxylic and hydroxyl groups (32). Estradiol contains phenol and hydroxyl functional groups therefore it exhibits donor and acceptor properties. In addition, the aromatic rings of estradiol are electron rich and may serve as a weak hydrogen acceptor. The bulk organic matter studied mainly contains biopolymer oxygenated functional groups (3). The NOM surrogates are composed of a wide range of functional groups, including carboxylic, phenolic and carboxyl groups (33) while the polysaccharides mainly contain hydroxyl moieties. Tannic acid primarily contains phenolic, catechol and gallic acid moieties (34). SDS is the only bulk organic studied which does not contain a bipolar functional group, however it has a monopolar sulfate moiety.

For all bulk organics the linearity of the sorption isotherms suggests that all sorbents act as partitioning sorbents and adsorption does not play a role (3). This linearity is consistent with previous findings for estradiol (35, 36).

At pH 7 the bulk organic matter-water partition coefficients were all similar, except for SDS and tannic acid, and ranged from log KOM 4.21 to 3.75 (Table 2). This range corresponds to a factor of

\[
\text{KOM} = \frac{C_{\text{OM}}}{C_W}
\]

KOM can be derived from the slope of the linear regression of C_{OM} as a function of C_W if sorption isotherms are linear. Since the concentration range is over several orders of magnitude the sorption isotherms were plotted on a log scale (Equation 3). The slope of the regression, n_s, indicates the deviation from linearity of the sorption isotherm (3). Since this value was close to 1 for all isotherms n_s was set to 1, which is equivalent to the linear relationship described by Equation 2.

\[
\log C_{\text{OM}} = \log K_{\text{OM}} + n_s \log C_W
\]
three of the partition coefficients with the exception of tannic acid and SDS, which were significantly higher (4.86±0.26) and lower (3.68±0.20) respectively.

The bulk organic-water partition coefficients for NOM surrogates ranged from 4.21±0.23 to 3.95±0.21 (Table 2). There was no significant difference in the partition coefficients. It was thought that the origin of the bulk organic would influence partitioning, as this can affect the aromaticity, structure and functional group content. For example, terrestrial humic acids, such as Aldrich HA, are typically more aromatic and contain more phenolic and carboxylic groups compared to aquatic humics, such as IHSS HA (37). However, there was no significant difference in partition coefficients for Aldrich HA and IHSS HA (4.21±0.23 and 3.99±0.22 respectively). It should be noted that Aldrich HA was not pre-treated prior to the sorption experiments. Also, Australian NOM was not purified, hence it contained salts common in surface water (25). Nevertheless Australian and IHSS NOM had very similar 

The tannic acid-water partition coefficients (Figure 1 K) were significantly higher than the other interactions, indicating tannic acid was the strongest sorbent (log KOM 4.86 ±0.26). This has been observed previously in other studies with estradiol (6, 18) and may be related to the large fraction of phenolic hydroxyl groups in tannic acid. A study by Jin et al. (38) indicated that phenolic content, as opposed to total aromaticity, determines sorption of hormones with a phenolic moiety, such as estradiol and estrone. In addition at pH 7 any carboxylic acid groups are deprotonated, so tannic acid is the only organic matter in the selection that is neutral and of moderate hydrophobicity (Log KOM 2.21) (39).

The bulk organic-water partition coefficients for the polysaccharides, alginic acid, dextran and cellulose, were all similar to the NOM surrogates, despite polysaccharides having lower aromaticity (SUVA values in Table 1). Log KOM ranged from 3.75±0.2 to 3.96±0.21 (Table 2). It has been suggested that bulk organic matter lacking aromatic functional groups, such as dextran, has little effect on the fate and behaviour of steroidal hormones (38). However, the similarity in polysaccharide partition coefficients to the NOM surrogates may be due their hydrophilicity, thus increasing the potential for hydrogen bonding. The elemental stoichiometric ratios (specifically (O+N)/C), which are an indirect method to measure polarity (40), suggest that the selected polysaccharides are more polar than polyphenol and NOM surrogates (18, 41). In addition, all of the polysaccharides contain hydroxyl functional groups, enabling them to act as both a hydrogen donor and acceptor. Therefore, despite the lower aromaticity of these bulk organics, they still interact strongly with estradiol through hydrogen bonding. The large variation in molecular weight of the different polysaccharides (162-210000 g/mol) (Table 1) suggests that molecular weight does not appear to have a significant influence on the interaction of estradiol with polysaccharides.

The SDS-water partition coefficient (Figure 1 J) was significantly lower than all other bulk organics (log KOM 3.68±0.20). SDS is an anionic surfactant, which contains a hydrophilic head, with a hydrophobic tail (42). Partitioning is primarily expected to occur through weak hydrogen bonding with the hydrophilic head of SDS, which is not bipolar but only a hydrogen acceptor (sulfate group). In contrast, all other bulk organics are bipolar, thus allowing both hydrogen donating and accepting activities. As the SDS concentration is below the critical micelle concentration (0.0082 M at 25°C) micelle formation is not a factor in partitioning. It is unlikely that the hydrophobic tail of SDS contributes significantly to the interaction, as research by Yamamoto et al. (18) has indicated that hydrogen bonding is the primary mechanism of interaction between bulk organic matter and estradiol, compared to non-specific interactions.

Effect of solution chemistry (pH): The interaction of estradiol with bulk organic matter was studied from pH 4 to 9. Figure 2 shows a decrease in partition coefficient from neutral to alkaline pH. With the exception of SDS and tannic acid, this difference was generally less than a factor of 3, and not considered to be significant. A decrease in partition coefficient at alkaline pH has been observed previously when studying the interaction of phenolic compounds with commercial humic acid using SPME (13, 43).

It is expected that this pH dependence of partition coefficient is related to the dissociation of bulk organic matter, not the speciation of estradiol. Estradiol is a weak acid (pKa 10.23) and therefore can be affected by pH. However, at pH 9 there is still 94% neutral species compared to almost 100% neutral species from pH 4 to 8. As the dissociation of estradiol is minimal, it is not expected to significantly contribute to a decrease in partitioning. In addition, any changes in fibre characteristics are unlikely to affect partitioning. Zeta potential measurements (mV) of the fibre indicated no change in charge in the studied pH range.

In contrast, most bulk organic matter is affected by pH in the investigated pH range. Phenolic functional groups deprotonate at alkaline pH values (pKa 9.9 of unsubstituted phenol), while carboxylic groups deprotonate under acidic conditions (pKa around 4.5) (33, 44). Due to the high carboxylic functional group content in the majority of bulk organic matter studied, most are negatively charged at neutral pH. pH changes can have implications on intramolecular bonding within the bulk organic matter, as well as molecular shape. In acidic solutions NOM surrogates can coil due to intramolecular hydrogen bonding, while at neutral and alkaline pH values the NOM has a linear structure (45). The conformational changes in alginate are similar, however it depolymerises above pH 8 (46). This change in bulk organic matter structure is expected to affect the interaction with estradiol.

Tannic acid is abundant with polyphenol structures (mainly gallic acid) and all carboxylic acid groups are deprotonated. The first pKa of gallic acid is 8.7 (34), therefore tannic acid will start to go from neutral to anionic around pH 7 to 8, which explains the large pH dependence of the KOM of one order of magnitude between pH 4 and 9.

Comparison with other quantification techniques: The interaction of estradiol with bulk organic matter has been quantified previously using FQ and SE. Yamamoto et al. (18) calculated the interaction between estradiol and Suwannee River (IHS) FA and HA, pre-filtered Aldrich HA and tannic acid using FQ, while the interaction between estradiol and IHSS HA, alginic acid, tannic acid and dextran was quantified using SE. The partition coefficients calculated using SPME were compared to these two methods in Figure 3. SPME, FQ and SE are very different techniques, and therefore have different advantages and limitations. As mentioned previously, SE can be limited by the concentration of bulk organic matter used in the experiments, while FQ partition coefficients are based on assumptions regarding static quenching compared to dynamic quenching (47). SPME was selected as it is a simple and sensitive technique which is suitable for low bulk organic concentrations (48, 49). Doll et al. (49) has indicated that this is due to limitations associated with SPME, as it can disturb contaminants weakly bound to the outer shell of bulk organic matter and lead to a higher concentration in the aqueous phase, therefore underestimating partitioning. Partition coefficients calculated using SPME and SE were similar for IHSS HA, tannic acid and alginic acid but significantly different for dextran, despite similar molecular weight fractions used.
in both experiments (Figure 3). The difference between the results is expected to be due to the dextran organic carbon concentration, which was as high as 263 mgC/L.

In conclusion, the SPME method has been shown to work for environmentally relevant concentrations of bulk organic matter and trace contaminants. The results of the present study demonstrate how the interaction of estradiol with bulk organic matter at environmentally relevant concentrations is influenced by the type of bulk organic matter and pH. Partition coefficients presented here can be applied directly to calculate the interaction with organic matter studied here. Further, the method can be applied to determine partition coefficients of other trace organics with any bulk organic matter. Knowledge of bulk organic matter-water partition coefficients are required for environmental models, such as the fugacity model (50), in order to understand the fate and behaviour of trace contaminants in the environment. While the typical nd-SPME assumptions were not applicable in experiments with environmentally realistic organic matter concentrations, the technique was suitable if the full mass balance of the system was used. The method is more tedious than typical nd-SPME, but is more generally applicable and versatile, and can in principle be used for a wide range of organic contaminants.

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Literature Cited


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<th>Charge at Neutral pH</th>
<th>Origin in Water</th>
<th>Carbon %</th>
<th>Carbon Composition % (13C NMR)</th>
<th>Molecular Weight (g/mol)</th>
<th>SUVA (L/mg/m)* (Specific ultraviolet absorption at 254 nm)</th>
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Figure 3

- SPME (results from this work)
- Fluorescence Quenching (FQ) (18)
- Solubility Enhancement (SE) (18)