Influence of Solute-Solute Interactions on Membrane Filtration

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Declaration

I declare that the thesis has been composed by myself and the work contained in it is my own, except where stated otherwise. Further, this work has not been submitted for any other degree or professional qualification except as specified.

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Abstract

An understanding of solute-solute interactions is essential for aquatic systems as this can affect the fate and behaviour of micropollutants in the environment and engineered systems. Despite the importance of solute-solute interactions there is a general lack of understanding which may be attributed to the fact that many engineering models overlook solute-solute interactions and that the quantification of such interactions is inherently difficult. When solute-solute interactions are considered, they are often studied at unrepresentative concentrations and do not consider the influence of organic matter type or solution chemistry. Steroidal hormones, such as estradiol and estrone, were selected as model micropollutants as they are ubiquitous in the aquatic environment due to constant introduction of wastewater effluent, and can have implications for growth and development of organisms including impaired fertility and behavioural abnormalities. The purpose of this study was to develop a methodology to quantify solute-solute interactions at environmental concentrations, and to determine the implications of such interactions in membrane filtration.

A solid-phase microextraction (SPME) technique was developed to quantify solute-solute interactions at environmental (low) concentrations. Using SPME, organic matter-water partition coefficients (log \( K_{OM} \)) were measured for a range of steroidal hormones including estradiol, estrone, progesterone and testosterone with different organic matter types such as humic acid. The dominant mechanism of hormone-organic matter interactions was identified as hydrogen bonding. In the case of estrone and progesterone the log \( K_{OM} \) values were significantly influenced by organic matter type and concentration, as well as solution chemistry. No difference was observed for estradiol and testosterone due to generally weaker sorption to organic matter.

Previous studies have indicated that the presence of organic matter can alter micropollutant retention in membrane filtration. Much of the current literature focuses on solute-membrane interactions, as the influence of solute-solute interactions are typically difficult to determine in membrane filtration. Therefore,
hormone-organic matter interactions were studied to determine if this interaction had an influence on hormone removal by ultrafiltration (UF) using a range of molecular weight cut-off (MWCO) membranes. The results indicated increased retention of estrone in the presence of humic acid, while organic matter concentration and solution chemistry influenced retention by affecting solute-solute interactions. The findings of this study indicate the importance of solute-solute interactions in membrane filtration and experimental log $K_{OM}$ results were used to quantify the findings and elucidate the influences of 1) membrane sorption, 2) solute-solute interactions and 3) solute-foulant interactions. Further, the removal of steroidal hormones using a magnetic ion exchange (MIEX®) resin with a range of MWCO UF membranes was studied as such sorbents can be used to improve micropollutant removal in wastewater treatment. Greater removal with IX-UF was observed compared to UF alone and the main hormone removal mechanisms were sorption to MIEX® and solute-fouling interactions.

The findings of this study indicate that it is indeed possible to quantify solute-solute interactions at environmental concentrations using SPME, with hydrogen bonding being the main mechanism of interaction for steroidal hormones and organic matter. Further, micropollutant retention by membrane filtration can be influenced by solute-solute interactions.
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In Preparation

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1 Introduction

Steroidal hormones such as estradiol, estrone, progesterone and testosterone are micropollutants which are naturally excreted by humans and many animals. These hormones are essential for growth and development, however, elevated concentrations in the environment can have adverse effects for organisms, such as developmental and reproductive abnormalities in fish (Jobling et al., 1998). Previous studies have indicated that steroidal hormones are not completely removed during conventional wastewater treatment, with estradiol, estrone and testosterone recorded in effluent at low nanogram per litre concentrations (Baronti et al., 2000; Johnson et al., 2005). This was further confirmed by a study of micropollutants in contaminated US streams in 1999-2000 which found median concentrations of testosterone and progesterone at approximately 100 ng/L, while estradiol and estrone were an order of magnitude less (Kolpin et al., 2002). In addition to point sources such as wastewater effluent, diffuse sources such as animal agriculture can also contribute to elevated concentrations of steroidal hormones in the aquatic environment (Kolodziej et al., 2004). A study by Finlay-Moore et al. (2000) demonstrated that land application of chicken broiler litter can lead to runoff concentrations of up to 2 µg/L for estradiol and testosterone. In addition, natural hormones such as estrogens and testosterone are used to increase the growth and feeding efficiency of cattle in countries such as the US (Orlando et al., 2004). Therefore, runoff from feedlots could potentially increase the concentration of hormones in water.

The fate and behaviour of steroidal hormones in the aquatic environment can be influenced by the interaction with organic matter which is considered ubiquitous in natural waters. This interaction is referred to as a solute-solute interaction. Bioavailability and hence toxicity of certain micropollutants decreases when bound to organic matter (Perminova et al., 2001). However, micropollutant degradation can be inhibited when it interacts with organic matter, therefore impeding remediation (Lindsey and Tarr, 2000). Further, several studies have indicated that solute-solute interactions can improve micropollutant transport within aqueous solutions (Backhus and Gschwend, 1990; Herbert et al., 1993). The majority of studies focus on
hydrophobic or apolar micropollutants such as polycyclic aromatic hydrocarbons (PAH) or polychlorinated biphenyls (PCB). Therefore knowledge of the fate and behaviour of polar micropollutants such as steroidal hormones is limited. This indicates the necessity for a better understanding of solute-solute interactions for steroidal hormones.

Solute-solute interactions can also have implications for micropollutant removal during water treatment processes. For example, micropollutants which interact with particulate organic matter can be removed to a greater extent through settling or coagulation processes in conventional treatment compared to freely dissolved micropollutants (Ballard and MacKay, 2005). Further, previous studies have suggested that solute-solute interactions can influence micropollutant removal during membrane filtration processes (Devitt et al., 1998; Schäfer et al., 2006). However, the removal can be highly variable and depend on organic matter type, solution chemistry and membrane properties.

Despite the importance of solute-solute interactions there are still large gaps in knowledge. This may be due to the fact that many engineering models reject solute-solute interactions. However, as demonstrated above, these interactions are critical for water and wastewater treatment. Such interactions are of particular importance when separation occurs at the molecular scale, which is often the case in advance treatment such as membrane technology. While solute-solute interactions can be an important mechanism for micropollutant removal in membrane filtration, they are typically overlooked in the literature as such interactions are difficult to quantify, particularly at environmentally realistic concentrations.

To improve knowledge, solute-solute interactions can be quantified using organic matter-water partition coefficients (log $K_{OM}$) which represent the equilibrium distribution of a micropollutant between two phases such as organic matter and water. Therefore, log $K_{OM}$ values can indicate if a micropollutant is more likely to remain in solution or interact with a particular organic matter type. Consequently, this is an important parameter in environmental modelling. However, quantification
of log $K_{OM}$ values can be difficult particularly for dissolved organic matter and the majority of studies focus on unrepresentative micropollutant concentrations (µg/L to mg/L). Consequently, the overarching aim of this thesis is to quantify solute-solute interactions and determine their influence on membrane filtration, which led to two primary aims:

1. Develop a technique to quantify solute-solute interactions at environmental concentrations for organic matter and steroidal hormones
2. Demonstrate the contribution of such solute-solute interactions towards micropollutant removal by ultrafiltration (UF)

This will be achieved by:

- Developing a solid-phase microextraction (SPME) technique to quantify the interaction of steroidal hormones with organic matter at low concentrations (Chapter 4)
- Determining the influence of organic matter type and concentration as well as solution chemistry (pH, ionic strength) on log $K_{OM}$ values and elucidating the interaction mechanisms (Chapter 5)
- Applying one-parameter and polyparameter linear free energy relationships (LFER) to determine if they are suitable to predict log $K_{OM}$ values for steroidal hormones and organic matter commonly found in water and wastewater (Chapter 6)
- Applying experimental log $K_{OM}$ values to quantify the influence of solute-solute interactions for hormone removal by stirred cell UF and compare results with experimental data (Chapter 7)
- Assessing micropollutant removal using an ion exchange-ultrafiltration (IX-UF) hybrid process (Chapter 8)

A schematic overview of the thesis is shown in Figure 1.1.
Introduction

In this thesis the mechanisms and quantification of solute-solute interactions will be reviewed in Chapter 2 as well as the implications of these interactions for membrane filtration. The materials and methods are described in Chapter 3 with specific method development for SPME outlined in Chapter 4. Quantification of log $K_{OM}$ values for several steroidal hormones takes place in Chapter 5 with validation using one-parameter and polyparameter LFER modelled log $K_{OM}$ values in Chapter 6. In Chapter 7 the impact of solute-solute interaction for steroidal hormone removal by UF will be determined using log $K_{OM}$ values, while the application of an IX-UF hybrid process for improved hormone removal will be explored in Chapter 8. The importance of solute-solute interactions in wastewater treatment and the wider research environment will be discussed in Chapter 9 followed by a summary of the results in Chapter 10.
2 Solute-Solute Interactions: A Review of the Interaction of Micropollutants with Organic Matter in Water and Effects on Membrane Filtration

In this chapter solute-solute interactions are discussed as they can influence the fate and behaviour of micropollutants in aqueous systems.

The interaction of steroidal hormones with different phases of organic matter will be discussed. While many studies have looked at the interaction of hormones with particulate and colloidal organic matter, few have considered the interaction with dissolved organic matter. Further, the majority of studies use unrepresentative micropollutant concentrations.

The solute-solute interaction mechanisms such as adsorption and partitioning will be discussed as well as specific organic matter-hormone interaction mechanisms. Different quantification techniques including solid-phase microextraction, equilibrium dialysis, solubility enhancement, fluorescence quenching and reverse phase partitioning will also be considered. This section will determine which technique is the most suitable for quantifying the interaction of steroidal hormones with organic matter at environmental concentrations.

Finally the implications of solute-solute interactions for membrane filtration will be considered. The impact of organic matter on micropollutant removal mechanisms such as membrane sorption, solute-foulant interactions and solute-solute interactions will be reviewed.
Solute-Solute Interactions

2.1. Introduction

Solute-solute interactions can be described as the interaction between two dissolved components such as organic matter and micropollutants within an aqueous solution. Organic matter includes a wide range of organic compounds such as natural organic matter (NOM), polyphenols, polysaccharides and surfactants. Micropollutants such as pesticides, natural and synthetic hormones and pharmaceutically active chemicals, are ubiquitous in the aquatic environment, and are of concern due to their potential ecological and health related impacts.

As discussed in Chapter 1 organic matter-water partition coefficients (log K_{OM}) can be applied to quantify solute-solute interactions. The partitioning of a micropollutant between the aqueous and organic matter phases is determined by the micropollutant fugacity. Fugacity is a thermodynamic property which represents the capacity of a micropollutant to escape from a particular phase (Schwarzenbach et al., 2003). It is based on the difference in chemical potentials. The micropollutant will have a greater preference for the phase with the lowest fugacity and the transfer will continue until the system is at equilibrium. The fugacity concept has been applied to develop models to understand micropollutant fate within the environment (e.g. MacKay and Paterson, 1991) as well as food webs (e.g. Campfens and Mackay, 1997). Consequently, fugacity is an important concept for solute-solute interactions.

The purpose of this chapter is to improve understanding of solute-solute interactions for steroidal hormones. This will be achieved by identifying solutes of interest, describing solute-solute interaction mechanisms, discussing different techniques to quantify these interactions and finally describing the implications of these interactions for the removal of micropollutants during membrane filtration.
2.2. Solutes in Water and Wastewater

2.2.1. Micropollutants

Micropollutants can be defined as natural and synthetic compounds found in the environment at picogram per litre (pg/L) to nanogram per litre (ng/L) concentrations (Schwarzenbach et al., 2006). Due to poor removal using conventional water treatment processes micropollutants are frequently detected at low concentrations (ng/L) in surface waters (Kolpin et al., 2002). The implications of micropollutants in the environment are wide ranging and can include feminisation of male fish by steroidal hormones (Jobling et al., 1998), increased bacterial resistance by antibiotics (Reinthaler et al., 2003) and significant risks for human health by inorganic micropollutants such as arsenic (Morris, 1995). Of particular concern are hormonally active micropollutants which include natural and synthetic hormones as well as a number of synthetic compounds such as pharmaceuticals which share hormonal properties (Sumpter, 2008). These can be considered endocrine disrupting chemicals and can influence the growth and development of organisms, with current studies linking certain hormonally active micropollutants to the increasing rate of reproductive disorders in humans (Sharp and Skakkebaek, 2003).

For this study steroidal hormones were selected as model micropollutants due to their presence in the aquatic environment and potency. While steroidal hormones may not be as persistent as some other micropollutants they are still of concern as they are always present in the aqueous environment due to constant introduction from sewage effluent and can be considered ‘pseudopersistent’ (Sumpter and Johnson, 2005). Natural steroidal hormones such as estrone, estradiol, progesterone and testosterone are excreted naturally by humans as well as other animals. For example, pre-menopausal women can naturally excrete between 25 and 100 µg of estrogens per day while pregnant women can excrete up to 30 mg of estradiol per day (Loose-Mitchell and Stancel, 2001). Another common source of estrogens in the aquatic environment is synthetic ethinylestradiol, which is the active estrogen component of the contraceptive pill. Approximately 40% of ethinylestradiol ingested is excreted
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from the body (Johnson and Williams, 2004), therefore contributing significantly to the concentration of steroidal hormones in aquatic systems.

Steroidal hormones, particularly estradiol and ethinylestradiol, are amongst the most potent micropollutant with quantities as small as parts per trillion (ng/L) demonstrated to have significant effects on aquatic organisms (Routledge et al., 1998; Tabata et al., 2001). As early as the mid 1990’s concentrations as low as 0.1 ng/L of ethinylestradiol were shown to stimulate vitellogenin production (yoke precursor associated with females) in male trout exposed to sewage effluent (Purdom et al., 1994). Since this early study there have been many others which have confirmed that low concentrations of steroidal hormones can indeed have adverse effects on aquatic organisms such as an increase in intersex characteristics and general reduced fertility (e.g. Jobling et al., 2002; Jobling et al., 1998).

Therefore, due to the ubiquitous and potent nature of steroidal hormones it is important to understand more about their interactions with organic matter and hence their transport and fate within aquatic systems.

2.2.2. Organic Matter

Organic matter is ubiquitous within aqueous systems and includes NOM surrogates such as fulvic and humic acid as well as non-humic fractions including polysaccharides, polyphenols and surfactants. The different forms of organic matter present in the surface water comes from a number of terrestrial and microbial sources including soils, plants and algal decay as well as anthropogenic sources (McKnight et al., 2001). Consequently, the varying origins, as well as temporal and geographic factors, can make organic matter difficult to characterise (Chen et al., 2002). Due to its heterogeneous nature NOM can vary significantly in terms of polarity, functional group content, molecular weight and hydrophobicity, and these properties may influence micropollutant partitioning (Chin et al., 1997; Schlautman and Morgan, 1993b; Yamamoto et al., 2003). Further, the fate of micropollutants within aquatic systems are also influenced by their interaction with different organic matter phases.
(Pankow and McKenzie, 1991). Within the aquatic environment organic matter can be divided into three distinct phases which include dissolved, colloidal and particulate (Figure 2.1). By studying the three phases separately this can provide a greater insight into organic matter and how it can interact with micropollutants. Therefore, the three phases will be discussed below.

<table>
<thead>
<tr>
<th>Size</th>
<th>1 nm</th>
<th>10 nm</th>
<th>100 nm</th>
<th>1 µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular Weight</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 kDa</td>
<td>10 kDa</td>
<td>100 kDa</td>
<td>1 MDa</td>
<td></td>
</tr>
</tbody>
</table>

| Dissolved Organic Matter | Colloidal Organic Matter | Particulate Organic Matter |

Figure 2.1: Size ranges of the three different phases of organic matter (Adapted from Buffle et al., 1998; Jeanneau et al., 2007; Santschi et al., 1997)

Dissolved organic matter (DOM) can be operationally defined as material which passes through a 0.45 µm filter (Santschi et al., 1997). The molecular weight of DOM can vary from less than 0.1 kDa to greater than 100 kDa as it can contain a mixture of low molecular weight compounds and macromolecules (Leenheer and Croue, 2003). DOM constitutes the largest fraction of organic matter (40-80%) (Müller et al., 2004). Due to its ubiquitous nature this project will mainly focus on the interaction of steroidal hormones with DOM. Quantification of log KOM values can be difficult as physical separation techniques such as filtration or centrifuge cannot completely separate DOM from the aqueous phase (Schwarzenbach et al., 2003). Therefore, a more complex approach is required and different quantification options will be discussed below in Section 2.4.

Particulate organic matter (POM) can be defined as single organic matter particles or aggregates of organic matter that are greater than 0.45 µm (Frimmel, 1998). Unlike the dissolved phase the size of the particulate phase allows it to settle out of solution.
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which has implications for micropollutant removal during water treatment. Similar to the other phases of organic matter, the properties of POM can alter the likelihood of interaction with micropollutants. The size of a particle can have implications for binding behaviour, with smaller particles exhibiting a greater tendency to sorb certain micropollutants due to a greater surface area to volume ratio (Holthaus et al., 2002).

In addition to POM, inorganic particles coated with organic matter can also have implications for micropollutant fate within aquatic systems. Typical inorganic particles include clays such as aluminosilicate and aluminium oxides (Buffle et al., 1998; Schlautman and Morgan, 1993a). The adsorption of organic matter can have an effect on the stability and surface charge of the inorganic particles (Gu et al., 1995). It has been suggested that the sorption of NOM surrogates such as humic and fulvic acid to inorganic particles will stabilise the particles, allowing them to carry micropollutants further within the aquatic environment (Buffle and Leppard, 1995). However, this is not the case for all organic matter types, as Buffle et al. (1998) has also demonstrated that the sorption of polysaccharides can destabilise inorganic particles. As inorganic particles bear the most similarity to the POM phase, it is expected that the likelihood of interaction with micropollutants would be similar to POM.

The third phase is colloidal organic matter (COM), and as shown in Figure 2.1 this phase is difficult to separate from dissolved and particulate phases. The size of COM can range between 10 nm to 1 μm, however, the majority are less than 0.45 μm (Buffle et al., 1998). COM behaves like DOM, yet has the physical and chemical properties of POM (Morel and Gschwend, 1987). Similar to POM, colloids have a large surface area, yet remain in solution similar to DOM (Burgess et al., 1996). Traditionally, the influence of COM in solute-solute interactions has been overlooked due to their unique properties mentioned above. However, recent studies by Bowman et al. (2002), Holbrook et al. (2004) and Liu et al. (2005) have suggested that colloids can interact strongly with steroidal hormones.
Further, colloidal polymers or nanoparticles are also present in the aquatic environment from a number of sources including packing materials, textiles and composite materials (Reijnders, 2006). Nanoparticles typically range in size from 1 to 100 nm and due to their stability they can be considered mobile in the environment (Leppard et al., 2004). A study by Koh et al. (2006) found that NOM sorbed strongly to polysulphone nanoparticles. Consequently, it may be possible for organic coated nanoparticles to influence the fate of micropollutants within the environment.

As organic matter can exist in a number of forms it is likely this will influence the mechanism of interaction. Consequently, Section 2.3 will address the different interaction mechanisms of the solutes discussed above.

2.2.3. Influence of Solution Chemistry

Variations in solution chemistry, namely pH and ionic strength, can have implications for the charge, conformation and solubility of both organic matter and micropollutants. Many natural hormones including estradiol and estrone contain dissociable functional groups and become negatively charged at high pH (>10). Further, several studies have indicated that the charge and conformation of a range of NOM surrogates and polysaccharides change with variations in pH and ionic strength (Avaltroni et al., 2007; Ghosh and Schnitzer, 1980; Piccolo, 2001). For example, Ghosh and Schnitzer (1980) demonstrated that humic acid can change from a rigid and coiled structure at low pH to flexible, linear polyelectrolytes in neutral and alkaline pH solutions due to charge repulsion.

Despite this very few studies have considered the implications of solution chemistry on the interaction of steroidal hormones with organic matter, with the majority of studies focusing on neutral pH. While the pH of surface water is generally considered to be between 6 and 9 (Ra et al., 2008), it can vary naturally. For example, natural surface water from a volcanic crater lakes can have pH values of less than 0.3 due to volcanic gases (Löhr et al., 2005). Further, the pH can also vary during water treatment with some chemical clarification processes requiring alkaline
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pH conditions (Semerjian and Ayoub, 2003). Therefore, an understanding of the influence of solution chemistry on solute-solute interactions is essential to determine the fate of hormones within the environment.

2.3. Solute-Solute Interaction Mechanisms

The interaction of all phases of organic matter with micropollutants is defined as sorption and this can be due to a number of interactions including chemical, electrostatic and physical (Grathwohl, 1998). Sorption is a phase transfer process where micropollutants can attach to the surface of a solid phase or penetrate into an organic matrix (Huang et al., 2003; Schwarzenbach et al., 2003). In organic matter-water systems sorption can be divided into two mechanisms, adsorption and partitioning (absorption), and these will be the focus of this section. Sorption is an extremely important process as the fate of micropollutants freely dissolved in solution can be very different to micropollutants sorbed to organic matter. This will be discussed in Section 2.5.2. Previously, there has been a great deal of uncertainty with terminology associated with sorption processes in the literature, which can impede the understanding of these mechanisms. The terminology in this study was selected for consistency with other reviews (Grathwohl, 1998; Schwarzenbach et al., 2003).

2.3.1. Adsorption

Adsorption is a surface interaction which involves the formation of physical and chemical bonds between the surface of the particulate phase of organic matter and the micropollutant (Rathbun, 2000). This interaction is demonstrated in Figure 2.2. Organic matter bound to inorganic particles and occasionally large colloids can also interact with micropollutants through this mechanism. As particles are solid the micropollutants cannot penetrate the structure (absorb) and therefore they can only adsorb to the surface. Adsorption to organic matter can be characterised by fast kinetics and competition for sorption sites (Luthy et al., 1997). The affinity of the micropollutant to adsorb to the POM is measured using the solid-water distribution
coefficient, $K_D$ (L/kg). This is often normalised for organic matter concentration ($K_{OC}$). The adsorption of micropollutants to POM is of significance in water and wastewater treatment. The large size of POM can increase the potential for micropollutant removal using microfiltration (MF) or ultrafiltration (UF) (Schäfer et al., 2002). Further, previous studies have indicated that coagulation is most suitable for the removal of large particles (Allpike et al., 2005), therefore any micropollutant bound to POM can be removed to a greater extent using conventional water treatment through coagulation and settling compared to micropollutants bound to the dissolved phase.

![Diagram of micropollutant adsorption](Image)

**Figure 2.2:** The adsorption of micropollutants represented by estradiol to particulate organic matter (Adapted from Rav-Acha and Rebhun, 1992)

### 2.3.2. Partitioning

Partitioning or absorption occurs through the process of dissolution, where the micropollutants transfer from the aqueous phase into the dissolved and submicron colloid phases of organic matter (Murphy et al., 1994). This mechanism is demonstrated in Figure 2.3. Partitioning is typically a fast process which lacks competition unlike adsorption (Luthy et al., 1997). The interaction between micropollutants and DOM and COM can be measured by $K_{OM}$ values (L/kg). Similar to adsorption, the partition coefficient is often normalised for organic carbon concentration. The process of partitioning can significantly influence the fate of micropollutants in the aquatic environment. For example, partitioning to certain types of DOM can increase the aqueous solubility of micropollutants with typically low solubility (Chiou et al., 1986) altering their behaviour in the aqueous system.
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This is due to the greater solubility of DOM. Further, partitioning of micropollutants to DOM and COM can lead to a decrease in the adsorption of micropollutants to POM due to increased competition (Bowman et al., 2002; Johnson et al., 1998).

Figure 2.3: The partitioning of micropollutants represented by estradiol to dissolved and colloidal organic matter (Adapted from Rav-Acha and Rebhun, 1992)

2.3.3. Sorption Isotherms

The relationship between freely dissolved and organic matter sorbed micropollutants can be demonstrated using sorption isotherms. These are applicable to all organic matter phases. Sorption isotherms can be considered linear or nonlinear. Linearity occurs when the affinity of a micropollutant for the sorbent remains constant over a concentration range (typically up to one order of magnitude) meaning there is no competition. Consequently, micropollutant fugacity is a linear function of micropollutant concentration in the dissolved and sorbed phases (Karickhoff, 1984). This typically occurs for partitioning due to the dissolution process, but adsorption isotherms may also be linear at low micropollutant concentrations or when the organic matter adsorption sites are not limited and hence there is no competition (Schwarzenbach et al., 2003). However, many adsorption isotherms are nonlinear. Nonlinear isotherms typically occur when the micropollutant concentration exceeds the number of surface adsorption sites leading to competition and eventual saturation of organic matter (Schwarzenbach et al., 2003). There are two main quantitative relationships used to describe adsorption isotherms, Freundlich and Langmuir isotherms.
Chapter 2

The Freundlich isotherm is a mathematical model which uses an empirical relationship to model nonlinear adsorption (Schwarzenbach et al., 2003). The Freundlich equation can be seen below in Equation 2.1 where $C_S$ was the concentration of micropollutants sorbed to POM at equilibrium, $C_W$ was the freely dissolved concentration of micropollutants in solution at equilibrium and $K_F$ was the Freundlich constant. The empirical constant $n$ was used to reflect nonlinearity (Hemond and Fechner-Levy, 2000). This constant can indicate adsorption restrictions, as shown in Figure 2.4. For example, when $n$ is less than 1 there is a reduction in the amount of organic matter binding sites available, which leads to a decrease in adsorption as micropollutant concentration increases (Tremblay et al., 2005). However, when $n$ is greater than 1 there is increased adsorption when the micropollutant concentration increases (Hemond and Fechner-Levy, 2000). If $n$ is equal to 1, the isotherm is linear.

\[ C_S = K_F C_W^n \]  

(2.1)

Figure 2.4: Freundlich isotherm and the influence of the nonlinearity constant ($n$) on sorption of micropollutants to organic matter (Adapted from Schwarzenbach et al., 2003)
The Langmuir isotherm is another empirical model commonly used to quantify the adsorption of micropollutants to POM. Compared to the Freundlich isotherm, the Langmuir isotherm is considered a simpler isotherm to demonstrate binding site limited adsorption (Huang et al., 2003). The Langmuir isotherm is represented by Equation 2.2, where \( q \) was the amount of micropollutant adsorbed, \( K_L \) was the Langmuir constant and \( q_{MAX} \) was the maximum amount of micropollutant adsorbed to the organic matter (Gu et al., 1995). However, this isotherm is limited to POM with homogeneous sorption sites, therefore this may not be relevant for NOM particles as homogeneity is unlikely (Huang et al., 2003). Therefore, when considering POM the Freundlich isotherm may be a more applicable model.

\[
q = \frac{K_L q_{MAX} C_W}{K_L C_W + 1}
\]  

(2.2)

Previous studies have suggested that the interaction of micropollutants with soil and sediment can occur through both adsorption and absorption (Weber et al., 1992). This is due to the heterogeneous nature of organic matter. Consequently, the traditional Freundlich and Langmuir isotherms are often not suitable for systems involving soil, black carbon, soot and sediment. Several author have applied dual models which include combinations of linear, Freundlich and Langmuir isotherms and found a good agreement with experimental data (Xia and Ball, 1999; Xing and Pignatello, 1997). However, as micropollutant sorption to DOM is expected to be linear due to partitioning such sorption isotherms will not be considered further.

2.3.4. Molecular Interaction Mechanisms

While the general sorption mechanisms are discussed above, this section focuses on the specific and non-specific molecular interaction mechanisms for steroidal hormones and organic matter. Based on previous studies, the mechanism of hormone sorption to organic matter appears to be a combination of non-specific (van der Waals) and specific interactions (e.g. hydrogen bonding). Van der Waals forces are
weak attractive forces which occur between any molecule (Goss and Schwarzenbach, 2003). Therefore, they are always present and as they are typically weak it is unlikely they will contribute significantly to sorption. Non-specific interactions will be discussed further in Chapter 6.

Hydrogen bonding is a specific interaction which can only occur between hydrogen donors and acceptors (Goss and Schwarzenbach, 2003). The polarity of a solute can be defined as apolar, monopolar or bipolar (Table 2.1). The majority of steroidal hormones are bipolar, meaning they contain both hydrogen donating and accepting functional groups. These include hydroxyl and phenolic moieties. However, progesterone only contains monopolar ketone groups, therefore it can act as a hydrogen acceptor only. Progesterone can still interact with organic matter through hydrogen bonding provided the organic matter contains bipolar or hydrogen donating groups. Finally, apolar molecules lack hydrogen accepting or donating functional groups and can only interact through non-specific interactions. The majority of organic matter types such as humic acid, fulvic acid, tannic acid and alginic acid contain a wide range of bipolar functional groups including phenolic, carboxylic, amino and hydroxyl moieties. Some anthropogenic organic matter types such as the surfactant sodium dodecyl sulphate (SDS) only contains a monopolar sulphate group, however, they can still interact with hormones.

<table>
<thead>
<tr>
<th>Polarity</th>
<th>Mechanism of Interaction</th>
<th>Functional Group</th>
<th>Steroidal Hormones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apolar</td>
<td>van der Waals forces</td>
<td>Alkanes</td>
<td>-</td>
</tr>
<tr>
<td>Monopolar</td>
<td>van der Waals forces and hydrogen bonding (donating or accepting)</td>
<td>Ketone, sulphate</td>
<td>Progesterone</td>
</tr>
<tr>
<td>Bipolar</td>
<td>van der Waals forces and hydrogen bonding (donating and accepting)</td>
<td>Phenolic hydroxyl, hydroxyl, carboxylic</td>
<td>Estradiol, estrone, testosterone</td>
</tr>
</tbody>
</table>

Further, several researchers have suggested π-electron interactions (aromatic-aromatic interactions) are an important sorption mechanism for steroidal hormones.
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(Holbrook et al., 2003; Yamamoto et al., 2003). \(\pi\)-electron are electron rich, and may contribute to strong interactions between phenolic groups in estradiol and estrone and certain phenolic rich organic matter types, particularly tannic acid. Such aromatic groups have increased polarizability which can increase non-specific interactions between the solutes. This will be discussed further in Chapter 6.

It is unlikely ‘hydrophobic’ or entropy-driven interactions play a large role in solute-solute interactions for steroidal hormones. Hydrophobicity is defined as ‘water-fearing’ and is often used to describe apolar compounds which cannot interact with water through hydrogen bonding. Octanol-water partition coefficients (log K\text{OW}) are an indicator of hydrophobicity. Much of the earlier work on micropollutant sorption focused on apolar micropollutants such as polycyclic aromatic hydrocarbons (PAH) and polychlorinated biphenyls (PCB) with strong correlations between log K\text{OW} and log K\text{OM} values suggesting hydrophobicity influenced partitioning (Kopinke et al., 1995). However, recent steroidal hormone sorption studies have found no correlation with log K\text{OW} values (Holbrook et al., 2004; Liu et al., 2005; Yamamoto et al., 2003). Therefore, it is unlikely that entropy-driven interactions are a significant interaction mechanism for steroidal hormones.

2.4. Quantification of Solute-Solute Interactions

Quantification of solute-solute interactions through the calculation of log K\text{OM} values can assist in determining the fate of micropollutants in the aquatic environment (Mott, 2002). Log K\text{OM} values are related to micropollutant fugacity in the water (\(\phi^w\)) and organic matter (\(\phi^{OM}\)) phases (Equation 2.3). There are several different techniques available to quantify this interaction. These include solid-phase microextraction, equilibrium dialysis, fluorescence quenching, reverse phase partitioning, solubility enhancement, and UF. With the exception of UF, the above techniques can be used to measure solute-solute interactions with DOM. The purpose of this section is to introduce the different techniques, and determine which is the most suitable for quantifying the interaction of steroidal hormones with DOM at environmental concentrations.
\[ K_{OM} = \frac{\phi^W}{\phi^{OM}} \]  

(2.3)

2.4.1. Solid-Phase Microextraction

Solid-phase microextraction (SPME) can quantify solute-solute interactions by measuring the freely dissolved concentration of micropollutants in solution. The use of SPME has increased in recent years mainly due to its ability to separate and sample the solution simultaneously. Further, SPME is a fast, inexpensive and simple technique (Heringa et al., 2002). SPME has been demonstrated as an appropriate technique to quantify the interaction of micropollutants with dissolved (Kopinke et al., 2001), colloidal (King et al., 2004) and particulate (Poerschmann et al., 1997) organic matter phases.

SPME applies a polymer coated fibre to extract micropollutants from solution, and is based on the principle that it is only possible to extract freely dissolved micropollutants while any bound to organic matter will remain in solution (Figure 2.5) (Lord and Pawliszyn, 2000). After the freely dissolved micropollutant has reached equilibrium with the fibre it can be analysed, either through chromatography methods such as gas chromatography or through liquid scintillation counting, provided the micropollutant is radiolabelled.

There are some disadvantages of SPME which may limit its application. Firstly, there is the possibility of fibre fouling by organic matter. There have been many opposing papers published as to whether fibre fouling does occur (e.g. Heringa et al., 2006; Urrestarazu Ramos et al., 1998), however, the consequences of fouling could result in inaccurate quantification of solute-solute interactions. Secondly, the SPME fibres can be susceptible to damage, which can lead to a reduction in the sensitivity of the results. It has been suggested that pH adjustment and high molecular weight compounds can damage the fibres, impeding the ability to extract micropollutants (Lord and Pawliszyn, 2000).
Previous studies have applied SPME to quantify partitioning of estradiol to proteins (Heringa et al., 2002) therefore this technique is suitable for steroidal hormones. Further, Urrestarazu Ramos et al. (1998) applied SPME to quantify the interaction of environmental concentrations (ng/L) of micropollutants such as DDT with organic matter. Consequently, SPME appears to be an appropriate technique to quantify the interaction of steroidal hormones with organic matter at low concentrations.

2.4.2. Equilibrium Dialysis

Equilibrium dialysis applies physical separation to quantify the interaction of micropollutants with organic matter. The advantages of equilibrium dialysis include ease of use and lack of complex equipment (Heringa and Hermens, 2003). Equilibrium dialysis is also suitable for the majority of micropollutants, and is not limited to volatile or fluorescing compounds. In addition, previous studies have indicated equilibrium dialysis is suitable to quantify solute-solute interactions at environmental concentrations (Carter and Suffet, 1982). It is generally used for DOM, however, a study by Gallé et al. (2005) applied equilibrium dialysis to quantify colloid phase solute-solute interactions.
The typical equilibrium dialysis arrangement includes two chambers separated by a membrane with a molecular weight cut off generally around 1000 Da. A known concentration of micropollutants is added to one chamber, while organic matter is added to the other chamber. The micropollutant is able to diffuse across the membrane, and can then interact with the organic matter or remain freely dissolved, while the organic matter cannot pass through the membrane (Figure 2.6). Equilibrium is reached when the chemical potential of the micropollutant is the same on both sides of the membrane. Alternatively, another option for equilibrium dialysis involves a dialysis bag containing organic matter being placed in a container containing micropollutants, with the dialysis bag acting as the membrane (Carter and Suffet, 1982).

![Figure 2.6: Schematic representation of equilibrium dialysis indicating diffusion of micropollutant, represented by estradiol, across the membrane at equilibrium](image)

There are several limitations associated with equilibrium dialysis. Firstly, it can be time consuming as the length of time required to reach equilibrium can range from days to several weeks depending on the studied micropollutant (Akkanen et al., 2005). This long equilibrium time may lead to micropollutant degradation adversely influencing the results. Retention of DOM by the dialysis membrane can be another limitation. Based on Figure 2.1 DOM can contain fractions smaller than 1 kDa, therefore, it is possible that organic matter can pass through the dialysis membrane. Further, previous studies have found significant losses (up to 15%) of organic matter.
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(Akkanen and Kukkonen, 2003; Carter and Suffet, 1982). Depending on dialysis membrane properties, the organic matter loss may be due to adsorption to the membrane, as Jucker and Clark (1994) found organic matter sorbs strongly to hydrophobic UF membranes. Further, it is also possible that micropollutants can sorb to the dialysis membrane.

Yamamoto et al. (2004) studied partitioning of estradiol to synthetic liposomes using equilibrium dialysis; therefore, this technique may be suitable to quantify partitioning of steroidal hormones to organic matter. However, due to the limitations described above, particularly the potential for solute loss due membrane sorption, equilibrium dialysis is not a suitable technique.

2.4.3. Fluorescence Quenching

Fluorescence quenching can quantify solute-solute interactions by measuring fluorescence intensity of micropollutants in solution. Fluorescence quenching is considered a simple, fast and sensitive technique to determine log $K_{OM}$ values (Backhus et al., 2003). Further, no separation of the sample is required eliminating problems associated with equilibrium dialysis and causing minimal disturbance to the equilibrium.

The fluorescence intensity of fluorescing micropollutants will decrease (become quenched) when they are bound to organic matter (Mackenzie et al., 2002) (Figure 2.7). Therefore, the free concentration of micropollutants in a solution is represented by the fluorescence of the solution. In order for fluorescence quenching to quantify solute-solute interactions several assumptions must be made. Firstly, fluorescence quenching assumes that the micropollutant is completely quenched when bound to the organic matter, but that may not always be the case (Backhus and Gschwend, 1990). Secondly, this method assumes that the fluorescence of the freely dissolved micropollutant will not be reduced due to other processes such as photodegradation or molecular oxygen (Backhus et al., 2003). It can be quite difficult to prove these assumptions, therefore they are potential sources of error.
Figure 2.7: Schematic representation of fluorescence quenching, which is based on the principle that fluorescing micropollutants, represented by estradiol, will cease to fluoresce when bound to organic matter.

There are also several other limitations associated with the use of fluorescence quenching. An important limitation is that this technique is only suitable for micropollutants which fluoresce (Gauthier et al., 1986), and consequently it is commonly used for PAHs. Further, the majority of studies use micropollutant concentrations in the µg/L to mg/L range (Chin and Gschwend, 1992; Holbrook et al., 2004) which are not representative of environmental concentrations.

The interaction of estradiol with both DOM and COM has been studied previously using fluorescence quenching (Holbrook et al., 2004; Yamamoto et al., 2003). Yamamoto et al. (2003) found incomplete quenching of estradiol in the presence of polysaccharides. Further Holbrook et al. (2004) suggested that partitioning of estradiol to colloids may be underestimated by the presence of any inorganic matter in colloids as this is unable to quench fluorescent micropollutants. Due to these limitations, fluorescence quenching is not a suitable technique to quantify solute-solute interactions at environmental concentrations.
2.4.4. Reverse Phase Partitioning

Reverse phase partitioning is a physical separation technique which quantifies solute-solute interactions through the retention of freely dissolved micropollutants in solution (Landrum et al., 1984). Micropollutants bound to organic matter are able to pass through a hydrophobic cartridge allowing the bound micropollutant concentration to be quantified experimentally. The freely dissolved concentration is calculated by considering the difference between the initial micropollutant concentration and the bound concentration (Backhus and Gschwend, 1990).

A schematic diagram representing reverse phase partitioning is shown in Figure 2.8. The cartridge, also referred to as Sep-Pak or C-18 cartridge, is a hydrophobic packing material (Heringa and Hermens, 2003) and separation occurs due to micropollutant polarity. This technique assumes that organic matter is too polar to be sorbed to the cartridge and therefore is not retained (Landrum et al., 1984). Therefore, in theory any micropollutants bound to the organic matter will also pass through the cartridge.

![Figure 2.8: Schematic representation of reverse phase partition, demonstrating the retention of micropollutants in the cartridge](image)

Figure 2.8: Schematic representation of reverse phase partition, demonstrating the retention of micropollutants in the cartridge
There are several important problems with reverse phase partitioning that prevent widespread use. Certain micropollutants are not completely retained by the cartridge and display a high breakthrough rate (Fan et al., 1997). This is related to the hydrophobic cartridge and its inability to retain polar micropollutants. For example, as steroidal hormones contain polar functional groups it is likely that freely dissolved hormones would not be completely retained.

Further, there are several limitations associated with the retention time. Despite the typical retention time being relatively short, desorption of bound micropollutants from the organic matter can occur, which leads to an underestimation of the log $K_{OM}$ value (Mott, 2002). However, this problem cannot be solved by reducing the retention time, as this can reduce the retention of freely dissolved micropollutants.

Therefore, due to the problems associated with reverse phase partitioning, particularly its unsuitability for polar steroidal hormones, this technique will not be considered to quantify steroidal hormone solute-solute interactions.

2.4.5. Solubility Enhancement

Solubility enhancement is related to the observation that DOM can increase the solubility of micropollutants in aqueous solution due to partitioning (Chiou et al., 1986). Therefore, solubility enhancement quantifies solute-solute interactions by determining the apparent solubility of a micropollutant in solutions containing different organic matter concentrations and comparing this to the solubility of the micropollutant in pure water. Solubility enhancement is considered by many researchers to be the most reliable quantification technique (Backhus et al., 2003; Tiller and Jones, 1997) as it has the least amount of potential experimental problems. This is because it is not subject to as many assumptions compared to other techniques such as fluorescence quenching.

However, there are several limitations associated with this technique. Firstly, the effectiveness of solubility enhancement in quantifying solute-solute interactions is
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dependent on properties of the organic matter. The organic matter should contain apolar functional groups and have a large molecular weight otherwise a significant increase in solubility will not be observed (Chiou et al., 1986). Similarly, dilute concentrations of organic matter are not expected to enhance solubility greatly. Therefore, organic matter concentrations in excess of 100 mg/L are often required to induce solubility enhancement (Chiou et al., 1986). The typical organic matter concentrations in surface water ranges from 0.2 to 30 mgC/L (Frimmel and Abbt-Braun, 1999; Gjessing et al., 1999). Yamamoto et al. (2003) applied solubility enhancement to quantify the interaction of estradiol with a range of DOM types. Concentrations of polysaccharide dextran as high as 263 mgC/L were required to induce solubility enhancement and this is significantly higher than environmental concentrations. As a result, it is unlikely that solubility enhancement is a suitable technique to quantify solute-solute interactions for steroidal hormones at environmental concentrations.

Within this section a number of solute-solute quantification techniques were compared to determine their applicability to quantify the interaction of steroidal hormones with organic matter at environmental concentrations. Many techniques are restricted to specific micropollutants and organic matter types. However, SPME appears to be a suitable technique as it can quantify solute-solute interactions at environmental concentrations.

2.5. Solute-Solute Interactions in the Literature

2.5.1. Experimental Hormone-Organic Matter Interactions

In Table 2.2 experimental partition and distribution coefficients ($10^3$ L/kg) from the literature were compared in order to assess if steroidal hormones have a greater likelihood of interacting with different organic matter phases. The log $K_{OM}$ and log $K_D$ values presented in Table 2.2 were quantified using a number of different techniques including cross-flow UF, fluorescence quenching and solubility enhancement. Differences between the techniques may, to a limited extent,
contribute to the differences in sorption prediction within the phases. However, Table 2.2 can still provide a general overview of the likelihood of interaction.

Table 2.2: Summary of experimental $K_{OM}$ and $K_D$ values for all phases of organic matter

<table>
<thead>
<tr>
<th>Dissolved Organic Matter</th>
<th>Organic Matter Origin</th>
<th>Hormone</th>
<th>$K_{OM}$ $(10^3 \text{ L/kg})$</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tannic acid</td>
<td>Estradiol</td>
<td>191</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>Suwannee River humic acid</td>
<td>Estradiol</td>
<td>83</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>Alginic Acid</td>
<td>Estradiol</td>
<td>6</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>Dextran</td>
<td>Estradiol</td>
<td>0.6</td>
<td>a</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Colloidal Organic Matter</th>
<th>Organic Matter Origin</th>
<th>Hormone</th>
<th>$K_{OM}$ $(10^3 \text{ L/kg})$</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>River Conwy, UK</td>
<td>Estradiol</td>
<td>22</td>
<td>b</td>
</tr>
<tr>
<td></td>
<td>River Conwy, UK</td>
<td>Estrone</td>
<td>14</td>
<td>b</td>
</tr>
<tr>
<td></td>
<td>Wastewater</td>
<td>Estradiol</td>
<td>&lt;1-179</td>
<td>c</td>
</tr>
<tr>
<td></td>
<td>River Ouse, UK</td>
<td>Estradiol</td>
<td>9</td>
<td>d</td>
</tr>
<tr>
<td></td>
<td>River Ouse, UK</td>
<td>Estrone</td>
<td>15</td>
<td>d</td>
</tr>
<tr>
<td></td>
<td>Tannic acid</td>
<td>Estradiol</td>
<td>191</td>
<td>e</td>
</tr>
<tr>
<td></td>
<td>Aldrich humic acid</td>
<td>Estradiol</td>
<td>83</td>
<td>e</td>
</tr>
<tr>
<td></td>
<td>Alginic Acid</td>
<td>Estradiol</td>
<td>0.4</td>
<td>e</td>
</tr>
<tr>
<td></td>
<td>River Ouse, UK</td>
<td>Estradiol</td>
<td>7-73</td>
<td>f</td>
</tr>
<tr>
<td></td>
<td>River Ouse, UK</td>
<td>Estrone</td>
<td>15-110</td>
<td>f</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Particulate Organic Matter</th>
<th>Organic Matter Origin</th>
<th>Hormone</th>
<th>$K_D$ $(10^3 \text{ L/kg})$</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>River Conwy, UK</td>
<td>Estradiol</td>
<td>0.2</td>
<td>b</td>
</tr>
<tr>
<td></td>
<td>River Conwy, UK</td>
<td>Estrone</td>
<td>0.2</td>
<td>b</td>
</tr>
<tr>
<td></td>
<td>Rivers Aire, Calder and Thames, UK</td>
<td>Estradiol</td>
<td>0.02-0.2</td>
<td>g</td>
</tr>
<tr>
<td></td>
<td>Mississippi River, USA</td>
<td>Estradiol</td>
<td>0.004</td>
<td>h</td>
</tr>
<tr>
<td></td>
<td>Mississippi River, USA</td>
<td>Estrone</td>
<td>0.002</td>
<td>h</td>
</tr>
<tr>
<td></td>
<td>Mississippi River, USA</td>
<td>Testosterone</td>
<td>0.005</td>
<td>h</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Inorganic Particulate Matter</th>
<th>Organic Matter Origin</th>
<th>Hormone</th>
<th>$K_D$ $(10^3 \text{ L/kg})$</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aluminium oxide with humic acid coating</td>
<td>Estradiol</td>
<td>0.04</td>
<td>i</td>
</tr>
<tr>
<td></td>
<td>Aluminium oxide with humic acid coating</td>
<td>Estrone</td>
<td>0.01</td>
<td>i</td>
</tr>
</tbody>
</table>

`^a`Yamamoto et al. 2003; `^b`Bowman et al. 2002; `^c`Holbrook et al. 2004; `^d`Liu et al. 2005; `^e`Yamamoto and Liljestrand, 2003; `^f`Zhou et al. 2007; `^g`Holthaus et al. 2002; `^h`Lee et al. 2003; `^i`Ra et al. 2008

Table 2.2 indicates that the likelihood of interaction of estradiol appears to be similar for both dissolved and colloidal phases. This is because both phases interact with
Solute-Solute Interactions

micropollutants through partitioning which is a dissolution process. POM has a reduced affinity for micropollutants and interacts with micropollutants through adsorption which is a surface effect. Therefore, the difference in the likelihood of interaction may be related to the different mechanisms of interaction between micropollutant and the different phases of organic matter. Further, log $K_{OM}$ values varied with organic matter type, with greater sorption of estradiol to tannic acid compared to alginate acid. Consequently, the influence of organic matter type for partitioning will be explored in Chapter 5.

The majority of studies with hormones focus on COM and POM, with very few studying DOM. This is due to the difficulty associated with determining micropollutant partitioning to DOM. As DOM is the most common organic matter phase it is essential that micropollutant interaction with DOM is better understood. In addition, the few studies that do consider DOM (e.g. Yamamoto et al., 2003) are at unrepresentatively high micropollutant concentrations (~700 μg/L). As steroidal hormones are typically found in the aqueous environment in the ng/L range it is important to study the interaction at low levels to ensure sorption studies are representative. Further, very few studies look at other natural hormones such as progesterone and testosterone. While these are not typically as potent as estradiol, they are often still found in the aquatic environment in significant concentrations (~100 ng/L) (Kolpin et al., 2002) meaning they still may have implications for endocrine disruption.

2.5.2. Fate of Micropollutants in the Aquatic Environment

The sections above have focused on identifying solutes of interest, describing interaction mechanisms and outlining quantification techniques. However, one of the most important reasons to study solute-solute interactions is to understand how such interactions can influence micropollutant fate within the aquatic environment. For example, transport of micropollutants can differ when they are sorbed to organic matter. Several studies have indicated that sorption to COM promotes transport in the environment provided the colloids are stable (Backhus and Gschwend, 1990; Buffle
et al., 1998). In contrast, micropollutants sorbed to POM are more likely to settle out or be removed by filtration. Consequently, micropollutant transport is dependent on organic matter properties.

Further, solute-solute interactions can influence bioavailability and bioaccumulation. Bioavailability is the fraction of micropollutant available for biological activity such as uptake, while bioaccumulation refers to the build up of micropollutants within an organism (Schwarzenbach et al., 2003). Uptake by organisms is also related to the fugacity of the micropollutant. Previous studies have indicated that sorption to organic matter will reduce the bioavailability of micropollutants and hence reduce bioaccumulation in organisms (Akkanen and Kukkonen, 2003; Landrum, 1989). Consequently, micropollutants which sorb weakly to organic matter are more likely to be bioavailable. Therefore, as solute-solute interactions have implications for toxicity and bioaccumulation in organisms it is important to be able to quantify this interaction.

2.6. Implications of Solute-Solute Interactions for Micropollutant Removal by Membrane Filtration

At present there is a limited understanding of the influence of solute-solute interactions in water and wastewater treatment. Previous studies have demonstrated incomplete removal of micropollutants during wastewater treatment (e.g. Baronti et al., 2000; Carballa et al., 2004; D’Ascenzo et al., 2003). Therefore, it is important to understand how solute-solute interactions can influence micropollutant removal. This section will focus on membrane filtration as high removal of steroidal hormones was observed in the literature (e.g. Nghiem et al., 2004b). Several studies have indicated that the presence of organic matter can alter micropollutant retention by membrane filtration (e.g. Agbekodo et al., 1996; Devitt and Wiesner, 1998; Hu et al., 2007; Ng and Elimelech, 2004). The majority of studies in this area focus on nanofiltration (NF) due to its high removal of NOM and some micropollutants. Within the literature, several removal mechanisms have been suggested including membrane sorption, solute-foulant interactions and solute-solute interactions (Figure 2.9). This
Solute-Solute Interactions

Figure 2.9: Micropollutant removal mechanisms in membrane filtration

2.6.1. Membrane Sorption

The influence of organic matter on membrane sorption is variable and is dependant on the properties of the micropollutant, organic matter and membrane. Some studies have found decreased micropollutant sorption in the presence of organic matter, and attributed this to competition for sorption sites (Chang et al., 2003; Comerton et al., 2007; McCallum et al., 2008). In contrast, other studies have demonstrated increased micropollutant sorption suggesting that organic matter leads to modification of the membrane, allowing for greater sorption (Hu et al., 2007). Further, Jin et al. (2007) observed different sorption behaviour with different organic matter types, indicating the influence of organic matter type on micropollutant removal. Solution chemistry can also have implications for membrane sorption. For example, Hu et al. (2007) found greater sorption of estrone in the presence of humic acid at pH 4 compared to pH 7. The decrease in sorption with increasing pH was related to changing properties of the studied organic matter such as charge and conformation. Membrane sorption can be quantified through experimental mass balance equations.
2.6.2. Solute-Foulant Interactions

Another common mechanism for micropollutant removal is solute-foulant interactions, which are typically indicated by flux decline. According to Aoustin et al. (2001) organic matter fouling can occur through one or more of the following mechanisms including accumulation of organic matter on the membrane to form a cake layer, sorption to the membrane surface or sorption into the membrane leading to pore blocking. Within the literature, the influence of solute-foulant interactions appears to be highly variable and dependent on properties of the micropollutant such as charge and molecular weight as well as organic matter properties and membrane material. Several studies have attributed improved steroidal hormone removal in the presence of organic matter to solute-foulant interactions (Hu et al., 2007; Jermann et al., 2009).

There are three main mechanisms which can influence micropollutant retention in fouled membranes including enhanced concentration polarisation, pore blocking and adsorption to the fouling layer (Nghiem and Hawkes, 2007). Whether micropollutant retention will increase or decrease appears to be primarily related to membrane pore size as well as experimental conditions such as the presence of inorganic ions.

Ng and Elimelech (2004) studied the retention of estradiol and progesterone by reverse osmosis (RO) membranes in the presence of colloidal fouling. The results indicated retention of the studied hormones decreased in the presence of colloidal fouling. This was attributed to the reduction in hormone back diffusion from the membrane surface due to the fouling layer. This led to an accumulation of hormones on the membrane causing a greater concentration gradient which assisted with the diffusion of hormones across the membrane to the permeate side (Ng and Elimelech, 2004). Nghiem and Hawkes (2007) also observed decreased micropollutant retention by increased concentration polarisation for tight NF membranes.

In contrast, several studies have indicated increased micropollutant retention due to organic fouling. For looser NF membranes pore blocking by organic matter has been
suggested to reduce micropollutant transport through the membrane leading to increased retention (Nghiem and Hawkes, 2007; Nghiem et al., 2008). Further, it has been suggested that the presence of the fouling layer will reduce the interaction of micropollutants with the membrane leading to a reduction in micropollutant diffusion through the membrane (Nghiem et al., 2008). Plakas et al. (2006) and McCallum et al. (2008) observed increased micropollutant retention by NF due to the presence of organic matter suggesting that the fouling layers acted as a second barrier.

Further, solute-foulant interactions can have implications for membrane modification which can consequently influence the retention and sorption of micropollutants. Childress and Elimelech (1996) studied the influence of humic acid on the charge of selected NF and RO membranes and found the membrane charge became more negative in the presence of humic acid. This was most prominent at acidic pH and in the presence of divalent ions, such as calcium and magnesium (Childress and Elimelech, 1996). The influence of an anionic surfactant, SDS, on NF and RO membranes was also studied and was found to influence membrane charge and hydrophobicity (Childress and Elimelech, 1996; Childress and Elimelech, 2000).

Due to the potentially altered membrane charge and hydrophobicity, the modification of membrane surfaces by organic matter is expected to have an influence on the removal of micropollutants. Xu et al. (2006) suggested modification of NF membranes by organic matter can have opposing consequences for micropollutant retention. Firstly, increased retention of charged micropollutants may be observed due to increased negativity of the fouled membrane (Donnan exclusion). Secondly, retention of non-ionic micropollutants may decreased due to pore expansion caused by the increased negative charge (Xu et al., 2006).

2.6.3. Solute-Solute Interactions

While the above two mechanism feature prominently in the literature, other studies have suggested that solute-solute interactions can lead to increased micropollutant removal (Dalton et al., 2005; Devitt et al., 1998; Schäfer et al., 2006). However,
these studies were unable to quantify such interactions. It was hypothesised that hydrogen bonding between the micropollutant and the organic matter contributes to increased micropollutant retention through size exclusion (Devitt and Wiesner, 1998). Previous studies have suggested that this interaction is influenced by organic matter type and concentration as well as solution chemistry (Agbekodo et al., 1996; Nghiem et al., 2004a; Nghiem et al., 2005).

Recently, several studies have applied log $K_{OM}$ values to quantify solute-solute interactions in membrane filtration (Hu et al., 2007; Jermann et al., 2009; Jin et al., 2007). However, these studies applied log $K_{OM}$ values calculated for different micropollutants and at different concentrations (Hu et al., 2007; Jin et al., 2007) or for hydrophobic membranes (Jermann et al., 2009) where other mechanisms such as membrane sorption and fouling will dominate. Therefore, using log $K_{OM}$ values calculated with the same experimental conditions used in the membrane filtration experiments it may be possible to quantify the influence of solute-solute interactions. This will be discussed further in Chapter 7.

2.6.4. Micropollutant Removal by Membrane Hybrid Processes

The majority of the above studies applied NF to remove micropollutants from solution. As NF requires high pressure, it may be possible to combine another membrane process such as UF with a sorbent to improve micropollutant removal while reducing energy consumption. Therefore, as an alternative steroidal hormone removal technique it may be possible to combine UF with an ion exchange (IX) resin. Anionic IX resins such as magnetic ion exchange (MIEX®) are used to adsorb weak organic acidic ions from water and were developed to remove NOM from raw drinking water (Allpike et al., 2005; Boyer and Singer, 2006; Humbert et al., 2007). Further, MIEX® has also been applied to remove other contaminants from solution, including chloride, bromide, nitrate and sulphate (Boyer and Singer, 2006; Humbert et al., 2005), as well as pesticides such as atrazine and isoproturon (Humbert et al., 2008; Humbert et al., 2005) and pharmaceuticals such as antibiotics (Choi et al., 2007).
The application of IX-UF hybrid processes to improve NOM removal has previously been studied in the literature (Humbert et al., 2007; Kabsch-Korbutowicz et al., 2008; Son et al., 2005; Zhang et al., 2007; Zhang et al., 2006). Son et al. (2005) demonstrated that an IX-UF process with a 100 kDa membrane led to significantly higher removal of dissolved organic carbon (69% removed) compared to a coagulation-UF process (40%). As MIEX® has been previously applied to remove micropollutants, it may be possible to enhance steroidal hormone removal using an IX-UF hybrid process, and this will be discussed further in Chapter 8.

2.7. Conclusions

Solute-solute interactions have implications for both natural and engineered aquatic systems, particularly when considering separation on a molecular level. Both organic matter and estrogenic micropollutants are ubiquitous in aquatic systems, and the interaction of these solutes can affect the distribution of the micropollutants. There is a higher likelihood of interaction when steroidal hormones interact with dissolved and colloidal phases of organic matter through partitioning, as opposed to adsorption to the particulate phase. The difference can be attributed to the different types of bonding, as partitioning occurs through stronger chemical bonds, while adsorption is mainly achieved through weaker physical interactions.

In order to determine log $K_{OM}$ values it is necessary to find a technique to quantify solute-solute interactions. While there appears to be a large number of quantitative techniques applicable to measure these interactions, the majority are limited to specific micropollutants and organic matter types and concentrations. As SPME is suitable for steroidal hormones and can quantify solute-solute interactions at environmental concentrations it appears to be the most suitable technique and will be discussed further in Chapters 3, 4, and 5.

While much of the focus in the current literature centres on membrane sorption and solute-foulant interactions, solute-solute interactions can also have implications for micropollutant removal by membrane filtration. The application of log $K_{OM}$ values to
quantify solute-solute interactions in membrane filtration will be discussed further in Chapter 7.
3 Materials and Methods

In this chapter the materials and methods applied in this project will be outlined. The materials included chemicals, organic matter, magnetic ion exchange (MIEX®) resin, solid-phase microextraction (SPME) fibre and ultrafiltration (UF) membranes.

The analytical equipment used in this study will be described and includes a liquid scintillation counter, total organic carbon analyser, solid-state $^{13}$C nuclear magnetic resonance (NMR) and focused ion beam scanning electron microscopy (FIB-SEM).

The experimental protocols used to determine organic matter-water partition coefficients ($\log K_{OM}$) will be described as will the filtration protocols used for stirred cell UF.

The chapter will conclude with error calculation techniques applied in this study. Further information regarding materials and methods can be found in Appendices 1, 2 and 3.
Materials and Methods

3.1. Chemicals and Background Electrolyte

All chemicals were of analytical grade and were purchased from Fisher Scientific (Loughborough, UK). The solution was buffered using 1 mM NaHCO₃ with 20 mM NaCl to act as a background electrolyte. In some experiments the influence of ionic strength was studied and concentrations of 0, 20, 50 and 100 mM NaCl were used. In 0 mM experiments a negligible NaCl concentration was present due to pH adjustment. pH was adjusted from 3 to 12 using 1 M NaOH and HCl. Pure water (18.2MΩ/cm) was obtained from Elga PURELAB Ultra (High Wycombe, UK).

3.2. Organic Matter

Several different types of organic matter were studied including natural organic matter (NOM) surrogates, polysaccharides, polyphenols and surfactants. These were selected as representatives of organic matter found in water and wastewater. Suwannee River standard humic acid II (HA) (Cat. No. 2S101H), fulvic acid (FA) (Cat. No. 1S101F) and NOM (Cat. No. 1R101N) were purchased from the International Humic Substances Society (IHSS) (St. Paul, USA). Australian NOM was concentrated using microfiltration (MF) and reverse osmosis (RO) from Brisbane Water National Park, Australia and was extensively characterised (Schäfer, 2001). Aldrich humic acid (HA) (sodium salt), alginic acid (sodium salt) from Macrocystis pyrifera, powder cellulose, colloidal cellulose, tannic acid, and sodium dodecyl sulphate (SDS) (≥96%) were all purchased from Sigma Aldrich (Gillingham, UK). Dextran was purchased from Acros Organics (Geel, Belgium). Aldrich HA may not be an accurate representative of a natural terrestrial humic acid (Malcolm and MacCarthy, 1986) and previous studies have indicated that it is really colloidal organic matter (COM) rather than dissolved organic matter (DOM) (Costa et al., 2006). However, it was still selected as it is frequently studied in the literature. Selected characteristics of the organic matter used in this study and their origins are provided in Table 3.1.
The majority of experiments focused on only three organic matter types, Aldrich HA, alginic acid and tannic acid. Humic acid stems from a wide range of sources including vegetation, peat, coal and soil, and can be considered ubiquitous in most aquatic environments (Thurman and Malcolm, 1981). Due to the high content of the strong acidic functional group carboxylic acid (Table 3.2), the acid dissociation constant (pKₐ) of Aldrich HA is around 4.26 (Shin et al., 1999). Alginic acid, a polysaccharide, is the main constituent of brown algae, therefore it is found in surface and wastewaters (Davis et al., 2003). Alginic acid is composed of mannuronic (~60%) and guluronic (~40%) acids (De Stefano et al., 2006) and the pKₐ for mannuronic and guluronic acids are 3.4 and 3.7 respectively (de Kerchove and Elimelech, 2006). Tannic acid is an abundant plant polyphenol, and is present in surface waters due to leaching from vegetation (Cruz et al., 2000). Due to the presence of phenolic hydroxyl groups (Table 3.2) the pKₐ of tannic acid is around 8.5 (Kraal et al., 2006).

The concentration of organic matter in natural waters can vary greatly, and can range from 0.2 to 30 mgC/L (Frimmel and Abbt-Braun, 1999; Gjessing et al., 1999). For most experiments, an organic matter concentration of 12.5 mgC/L was used. The influence of organic matter concentration was also studied in some experiments with concentrations of 12.5, 25, 50 and 125 mgC/L.
## Materials and Methods

**Table 3.1: Characteristics of the organic matter**

<table>
<thead>
<tr>
<th>Organic Type</th>
<th>Molecular Formulae</th>
<th>Category</th>
<th>Charge at pH 7</th>
<th>Origin in Water</th>
<th>Carbon %</th>
<th>Carbon Composition % ($^{13}$C NMR)</th>
<th>Molecular Weight (g/mol)</th>
<th>SUVA* (L/mg.m)</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alginic acid Powder and colloidal cellulose</td>
<td>$[C_{6}H_{7}NaO_{6}]<em>n$ $[C</em>{6}H_{10}O_{5}]_n$</td>
<td>Polysaccharide</td>
<td>Brown seaweed and algae</td>
<td>Toilet paper, plant decay</td>
<td>36</td>
<td>-</td>
<td>12000-80000</td>
<td>-</td>
<td>a,b</td>
</tr>
<tr>
<td>Dextran</td>
<td>$[C_{6}H_{10}O_{5}]_n$</td>
<td>Polysaccharide</td>
<td>Neutral</td>
<td>Bacterial product</td>
<td>44</td>
<td>-</td>
<td>81026-810264</td>
<td>-</td>
<td>c</td>
</tr>
<tr>
<td>IHSS Fulvic Acid</td>
<td>-</td>
<td>NOM surrogate</td>
<td>Peat and decomposing vegetation</td>
<td></td>
<td>53</td>
<td>Aliphatic: 49 Aromatic: 24 Carboxyl: 20 Carbonyl: 7</td>
<td>1000-2300</td>
<td>3.30</td>
<td>f,g,h,i</td>
</tr>
<tr>
<td>IHSS Humic Acid Aldrich Humic Acid</td>
<td>-</td>
<td>NOM surrogate</td>
<td>Peat and decomposing vegetation (IHSS HA) and soil or peat (AHA)</td>
<td></td>
<td>53-56</td>
<td>Aliphatic: 37 (IHSS); 59 (AHA) Aromatic: 37 (IHSS); 26 (AHA) Carboxyl: 19; (IHSS); 9 (AHA) Carboxyl: 8 (IHSS); 6 (AHA)</td>
<td>600-60000</td>
<td>4.07 (AHA), 4.22 (IHSS HA)</td>
<td>f,g,h,i,j</td>
</tr>
<tr>
<td>IHSS NOM Australian NOM†</td>
<td>-</td>
<td>NOM surrogate</td>
<td>Peat, soil and decomposing vegetation</td>
<td></td>
<td>6.3-53</td>
<td>Aliphatic: 49 (IHSS) Aromatic: 23 (IHSS) Carboxyl: 20 (IHSS) Carboxyl: 8 (IHSS)</td>
<td>1381 (Aus NOM)</td>
<td>3.05 (IHSS NOM), 2.58 (AUS NOM)</td>
<td>g, i</td>
</tr>
<tr>
<td>SDS Tannic Acid</td>
<td>$CH_{3}(CH_{2})<em>{11}OSO</em>{3}Na$ $C_{7}H_{5}O_{6}$</td>
<td>Surfactant</td>
<td>Detergent</td>
<td>Plants</td>
<td>50</td>
<td>-</td>
<td>288</td>
<td>-</td>
<td>k</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>54</td>
<td>-</td>
<td>1701</td>
<td>4.23</td>
<td>e</td>
</tr>
</tbody>
</table>

* Specific ultraviolet absorption at 254 nm (Measured data); † Salt composition (Schäfer, 2001)

_aDavis et al. 2003; _bLee and Elimelech, 2007; _cXing et al. 1994; _dBuffle et al. 1998; _eYamamoto et al. 2003; _fChin et al. 1994; _gSchäfer, 2001; _hSimpson, 2001; _iThorn et al. 1989; _jMalcolm and MacCarthy, 1986; _kSingh and Song, 2006_
Table 3.2: Selected characteristics of Aldrich HA, alginic acid and tannic acid

<table>
<thead>
<tr>
<th></th>
<th>(pK_a)</th>
<th>Total acidity (meq/g)</th>
<th>Carboxylic groups (meq/g)</th>
<th>Hydroxyl groups (meq/g)</th>
<th>Conformation changes with pH</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldrich Humic Acid</td>
<td>4.3</td>
<td>7.06</td>
<td>4.80</td>
<td>2.26</td>
<td>Low pH: Coiled and rigid</td>
<td>a, b, c</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>High pH: Linear and flexible</td>
<td></td>
</tr>
<tr>
<td>Alginic acid</td>
<td>3.4, 3.7</td>
<td>8.65</td>
<td>7.02</td>
<td>1.63</td>
<td>Low pH: Coiled at pH 3, but size and flexibility increases from pH 4</td>
<td>d, e, f, g</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>High pH: Depolymerises above pH 8</td>
<td></td>
</tr>
<tr>
<td>Tannic Acid</td>
<td>8.5</td>
<td>11.4</td>
<td>1.88</td>
<td>9.55</td>
<td>pH &gt; 6.5: Hydrolysis to gallic acid which increases with pH</td>
<td>h, i, j</td>
</tr>
</tbody>
</table>

\(\text{aShin et al. 1999; \text{bKim et al. 1990; \text{cGhosh and Schnitzer, 1980; \text{dDavis et al. 2003; \text{eJeon et al. 2002; \text{fAvaltroni et al. 2007; \text{gde Kerchove and Elimelech, 2006; \text{hKraal et al. 2006; \text{iFlores-Céspedes et al. 2006; \text{jOsawa and Walsh, 1993.}}}}}}}}

3.3. Steroidal Hormones

A range of steroidal hormones were studied. Tritium labelled \([2,4,6,7-^3\text{H}]17\beta\)-estradiol (3.15 TBq/mmol), \([1,2,6,7-^3\text{H}]\)progesterone (3.52 TBq/mmol) and \([1,2,6,7-^3\text{H}]\)testosterone (2.70 TBq/mmol) were purchased from GE Healthcare (Little Chalfont, UK). \([2,4,6,7-^3\text{H}]\)estrone (3.55 TBq/mmol) purchased from GE Healthcare (Little Chalfont, UK) was used in Chapters 4 and 5, while \([2,4,6,7-^2\text{H}]\)estrone (2.449 TBq/mmol) purchased from Perkin Elmer (Beaconsfield, UK) was used in Chapters 7 and 8. Radiolabelled hormones were used in all experiments due to their sensitivity and ease of detection using liquid scintillation counters. 100 µg/L stock solutions were prepared in methanol from the initial stock. The properties of the studied hormones are shown in Table 3.3. The studied hormone concentration range was from 100 ng/L to 1000 µg/L. For concentrations greater than 100 ng/L, radiolabelled hormones were mixed with non-labelled hormones (\(\geq 98\%\) purity) (Sigma Aldrich, Gillingham, UK).

While structurally similar, steroidal hormones vary in terms of functional group content, hydrogen bonding ability, hydrophobicity and solubility. Estradiol and estrone contain a phenolic hydroxyl functional group in the C-3 position, therefore
they dissociate in alkaline solutions. The pKₐ values for estradiol and estrone are 10.23 and 10.34 respectively (Kwon et al., 2006). As progesterone and testosterone do not contain functional groups which dissociate within the studied range (pH 3 to 12) they remain neutrally charged. Octanol-water partition coefficients (log K<sub>OW</sub>), which can indicate hydrophobicity, are also shown in Table 3.3 for all hormones. Further, the studied hormones contain different functional groups which will affect their hydrogen bond ability and consequently their partitioning to the solid-phase microextraction (SPME) fibre and organic matter.

### Table 3.3: Properties of studied steroidal hormones

<table>
<thead>
<tr>
<th></th>
<th>Estradiol</th>
<th>Estrone</th>
<th>Progesterone</th>
<th>Testosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structure</td>
<td><img src="image" alt="Structure" /></td>
<td><img src="image" alt="Structure" /></td>
<td><img src="image" alt="Structure" /></td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>Formula</td>
<td>C₁₈H₂₄O₂</td>
<td>C₁₈H₂₂O₂</td>
<td>C₂₁H₃₀O₂</td>
<td>C₁₉H₂₈O₂</td>
</tr>
<tr>
<td>Molecular Weight (g/mol)</td>
<td>272.4</td>
<td>270.4</td>
<td>314.5</td>
<td>288.4</td>
</tr>
<tr>
<td>pKₐ&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.23</td>
<td>10.34</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>log K&lt;sub&gt;OW&lt;/sub&gt; (pH 7)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.01</td>
<td>3.13</td>
<td>3.87</td>
<td>3.32</td>
</tr>
<tr>
<td>Polarity</td>
<td>Bipolar</td>
<td>Bipolar</td>
<td>Monopolar</td>
<td>Bipolar</td>
</tr>
</tbody>
</table>

<sup>a</sup>Kwon et al. 2006; <sup>b</sup>Hansch et al. 1995

### 3.4. SPME Fibre Characterisation

#### 3.4.1. Fibre Type

Polyacrylate (PA) fibre was purchased from Polymicro Technologies (Phoenix, USA), while polydimethylsiloxane (PDMS) fibre was provided by the Escher group at Eawag. The PDMS fibres were originally from Supelco (Bellefonte USA). Properties of the fibres are shown in Table 3.4. Polar PA is suitable for polar micropollutants such as steroidal hormones, while apolar PDMS is more applicable to apolar micropollutants such as polycyclic aromatic hydrocarbons (PAH) (Lord and Pawliszyn, 2000). Environmental scanning electron microscope (ESEM) images of
PA fibre are shown in Figure 3.1. In all SPME experiments 5 cm of polymer fibre was used, giving a volume of 0.77 µL for PA and 0.63 µL for PDMS.

Table 3.4: Characteristics of PA and PDMS fibres

<table>
<thead>
<tr>
<th></th>
<th>Polyacrylate (PA)</th>
<th>Polydimethylsiloxane (PDMS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polarity</td>
<td>Polar</td>
<td>Apolar</td>
</tr>
<tr>
<td>Analyte uptake method</td>
<td>Absorption</td>
<td>Absorption</td>
</tr>
<tr>
<td>Polymeric structure</td>
<td>Non-porous amorphous</td>
<td>Non-porous amorphous</td>
</tr>
<tr>
<td>Thickness (µm)</td>
<td>34.5 µm</td>
<td>29 µm</td>
</tr>
<tr>
<td>Volume (µL)</td>
<td>0.77 µL</td>
<td>0.63 µL</td>
</tr>
</tbody>
</table>

*a* Lord and Pawliszyn 2000; *b* Heringa *et al.* 2006; *c* Chen 2004

Figure 3.1: a) Planar view of PA fibre with glass core exposed; b) Top view of PA fibre using an ESEM

3.4.2. Fibre Charge

The influence of pH on the zeta potential of the PA fibre is shown in Figure 3.2. Fibres were cut into 2 mm pieces and placed in a cell with a platinum electrode on either side. Electrolyte solution (0.1 mM KCl) was passed through the cell from 0.13 to 0.4 Bar (original units 10 to 30 cm Hg). The potential was applied using a potentiostat (AMEL, Milan, Italy). Figure 3.2 indicated no significant difference in zeta potential (mV) within the studied range. Further, pressure did not have a significant influence on zeta potential. However, the absolute value of zeta potential shown in Figure 3.2 is too low to be considered accurate.
3.5. MIEX®

The ion exchange (IX) resin used in this study was a strong anion exchange resin (AER) called magnetic ion exchange (MIEX®) resin. MIEX® samples (batch number 16.221) were supplied by Orica Watercare (Melbourne, Australia). MIEX® has a magnetic iron oxide core with a macroporous polyacrylate shell that contains quaternary amide functional groups to assist with ion exchange (Johnson and Singer, 2004). Compared to conventional IX resins, MIEX® has a relatively high specific surface area, a fast reaction rate with NOM and magnetic properties (Son et al., 2005). Prior to the experiments MIEX® was introduced into a 10 mL syringe and allowed to settle for 10 minutes. Syringes were required as MIEX® is stored in a slurry form. A concentration of 10 mL/L was used in all experiments.

3.6. Membranes

Regenerated cellulose ultrafiltration (UF) membranes with a polypropylene or polyethylene support layer were supplied by Millipore (Bedford, US). PL series membranes with a polypropylene support layer were used in Chapter 7 and included
Chapter 3

PLAC, PLBC, PLCC, PLGC, PLTK and PLHK. The molecular weight cut-off (MWCO) ranged from 1 to 100 kDa. The ion exchange-ultrafiltration (IX-UF) experiments in Chapter 8 used PLC series membranes PLCCC, PLCGC, PLCTK and PLCHK instead of PLCC, PLGC, PLTK and PLHK. PLC membranes are regenerated cellulose with polyethylene support and offer greater mechanical stability compared to the PL series. Pure water flux and calculated pore diameters for all membranes is shown in Table 3.5. The membrane pore diameters and molecular diameters of the studied organic matter types and steroidal hormones were estimated using Equation 3.1 where \( r \) was the pore radius (m) and \( M \) was molecular weight (Da). This equation was determined by Schäfer (2001) and adapted the Stokes-Einstein equation (Equation 3.2) using a diffusion constant \( (D_L) \) equation (Equation 3.3) developed by Worch (1993) where:

\[
r = 2.037 \times 10^{-11} M^{0.53}
\]  
\[
r = \frac{K_BT}{6\pi\eta D}
\]  
\[
D_S = 3.595 \times 10^{-14} \frac{T}{\eta M^{0.53}}
\]

\( K_B \) was Boltzmann’s constant (J.K)  
\( T \) was temperature (K)  
\( \eta \) was dynamic viscosity (Pa.s)  
\( D \) was diffusivity (m²/h)  
\( D_S \) was solute diffusion constant (m²/s)

Regenerated cellulose was selected as it is hydrophilic (contact angle 26±3° (Pieracci et al., 1999)); therefore, minimal organic sorption was expected. Prior to the experiment the membranes were soaked overnight in deionised water.
### Table 3.5: Studied membrane properties with standard deviation (S.D) for pure water flux and permeability

<table>
<thead>
<tr>
<th>Membrane Type</th>
<th>MWCO (kDa)</th>
<th>Pore Diameter (nm)*</th>
<th>Pressure (Bar)</th>
<th>Pure Water Flux ± S.D (L/m².h)</th>
<th>Permeability ± S.D (L/m².bar)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLAC</td>
<td>1</td>
<td>1.58</td>
<td>5</td>
<td>21 ± 2</td>
<td>4</td>
</tr>
<tr>
<td>PLBC</td>
<td>3</td>
<td>2.84</td>
<td>5</td>
<td>32 ± 3</td>
<td>6</td>
</tr>
<tr>
<td>PLCC</td>
<td>5</td>
<td>3.72</td>
<td>5</td>
<td>50 ± 6</td>
<td>10</td>
</tr>
<tr>
<td>PLGC</td>
<td>10</td>
<td>5.37</td>
<td>5</td>
<td>80 ± 6</td>
<td>16</td>
</tr>
<tr>
<td>PLTK</td>
<td>30</td>
<td>9.61</td>
<td>1</td>
<td>281 ± 41</td>
<td>281</td>
</tr>
<tr>
<td>PLHK</td>
<td>100</td>
<td>18.20</td>
<td>0.5</td>
<td>348 ± 32</td>
<td>697</td>
</tr>
<tr>
<td>PLCCC</td>
<td>5</td>
<td>3.72</td>
<td>3</td>
<td>30 ± 3</td>
<td>10</td>
</tr>
<tr>
<td>PLCGC</td>
<td>10</td>
<td>5.37</td>
<td>3</td>
<td>306 ± 20</td>
<td>102</td>
</tr>
<tr>
<td>PLCTK</td>
<td>30</td>
<td>9.61</td>
<td>1</td>
<td>197 ± 3</td>
<td>197</td>
</tr>
<tr>
<td>PLCHK</td>
<td>100</td>
<td>18.20</td>
<td>0.5</td>
<td>392 ± 51</td>
<td>784</td>
</tr>
</tbody>
</table>

* Measured using Equation 3.1.

### 3.7. Stainless Steel Stirred Cells

Three stainless steel cells were used in parallel and are shown in Figure 3.3. The volume of the cell was 990 mL, with internal diameter of 70 mm (membrane surface area 38.48 cm²). Stirred cell schematic diagrams are shown in Appendix 1. All cells contained a magnetic stirrer assembly (Millipore, Watford, UK) and the cells were stirred at 300 RPM using a magnetic stirrer table (Fisher Scientific, Loughborough, UK). The cells were pressurised using compressed lab air. Permeate mass was measured using an Ohaus Adventurer Pro electronic balance (Leicester, UK). The temperature and pressure within each cell was measured using a thermocouple and pressure transducer and the data was collected using a data acquisition module (OMB-DAQ-56) which were all purchased from Omega Engineering (Irlam, UK). The data from the data acquisition module and the balances were collected using LabView 8.0 (National Instruments, Newbury, UK).
3.8. Analytical Equipment

3.8.1. Liquid Scintillation Counter

The hormone concentration in the SPME experiments, and the feed, permeate and concentrate samples in the stirred cell experiments were measured using a Beckman LS 6500 liquid scintillation counter (Fullerton, USA). Radioactivity was measured in disintegrations per minute (dpm). In 20 mL glass scintillation vials the hormone sample was dissolved in 7 mL of Ultima Gold LLT liquid scintillation cocktail (Perkin Elmer, Beaconsfield, UK). The samples were then counted for 10 minutes in triplicate to ensure accuracy. The activity in dpm was converted to hormone concentration in ng/L based on a calibration curve which used hormone concentrations from 0.01 to 1000 ng/L (Figure 3.4). The average detection limit for estradiol was 1.14 ng/L. The other studied hormones had similar detection limits (refer to Appendix 2).
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3.8.2. Total Organic Carbon Analyser

The organic matter concentration in the UF feed, permeate and concentrate samples were measured using a total organic carbon analyser (TOC-V CPH) in non-purgeable organic carbon (NPOC) mode (Shimadzu, Milton Keyes, UK). Prior to analysis the samples were acidified using 2 M HCl and sparged for 1:30 minutes to remove inorganic carbon. The oxidation efficiency of the TOC analyser is discussed in Appendix 2.

3.8.3. UV-Visible Spectrophotometer

Organic matter absorbance at 254 nm was measured using a Cary 100 Scan UV-visible spectrophotometer (Palo Alto, USA). This was used to determine the specific ultraviolet absorbance (SUVA) by dividing absorption at 254 nm by organic carbon concentration. SUVA was determined for most studied organic matter types and is an indicator of aromaticity (Weishaar et al., 2003).
3.8.4. Particle Size Analyser

The particle size and zeta potential of Aldrich HA and MIEX® were measured using a ZetaPALS Zeta Potential analyser (Brookhaven Instruments, Holtsville, USA). The charge and molecular size of Aldrich HA were determined as a function of solution chemistry. To study the influence of pH a solution containing 1 mM NaHCO₃, 20 mM NaCl and 12.5 mgC/L Aldrich HA was adjusted to pH 3, 8 and 12, while ionic strength was studied using 1 mM NaHCO₃ with NaCl concentrations of 0, 20, 50 and 100 mM at pH 8. The average effective diameter and zeta potential were measured based on 10 runs. To measure zeta potential a palladium electrode was inserted into the cell. The particle size and zeta potential of MIEX® was measured using a background electrolyte of 1 mM NaHCO₃, 20 mM NaCl background electrolyte and 10 mL/L MIEX®. The effective diameter was measured at pH 8, while zeta potential was measured at pH 8 and 11.

3.8.5. Optical Microscope

MIEX® was imaged using an optical microscope (Zeiss Axioskop 2) at 10 times magnification (Figure 3.5). Polarised light was used. As MIEX® is in a slurry form a recessed slide was used to image the sample.

![Figure 3.5: Optical microscope image of MIEX® beads at 10x magnitude (Zeiss Axioskop 2, polarized light)](image)
Organic matter and PA fibres were analysed using solid-state $^{13}$C cross-polarisation magic-angle spinning (CPMAS) nuclear magnetic resonance (NMR). Solid-state $^{13}$C NMR is spectroscopic technique which can quantify carbon functional groups such as aromatic, carbohydrate and aliphatic. A Varian VNMRS spectrophotometer (Palo Alto, USA) was operated at 100.56 MHz with a 4 mm rotor probe. Prior to analysis, 5 cm PA fibres were shaken for 48 hours in 100 mL flasks containing 12.5 mgC/L Aldrich HA or alginic acid with 1 mM NaHCO$_3$ 20 mM NaCl background electrolyte. The fibres were then cut into 1 cm lengths so they could fit in the rotor. The organic matter was not pre-treated before analysis. The $^{13}$C NMR spectra for Aldrich HA, alginic acid and tannic acid are shown in Figure 3.6.

![Figure 3.6: Solid state $^{13}$C NMR spectra for a) Aldrich HA, b) alginic acid and c) tannic acid](image-url)
3.8.7. Atomic Force Microscopy

Atomic force microscopy (AFM) was used to characterise the surface of the regenerated cellulose membranes. AFM can analyse the surface by applying a metal tip (cantilever) to the membrane (Bowen et al., 1996). A Nanowizard AFM (JPK Instruments AG, Berlin, Germany) was used to analyse samples in air in tapping mode. All membranes were stored in deionised water prior to analysis. The scanning size was 1 µm by 1 µm. The smaller membranes (1 to 5 kDa) shrunk rapidly when exposed to air, therefore only 10 to 100 kDa membranes (PLGC, PLTK and PLHK) were studied. Figure 3.7 indicates that the membrane surface of all studied membranes was smooth with root mean square (RMS) roughness values from 2.7 to 3.1 nm.

Figure 3.7: Surface topography of regenerated cellulose UF membranes using AFM a) PLGC (10 kDa), b) PLTK (30 kDa) and c) PLHK (100 kDa)
Materials and Methods

3.8.8. Environmental Scanning Electron Microscopy

The PA fibres were imaged using a Quanta 200F environmental scanning electron microscope (ESEM) at 20 kV (FEI, Hillsboro, USA) (Figure 3.1). The samples were cooled to 4°C prior to analysis.

3.8.9. Focused Ion Beam Scanning Electron Microscopy

Membrane fouling by MIEX® was assessed using focused ion beam scanning electron microscopy (FIB-SEM) at 15.00 kV (FEI, Hillsboro, USA). Prior to analysis the samples were frozen by plunging into slush liquid nitrogen. The samples were then cut with scissors for cross-section analysis and immediately analysed. In Figure 3.8 MIEX® particles can be observed on the surface of a 10 kDa regenerated cellulose membrane (PLCGC). Cross-section FIB-SEM will be applied in Chapter 8 to assess fouling mechanisms.

Figure 3.8: FIB-SEM image of 10kDa MWCO membrane following IX-UF experiments (1 mM NaHCO₃ 20 mM NaCl, pH 8, 10 mL/L MIEX®)
3.8.10. Energy Dispersive X-Ray

An energy dispersive X-ray (EDX) detector (Oxford Instruments, Abingdon, UK) was coupled with FIB-SEM for elemental analysis of the membrane. In Figure 3.9 the spectrum of the membrane surface in Figure 3.8 is shown. As the active layer of the membrane is regenerated cellulose the most prominent functional groups are carbon and oxygen. However, iron (Fe) is also detected confirming the presence of MIEX® on the surface.

![Figure 3.9: EDX spectra for 10kDa MWCO membrane shown in Figure 3.8 (1 mM NaHCO₃, 20 mM NaCl, pH 8, 10 mL/L MIEX®)](image)

3.9. Experimental Protocols

3.9.1. Fibre-Water Partitioning

The fibre-water partition coefficient (K_FW) is an important parameter required to determine the freely dissolved hormone concentration in aqueous solution. It is a dimensionless physico-chemical property and is not influenced by the presence of organic matter. It is calculated by kinetic uptake curves as function of pH. The concentration of freely dissolved micropollutant extracted by the fibre will increase with time until equilibrium is reached.
Materials and Methods

To determine $K_{FW}$ 100 ng/L of tritium labelled hormone was added to flasks containing 100 mL deionised water with 1 mM NaHCO$_3$ 20 mM NaCl background electrolyte. The pH was adjusted from 3 to 12 using 1 M HCl and NaOH. Seven 5 cm PA fibres were added to each flask and was shaken using a Sartorius Certomat BS-1 incubator shaker (Göttingen, Germany) at 200 RPM and a temperature of 25°C. The fibres were removed using stainless steel tweezers after 0.5, 1, 2, 5, 8, 24 and 48 hours and cut into two pieces and added to 20 mL glass scintillation vials with 7 mL Ultima Gold LLT scintillation cocktail. These were allowed to desorb overnight after being shaken briefly. The mass of estradiol adsorbed to the fibre was measured using a Beckman LS 6500 liquid scintillation counter.

In the majority of SPME experiments in the literature the fibres are held in place using a septum. However, in this study the fibres were free floating. The advantage of free floating fibres is it enables the whole fibre volume to be utilized for extraction. Without a septum it possible that the fibres could stick to the walls of the flask, however, shaking at 200 RPM prevented this.

Using the experimental uptake curve over 48 hours the calculated mass of steroidal hormone, $m_F$ (ng), on the fibre at equilibrium was determined by fitting a one compartment model (Equation 3.4). The parameters $K_{1}$ (ng.min) and $K_2$ (min) represent the uptake and release rates and were determined using Solver which is an optimisation method (Microsoft Excel), while $t$ was time (h).

$$m_F = \frac{K_1}{K_2} \left( 1 - e^{(-K_2t)} \right)$$

(3.4)

The hormone uptake rate $K_1$ (min) was calculated using Equation 3.5 where:
Chapter 3

\[ K_I = K_I^l \frac{V_W}{m_W \cdot V_F} \] (3.5)

\( m_W \) was the mass of freely dissolved hormone in aqueous solution at equilibrium (ng)
\( V_W \) was the volume of aqueous solution (L)
\( V_F \) was the fibre volume (L)

With the mass extracted by the SPME fibre at equilibrium now known \( K_{FW} \) was calculated using Equation 3.6. Alternatively, \( K_{FW} \) could be calculated by dividing the concentration of hormone in the fibre, \( C_F \) (ng/L), by the freely dissolved hormone concentration in aqueous solution, \( C_W \) (ng/L).

\[ K_{FW} = \frac{m_F \cdot V_W}{m_W \cdot V_F} = \frac{C_F}{C_W} \] (3.6)

To ensure depletion of the steroidal hormones from the solution was indeed negligible Equation 3.7 can be applied to determine extraction efficiency (Vaes et al., 1997).

\[ K_{FW} \frac{V_F}{V_W} \ll 1 \] (3.7)

For all studied hormones the extraction efficiency was significantly less than 1 with most less than 0.1 (Table 3.6). Therefore the condition of negligible depletion was met by the experimental methodology.
Materials and Methods

Table 3.6: Fibre extraction efficiency based on Equation 3.7 of for estradiol, estrone, progesterone and testosterone as at different pH values

<table>
<thead>
<tr>
<th></th>
<th>pH 3</th>
<th>pH 4</th>
<th>pH 5</th>
<th>pH 6</th>
<th>pH 7</th>
<th>pH 8</th>
<th>pH 9</th>
<th>pH 10</th>
<th>pH 11</th>
<th>pH 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol</td>
<td>0.03</td>
<td>0.04</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>0.02</td>
<td>0.01</td>
<td>0.001</td>
</tr>
<tr>
<td>Estrone</td>
<td>0.09</td>
<td>0.12</td>
<td>0.05</td>
<td>0.02</td>
<td>0.07</td>
<td>0.06</td>
<td>0.06</td>
<td>0.02</td>
<td>0.01</td>
<td>0.001</td>
</tr>
<tr>
<td>Progesterone</td>
<td>0.05</td>
<td>0.04</td>
<td>0.05</td>
<td>0.04</td>
<td>0.04</td>
<td>0.05</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Testosterone</td>
<td>0.006</td>
<td>0.005</td>
<td>0.005</td>
<td>0.006</td>
<td>0.006</td>
<td>0.005</td>
<td>0.006</td>
<td>0.005</td>
<td>0.005</td>
<td>0.005</td>
</tr>
</tbody>
</table>

3.9.2. Organic Matter-Water Partition Coefficients

Organic matter-water partition coefficients, $K_{OM}$ (L/kg), are used to relate the micropollutant concentration sorbed to organic matter to the freely dissolved micropollutant concentration in solution (Heringa et al., 2006). To determine $K_{OM}$, radiolabelled hormones with concentrations from 100 ng/L to 100 µg/L were added to 100 mL pH adjusted flasks containing the studied organic matter (typical concentration was 12.5 mgC/L). The solutions were shaken for 24 hours at 200 RPM to ensure equilibrium between the organic matter and hormone was reached. After 24 hours 1 mL of solution was removed to measure the initial concentration and transferred to a 20 mL glass scintillation vial with 7 mL Ultima Gold LLT scintillation cocktail. 5 cm of PA fibre was then added to each flask and shaken for 48 hours. The fibres were removed using tweezers, cut into two pieces and added to scintillation vials with 7 mL scintillation cocktail and allowed to desorb overnight after being shaken briefly. The scintillation vials were counted in a Beckman LS 6500 liquid scintillation counter.

The freely dissolved concentration of hormone in solution was calculated using Equation 3.8 using the calculated $K_{FW}$ value for the studied pH. To reduce experimental artifacts $m_w$ was calculated using in the same experimental conditions as the $K_{FW}$ experiments including the same background electrolyte, buffer solution and temperature (25°C). Further, a single PA fibre batch was used.
With the freely dissolved hormone mass calculated, it was possible to determine the mass of hormone sorbed to the organic matter, $m_{OM}$ (ng). In the majority of experiments a relatively low concentration of organic matter (12.5 mgC/L) was used to measure log $K_{OM}$ values at environmental concentrations. As a result the difference between the initial mass of hormone in solution, $m_{TOT}$ (ng), and $m_{W}$ could be small resulting in higher error compared to the method applied to higher organic matter concentrations. When determining $m_{OM}$ the majority of studies do not take into account $m_{F}$ as this is assumed to be negligible. However, for some weakly sorbing hormones $m_{F}$ was similar to the $m_{OM}$, therefore a full mass balance was required (Equation 3.9). As hormones are non-volatile the concentration of hormone in air was not considered.

$$m_{W} = \frac{m_{F} V_{W}}{K_{FW} V_{F}} \quad (3.8)$$

With $M_{OM}$ and $M_{W}$ known it was possible to calculate $K_{OM}$ (L/kg) using Equation 3.10. The total mass of DOM was $m_{DOM}$ (kg), while $C_{OM}$ was the concentration of hormone sorbed to the organic matter (ng/kg).

$$m_{TOT} = m_{F} + m_{W} + m_{OM} \quad (3.9)$$

$$K_{OM} = \frac{C_{OM}}{C_{W}} = \frac{m_{OM}}{m_{DOM}} \frac{V_{W}}{m_{W}} \quad (3.10)$$

Due to the potential error associated with the individual $K_{OM}$ measurements it was necessary to determine $K_{OM}$ over the entire studied concentration range (100 ng/L to 100 µg/L). $K_{OM}$ can be derived from the slope of the linear regression of $C_{OM}$ as a function of $C_{W}$ if the sorption isotherms are linear. The sorption isotherms were plotted on a log scale (Equation 3.11) as the studied concentration range was over
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three orders of magnitude (Figure 3.10). The slope of the regression, \( n_i \), indicated the linearity of the sorption isotherm with a slope of 1 considered linear (Schwarzenbach et al., 2003). As \( n_i \) was close to 1 for all isotherms it was set to 1, which was equivalent to the linear relationship described by Equation 3.11.

\[
\log C_{OM} = \log K_{OM} + n_i \log C_w
\]  

(3.11)

![Graph showing log C_{OM} vs log C_w](image)

Figure 3.10: Tannic acid-water sorption isotherm for estradiol. The slope is 1.05 which is close to 1 indicating linearity (1mM NaHCO₃, 20 mM NaCl, pH 7, 100 ng/L estradiol, 12.5 mgC/L tannic acid)

3.9.3. Stirred Cell Ultrafiltration

A 400 mL feed solution containing 100 ng/L hormone, 12.5 to 125 mgC/L organic matter and 1 mM NaHCO₃, 20 mM NaCl was stirred at 200 RPM using a magnetic stirrer table for 16 hours prior to the experiment. To determine hormone retention by the membrane itself, experiments were also conducted without organic matter. Deionised water was filtered through the membrane for 30 minutes at 0.5 to 5 bar depending on membrane MWCO. Pure water flux was measured for 60 minutes, with the exception of the 100 kDa membrane where only 30 minutes could be
measured due to the high flux. Flux was calculated using Equation 3.12 where \( J \) was flux (L/m\(^2\).h), \( V \) was permeate volume (L), \( A \) was membrane area in m\(^2\) and \( t \) was filtration time (h).

\[
J = \frac{1dV}{A \cdot dt}
\]  
(3.12)

Following pure water flux, a 50 mL feed sample was collected, and 350 mL was introduced to the cell. The pressure used for each membrane is shown in Table 3.5. Six 50 mL permeate samples were collected during the experiment at time intervals ranging from 2 to 40 minutes depending on the membrane MWCO, as well as a 50 mL concentrate sample. Flux decline, which is an indicator of membrane fouling, was calculated using Equation 3.13 where \( J_0 \) was pure water flux (L/m\(^2\).h).

\[
\text{Flux Decline} = \frac{J}{J_0}
\]  
(3.13)

Following the experiment, the flux was measured for 30 minutes. The membranes were cleaned by filtering with a 0.1 M NaOH solution for 30 minutes followed by deionised water for a further 30 minutes. Flux was measured after cleaning to ensure there was no fouling. The membranes were stored in 0.5% sodium metabisulfite and reused up to 5 times.

3.9.4. Ion Exchange-Ultrafiltration

To determine when equilibrium was reached between the hormone and MIEX\textsuperscript{®} sorption experiments were conducted. In 100 mL flasks deionised water was added with 1 mM NaHCO\(_3\) 20 mM NaCl background electrolyte solution and 100 ng/L hormone. Before the experiment, 1 mL was removed from the flask for analysis then 1 mL of MIEX\textsuperscript{®} was added from a 10 mL syringe giving a concentration of 10 mL/L. The resin was allowed to settle for 10 minutes before being added to the 100 mL
Filtration experiments were conducted in three stainless steel stirred cells. The feed solution contained deionised water, 1 mM NaHCO₃, 20 mM NaCl background electrolyte solution and 100 ng/L hormone. For experiments with NOM, concentrations of 12.5, 25, 50 and 125 mgC/L were added. A 25 mL sample was removed to determine hormone concentration prior to MIEX® addition. 3.75 mL of MIEX® was added to the remaining 375 mL feed solution. The solution was shaken in an incubator shaker for 1 hour (optimum time as determined from sorption kinetic experiments in Section 8.3 of Chapter 8). Another 25 mL sample was removed to determine the hormone concentration of the MIEX® feed. The 350 mL solution was then introduced into the stirred cell and stirred at 300 RPM. Six 50 mL permeate samples were collected as well as a 50 mL concentrate sample.

3.9.5. Stirred Cell Ultrafiltration and Ion Exchange-Ultrafiltration Analysis

Hormone concentration in the stirred cell was calculated as a function of permeate volume using Equation 3.14 where:

\[
C_{Bi} = \frac{C_{FD}V_{FD} - \sum C_{Pi}V_{Pi}}{V_{B}}
\]  

(3.14)

\(C_{Bi}\) was concentration within the cell (ng/L)
\(C_{FD}\) was the feed concentration (ng/L)
\(C_{Pi}\) was the permeate concentration based on the 4\textsuperscript{th} permeate sample (ng/L)
\(V_{B}\) was the volume in remaining in the cell, and was calculated by subtracting the cumulative permeate volume from the feed volume (L)
\(V_{FD}\) was initial feed volume (L)
\(V_{Pi}\) was the permeate volume (L)
In the MIEX® experiments $C_{FD}$ was based on the feed concentration prior to the addition of MIEX®. The hormone concentration in the permeate and within the stirred cell is shown in Figure 3.11 in the presence and absence of organic matter. In the presence of Aldrich HA the hormone concentration in the stirred cell is greater than estrone alone.

![Figure 3.11: Hormone concentration in the permeate and within the stirred cell](image)

Hormone retention ($R_\%$) by the membrane was calculated using Equation 3.15:

$$R_\% = (1 - \frac{C_P}{C_{Bi}})100\%$$  \hspace{1cm} (3.15)

The mass of hormone adsorbed per unit membrane area ($m_{ADS}$) was determined using Equation 3.16 where $C_C$ was the concentrate concentration in the remaining 50 mL (ng/L), $V_C$ was the concentrate volume (L) and $A$ was the membrane area (cm$^2$). Organic matter concentration, retention ($R_{OM\%}$) and membrane adsorption was also determined using Equations 3.14, 3.15 and 3.16, however, the units for $C_F$, $C_C$ and $C_{Pi}$ were mgC/L.
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\[ m_{ADS} = \frac{V_F C_F - V_C C_C - \Sigma V_{Pi} C_{Pi}}{A} \]  (3.16)

3.9.6. Quantifying the Influence of Solute-Solute Interactions in Ultrafiltration

Using log \( K_{OM} \) values hormone retention by solute-solute interactions in stirred cell UF could be predicted. In the SPME experiments 24 hours was allowed for equilibrium between the solutes to be reached, while the UF feed solution was stirred for 16 hours (overnight) prior to the experiment. During method development retention of estrone in the presence of organic matter was compared after no stirring, 1 hour, overnight and 24 hours stirring. No significant difference was found between overnight and 24 hours stirring, while retention was lower without stirring and after only 1 hour. Therefore, overnight stirring appears to be a sufficient amount of time for equilibrium between steroidal hormones and DOM to be reached.

First, \( m_W \) was determined for the stirred cells using Equation 3.17, where \( m_{TOT} \) was 40 ng (based on 100 ng/L in 0.4 L) and \( f_W \) was the freely dissolved hormone fraction in solution at equilibrium (%). This was determined experimentally from SPME experiments.

\[ m_W = m_{TOT} f_W \]  (3.17)

Using Equation 3.18 \( m_{OM} \) could be estimated. This is a rearrangement of Equation 3.10.

\[ m_{OM} = m_W \frac{K_{OM}}{V_W} m_{DOM} \]  (3.18)
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With $m_{OM}$ estimated it was possible to predict hormone retention due to solute-solute interactions ($R_{P\%}$) using Equation 3.19. $R_{OM\%}$ was organic matter retention (%) calculated using Equation 3.15. Due to the error associated with the different calculation methods, $R_{P\%}$ is not expected to be precise, but rather a general estimation.

$$R_{P\%} = \frac{m_{OM}}{m_{TOT}} R_{OM\%}$$  \hspace{1cm} (3.19)

3.10. Error Calculation

3.10.1. Organic Matter-Water Partition Coefficients

The total random error ($%E_{total}$) associated with the SPME extraction, calculated using error propagation (Equation 3.20), was 5.4%. This considers relative errors ($%E$) including the variability associated with fibre length as well as errors associated with laboratory equipment such as the liquid scintillation counter, micropipettes, Hamilton syringes and electronic balances. Further information about the relative error is shown in Appendix 3.

$$%E_{total} = \sqrt{(%E_1)^2 + (%E_2)^2 + (%E_3)^2}$$  \hspace{1cm} (3.20)

For each $K_{OM}$ value derived by linear regression standard deviation and confidence interval was calculated. Further, to determine if there was any significant difference between $K_{OM}$ values calculated as a function of solution chemistry or organic matter type an independent two-sample t-test was used (Equation 3.21). This tested the null hypothesis that there was no significant difference between log $K_{OM}$ values determined for different pH values or organic matter types. If the two-sample t-test statistic ($|t|$) was greater than the t-test critical value determined from t-distribution table then the null hypothesis is rejected. The log $K_{OM}$ values to be tested are represented by $\chi_1$ and $\chi_2$ and $n_1$ and $n_2$ are the respective sample sizes. $s$ was
calculated using Equation 3.22 where \( s_1^2 \) and \( s_2^2 \) are the respective standard deviations (Miller and Miller, 2000).

\[
|t| = \frac{(\chi_1 - \chi_2)}{s \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} \quad (3.21)
\]

\[
s^2 = \frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{(n_1 + n_2 - 2)} \quad (3.22)
\]

3.10.2. Ultrafiltration

Error propagation was applied to determine the variability associated with stirred cell experiments (Equation 3.20). Firstly, it was necessary to determine \( %E \) for analytical instruments and experimental parameters such as flux. \( %E \) was calculated using the coefficient of variance \( (C_V) \) (Equation 3.23) where \( X \) was the sample mean (Miller and Miller, 2000). Further detail including \( %E \) and \( %E_{\text{total}} \) are included in Appendix 3.

\[
C_V = \frac{s^2}{X} = %E \quad (3.23)
\]
4 Solid-Phase Microextraction (SPME) Methodology Development

In this chapter a methodology suitable for quantifying the interaction of steroidal hormones with organic matter will be developed using solid-phase microextraction (SPME). The theoretical concepts of SPME will be discussed, and the advantages and limitations of the technique will be highlighted as well as the implications of solution chemistry on this technique.

This chapter will focus on developing a SPME methodology by finding an appropriate fibre type and determining when equilibrium is reached between the fibre and solution. Fibre-water partition coefficients \((K_{FW})\) will be calculated for estradiol, estrone, progesterone and testosterone.

The results indicated that polyacrylate fibre is suitable to extract steroidal hormones and fibre-water equilibrium was reached after 48 hours. The presence of organic matter reduced hormone removal by the fibre suggesting that some hormones are interacting with the organic matter, and therefore are not freely dissolved. This suggests that SPME is indeed suitable to quantify solute-solute interactions at environmental concentrations.

Finally, to ensure that the methodology is robust the technique will be validated by considering fibre reproducibility, hormone degradation and the potential for fibre fouling. These studies indicate high reproducibility with no detectable fouling by organic matter.
4.1. Introduction

As discussed in Chapter 2 there are few techniques which can quantify the interaction of micropollutants with dissolved organic matter (DOM) at environmental concentrations. The aim of this section is to develop such a methodology and apply it specifically to steroidal hormones. Based on the literature review in Chapter 2, solid-phase microextraction (SPME) was selected as a promising quantification technique. In this chapter the theoretical concepts of SPME are discussed and a methodology suitable for steroidal hormones will be devised. This includes determining the appropriate SPME fibre type, fibre-water equilibrium and the influence of organic and inorganic matter on hormone uptake. In addition, potential limitations of the methodology such as reproducibility will be tested to ensure the technique is suitable for quantifying solute-solute interactions.

4.2. Theoretical Concepts of Solid-Phase Microextraction (SPME)

SPME was developed in the early 1990’s as a technique to quantify the concentration of micropollutants in solution (Arthur and Pawliszyn, 1990). SPME is a simple technique where micropollutants partition from solution to a polymer coated fibre. Compared to other techniques available at the time, such as solid phase extraction and liquid-liquid extraction, SPME had the advantage of equilibrium extraction, opposed to exhaustive extraction, making it suitable for small volumes and environmental samples. While much of the early work on SPME focused on quantifying the interaction of micropollutants with SPME fibres (e.g. Vaes et al., 1996a), many studies have applied SPME to quantify the interaction of micropollutants with a third phase, such as natural organic matter (NOM) or proteins (Heringa et al., 2002; Poerschmann et al., 1997; Urrestarazu Ramos et al., 1998). SPME can measure organic matter-water partition coefficients (log K_{OM}) based on the assumption that only freely dissolved micropollutants can be extracted from solution (Poerschmann and Kopinke, 2001).
4.2.1. Mechanisms and Theory

SPME is a flexible technique with different extraction modes depending on the studied micropollutant. Micropollutants can either be extracted using headspace or direct immersion modes depending on their volatility. For volatile micropollutants headspace mode can be used where the fibre is suspended above the solution preventing any contact with the aqueous phase (Mackenzie et al., 2002). This method is not suitable for non-volatile micropollutants such as steroidal hormones which need to be extracted by the fibre through direct immersion. The interaction of organic matter with the fibre is a potential limitation associated with this method, and this will be discussed further in Section 4.2.4.

As mentioned earlier SPME is an equilibrium extraction process. This section will focus on negligible depletion SPME (nd-SPME), a technique which only removes approximately 5% of freely dissolved micropollutants, thus causing negligible disturbance to the solution equilibrium. Despite non-exhaustive extraction, the freely dissolved concentration in solution can be determined using the mass extracted by the fibre and the fibre-water partition coefficient \( K_{FW} \) as outlined in Chapter 3. For nd-SPME to be applied there are several criteria that must be met. These were first developed by Vaes et al. (1996b) and include:

1. The SPME extraction must not alter the equilibrium between the micropollutant and organic matter (therefore less than 5% depletion of micropollutant)
2. The organic matter must not bind to the SPME fibre as this can influence the measurement
3. The presence of organic matter should not influence the uptake equilibrium of the freely dissolved micropollutants

Another form of SPME is matrix-SPME which uses soil or sediment as an equilibrium reservoir (Mayer et al., 2000) so the sorption of micropollutant to the organic matter is expected to be considerably higher than in dilute systems.
However, as the present study only considers DOM matrix SPME will not be discussed further.

The sensitivity and extraction efficiency of SPME is dependent on properties of both the fibre and the micropollutant including the material and volume of the fibre coating, and hydrophobicity and hydrogen bond ability of micropollutant. There are several different types of polymer coating including polyacrylate (PA), polydimethylsiloxane (PDMS), carbowax/divinylbenzene (DVB) and carbowax/templated polymer resin (TPR) which vary in polarity, porosity and sorption behaviour. For example, PA acts as a liquid and micropollutants absorb into the fibre, while carbowax/DVB acts as a solid and micropollutants adsorb onto the surface (Górecki et al., 1999). With adsorption, there can be extraction problems if there is competition for adsorption sites (Poerschmann and Kopinke, 2001).

The volume of the fibre coating also has implications for uptake. This is related to both the thickness of the fibre coating and the fibre length. SPME fibres typically have small volumes between 0.01 to 0.5 µL (Heringa and Hermens, 2003). This is important for negligible depletion as the mass of freely dissolved micropollutant extracted by the fibre is related to the small fibre volume compared to the total solution volume (Heringa et al., 2004). In this study fibre volumes of 0.63 to 0.77 µL were used. However, micropollutant depletion from the system was still considered negligible (Table 3.6 in Chapter 3) as a considerably larger total solution volume (100 mL) was used. The advantage of a larger fibre volume is reduced interference from the organic matrix (Zeng and Noblet, 2002). However, larger fibre volumes will increase fibre-water equilibrium time.

Properties of the micropollutant including hydrogen bond polarity and hydrophobicity will influence uptake to the fibre. Vaes et al. (1996b) demonstrated that hydrophobic micropollutants, indicated by large octanol-water partition coefficients (log $K_{OW}$), had greater log $K_{FW}$ values. Hydrogen bonding between PA fibre and the micropollutant is also important for micropollutant extraction, with
Vaes *et al.* (1996a) suggesting polar micropollutants have greater log $K_{FW}$ values compared to apolar contaminants when they both have similar log $K_{OW}$ values.

### 4.2.2. Influence of Solution Chemistry

The uptake of micropollutants to the polymer fibre can be influenced by the solution chemistry such as pH and ionic strength. Solution chemistry can alter properties of the micropollutant including charge and solubility which in turn can influence uptake. Several studies have demonstrated SPME fibres have a limited ability to extract micropollutants in pH solutions above their acid dissociation constant ($pK_a$) (Escher *et al.*, 2002; Holten Lützhøft *et al.*, 2000). Holten Lützhøft *et al.* (2000) found significantly decreased extraction of charged micropollutants by carbowax/TPR fibres. This has also been observed for PA fibres (Okeyo and Snow, 1998).

Further, decreasing micropollutant solubility can lead to improved fibre extraction efficiency. Increasing ionic strength can lead to ‘salting out’ which can cause a decrease in solubility of certain apolar and polar micropollutants in the presence of inorganic ions such as $Na^+$ at concentrations typically greater than 0.1 M (Schwarzenbach *et al.*, 2003). Boyd-Boyer and Pawliszyn (1995) found increased extraction by PA fibres with NaCl concentrations of 2 and 4 M for the majority of studied micropollutants. Further, ionic strength can also shield micropollutant charge, which may result in extraction of negatively charge micropollutants at high NaCl concentrations. In addition, solution chemistry may also have implications for organic matter-water partitioning as discussed in Chapter 2.

### 4.2.3. Advantages of SPME

There are many advantages of SPME which have led to its wide use in the literature. The main advantage of SPME is the ability to separate and sample simultaneously (Heringa *et al.*, 2002). This eliminates the use of solvents and their associated disposal issues, and prevents sample loss as no pre-treatment such as filtration is
required. Problems associated with the pre-treatment of solutions containing steroidal hormone will be discussed in Appendix 4. In addition, equilibrium between the micropollutant and fibre can be reached quickly, with studies indicating equilibrium can be reached in less than 10 minutes (Vaes et al., 1996b). Rapid equilibrium is related to the cylindrical geometry of the fibre which allows for fast absorption and desorption (Arthur and Pawliszyn, 1990). However, equilibrium kinetics will depend on fibre volume.

An important advantage of nd-SPME is limited disturbance to the equilibrium, which makes it applicable to environmental samples as there should be no change in the binding between the micropollutant and organic matter (Heringa et al., 2004). Therefore, the chance that bound micropollutants will desorb from the organic matter is minimised, ensuring that only the freely dissolved concentration is measured. Further, SPME can be analysed directly using a number of techniques including gas chromatography, high performance liquid chromatography or liquid scintillation counting. This gives SPME greater analytical flexibility compared to many other quantitative techniques. Further, these analysis techniques can often detect to sub-nanogram per litre level, therefore it can be applied to measure partitioning at environmentally realistic concentrations. Due to the above advantages SPME can be considered a simple and effective quantification technique.

4.2.4. Limitations of SPME

There are several important limitations of SPME which need to be considered prior to use. Firstly, there is the issue of fibre fouling by organic matter in solution. Fouling is a problem as it can underestimate log $K_{OM}$ values as there may be organic matter bound micropollutants sorbed to the fibre. Fouling can be avoided if headspace mode is applied, however, this is only suitable for volatile micropollutants. Currently there is limited knowledge of fibre fouling, with several studies indicating conflicting results. Urrestarazu Ramos et al. (1998) studied uptake of polychlorinated biphenyls (PCB) by SPME in the presence and absence of humic acid (180 mg/L) and found that humic acid did not influence the extraction of
micropollutants. However, this was after a brief (1 minute) sampling time. Zhang et al. (1996) observed significant fouling of SPME fibres in the presence of humic acid after 1 hour which was indicated by fibre colour change. In contrast, Ter Laak et al. (2005) studied uptake of polycyclic aromatic hydrocarbons (PAH) in the presence of humic acid from 7 to 14 days and found no evidence of organic matter fouling. All of the above examples used PDMS fibres. It is likely that the fibre polymer type, organic concentration and sampling time will influence the potential for fibre fouling.

Secondly, SPME is not suitable to measure partitioning of ionic micropollutants to organic matter, at least not without adjusting the equilibrium time or fibre length. This is important to consider when quantifying solute-solute interactions above the pKa of a micropollutant.

Thirdly, the SPME fibres are susceptible to damage, which can lead to a reduction in sensitivity of results. Arthur and Pawliszyn (1990) observed that fibres became fragile after thermal desorption which is required for analysis using chromatographic techniques. However, single use of fibres can reduce this potential limitation.

4.3. Fibre Types

Determining a suitable polymer fibre coating is necessary to maximise extraction of micropollutants (Boyd-Boland et al., 1994). In this study two polymers were trialled; PA and PDMS. These were selected as both act as a liquid which facilitates the absorption of the micropollutant (Heringa et al., 2006). While the fibre selection is often trial and error the most appropriate fibre material can generally be selected based on micropollutant polarity. The suitability of the polymers were assessed by measuring estradiol uptake over 5 hours. PA and PDMS fibres were added to 100 mL flasks containing 100 ng/L estradiol and were removed at 0.25, 0.5, 1, 2, 3.5 and 5 hours. The uptake of estradiol to the fibres was studied as a function of pH (Figure 4.1). Equilibrium between estradiol and the fibre was not reached after 5 hours for either fibre. However, 5 hours was enough time to determine that PDMS was not a
suitable fibre coating for steroidal hormones (Figure 4.1b). The mass extracted was over an order of magnitude less than PA, and no obvious influence of pH was observed. This was due to the apolar nature of PDMS which was not suitable to extract polar estradiol. Based on this result all log $K_{FW}$ and organic matter-water partition coefficients ($K_{OM}$) will be determined using PA coated fibres.

Figure 4.1: Uptake of estradiol to a) PA and b) PDMS coated fibres as a function of pH (1mM NaHCO$_3$, 20 mM NaCl, 100 ng/L estradiol)

4.4. Fibre-Water Equilibrium Time

To calculate log $K_{FW}$ and log $K_{OM}$ values it is necessary to determine when equilibrium between the fibre and hormone is reached. Equilibrium is difficult to predict as it can depend on fibre volume, thickness and properties of the micropollutant, therefore it can only be determined through uptake kinetic experiments. In a 100 mL flask 50 ng/L of estradiol was added and the pH was
adjusted to 7. Fibres were removed at 0.5, 1, 2, 5, 8, 24, 33, 96 and 133 hours. Figure 4.2 indicates equilibrium was reached after approximately 48 hours. Within the first 8 hours there was rapid removal of estradiol by the fibre which levelled out until equilibrium was reached at approximately 48 hours. This was a considerably long equilibrium time for a polar micropollutant, particularly as Heringa et al. (2002) reached equilibrium between estradiol and PA after 180 minutes. The long equilibrium time in this study was related to the volume of the PA fibre. The majority of studies use fibre lengths of 1 cm or less, while 5 cm fibres were used in all experiments. Therefore, the increase in fibre volume led to longer equilibrium times. Consequently, in all log $K_{FW}$ and log $K_{OM}$ experiments an equilibrium time of 48 hours was used.

![Figure 4.2: Equilibrium between estradiol and PA (1mM NaHCO$_3$ 20 mM NaCl, pH 7, 50 ng/L estradiol)](image)

4.5. Fibre-Water Partition Coefficients ($K_{FW}$)

The estradiol uptake curves used to calculate log $K_{FW}$ is shown in Figure 4.3a as a function of pH. The results indicated that the mass of estradiol absorbed by the fibres at equilibrium was relatively similar from pH 3 to 9 then decreased steadily to negligible removal at pH 12 (Figure 4.3b). The reduced extraction was due to the dissociation of estradiol at high pH. The pK$_a$ of estradiol is 10.23, meaning it is 50%
dissociated at this pH value. At pH 10 estradiol is 37% dissociated and increases to 98% dissociated at pH 12. SPME fibres have limited capacity for negatively charged micropollutants, therefore estradiol extraction was over an order of magnitude lower at pH 12 compared to when estradiol was neutral.

Figure 4.3: a) Estradiol uptake by PA coated fibre over 48 hours and b) mass of estradiol on the fibre after 48 hours as a function of pH (1mM NaHCO₃, 20 mM NaCl, 100 ng/L estradiol). This was used to determine log $K_{FW}$.

The log $K_{FW}$ values for estradiol, estrone, progesterone and testosterone were shown in Figure 4.4. Log $K_{FW}$ values for estradiol and estrone remained relatively constant until around pH 9 and 10, when it decreased rapidly, while there was no difference in log $K_{FW}$ values for progesterone and testosterone as a function of pH. Estrone has a pKₐ value of 10.34, while progesterone and testosterone do not dissociate within the studied pH range. Log $K_{FW}$ values also varied based on hormone type. Several studies have suggested that hydrophobicity influences $K_{FW}$ values with more hydrophobic micropollutants exhibiting greater fibre-water partitioning (Vaes et al., 1996b; Verbruggen et al., 2000). This was not the case in this study as hormones with the greatest log $K_{OW}$ values did not have the largest $K_{FW}$ values. For example, based on log $K_{OW}$ values from Hansch et al. (1995) testosterone should be more hydrophobic than estrone (Table 3.3 in Chapter 3), however, this trend was not reflected in the results. Therefore, it was likely that the strength of fibre-water partitioning was due to hydrogen bonding with the polyacrylate fibre as the hydrophobicity of the hormones was relatively similar (log $K_{OW}$ 3.13 to 4.01) (Vaes
et al., 1996a). Progesterone and estrone had the greatest log $K_{FW}$ values and both contain strong hydrogen accepting ketone groups in the C-20 and C-17 positions respectively.

![Graph showing log $K_{FW}$ values for estradiol, estrone, progesterone and testosterone as a function of pH (1mM NaHCO₃ 20 mM NaCl, 100 ng/L hormone concentration)]

**Figure 4.4: Log $K_{FW}$ values for estradiol, estrone, progesterone and testosterone as a function of pH (1mM NaHCO₃ 20 mM NaCl, 100 ng/L hormone concentration)**

Experimental log $K_{FW}$ values were compared to log $K_{FW}$ values from the literature (Figure 4.5). The literature log $K_{FW}$ values included micropollutants such as estradiol (Heringa et al., 2002), aniline, nitrobenzene, 4-chloro-3-methylphenol and 4-n-pentylphenol (Vaes et al., 1996b). All experiments used PA fibre and a pH of approximately 7. The log $K_{FW}$ value for estradiol (Heringa et al., 2002) was slightly higher than the experimental result. The increase could be attributed to the differences in fibre volume, as discussed in Section 4.2.1. In a study by Vaes et al. (1996b) log $K_{FW}$ values were calculated for a number of polar micropollutants with different hydrophobicities, all considerably lower than estradiol with the exception of 4-n-pentylphenol (log $K_{OW}$ 4.24). With the other micropollutants (aniline, nitrobenzene and 4-chloro-3-methylphenol) $K_{FW}$ decreased as the hydrophobicity of the micropollutant decreased. Therefore, unlike the observation for steroidal hormones in Figure 4.4 which all had similar log $K_{OW}$ values, hydrophobicity
appears to influence fibre-water partitioning when there was a considerable difference between log $K_{OW}$ values of micropollutants.

![Graph showing log KFW values for various compounds](image)

**Figure 4.5:** Experimental estradiol log KFW values compared with literature log KFW values for estradiol (Heringa et al., 2002) and aniline, nitrobenzene, 4-chloro-3-methylphenol and 4-n-pentylphenol (Vaes et al., 1996b)

### 4.6. Influence of Organic and Inorganic Matter

As low concentrations of both hormones and organic matter will be used to measure log $K_{OM}$ values it was important to determine if SPME could indeed be used to quantify the interaction. If estradiol interacts with organic matter there should be a decrease in freely dissolved hormone concentration, and therefore reduced estradiol removal by the fibre. The extraction of estradiol in the presence of organic matter was assessed using a range of organic matter types including NOM surrogates such as Aldrich humic acid (HA) and polysaccharides. The concentration of organic matter used in all experiments was 12.5 mgC/L.

The influence of inorganic matter on estradiol extraction by SPME was also studied. Previous studies have indicated increased organic matter sorption to polymeric membranes in the presence of calcium as it can act as a bridge between the organic matter and the membrane (Hong and Elimelech, 1997). If calcium bridging occurs
between organic matter and PA fibres this may increase estradiol uptake as any estradiol bound to the organic matter may sorb to the fibre. Consequently, the influence of CaCl$_2$ concentration on estradiol uptake in the presence of organic matter was studied. In 100 mL flasks 50 ng/L of radiolabelled estradiol was added with 12.5 mgC/L of Australian NOM with CaCl$_2$ concentrations from 0 to 4 mM. The pH was adjusted from 3 to 12. The solution was shaken from 24 hours, then SPME fibres were added and the solution was shaken for a further 48 hours.

The extraction of estradiol by PA fibre in the presence and absence of organic matter as a function of pH is shown in Figure 4.6. The results indicate greater extraction of estradiol alone compared to estradiol in the presence of organic matter. This suggests, assuming no interaction of organic matter with the fibre, that some estradiol was bound to the organic matter thus it was not freely dissolved and could not be removed by the fibre. Therefore, despite the low concentrations of estradiol and organic matter used SPME may be useful technique to quantify log K$_{OM}$ values at environmental concentrations. The influence of organic matter type on log K$_{OM}$ values will be discussed further in Chapter 5.

The addition of inorganic calcium appeared to have no significant influence on estradiol uptake by SPME fibre in the presence of Australian NOM with no difference observed with increasing calcium concentration (Figure 4.7). While calcium can increase organic matter sorption to membranes, it appears that calcium does not affect the SPME process.
Figure 4.6: Uptake of estradiol by PA fibre in the presence of organic matter as a function of pH (1mM NaHCO₃ 20 mM NaCl, 100 ng/L estradiol, 12.5 mgC/L organic matter)

Figure 4.7: Uptake of estradiol by PA fibre with calcium chloride and Australian NOM as a function of pH (1mM NaHCO₃ 20 mM NaCl, 0, 0.5, 2.5 and 4 mM CaCl₂, 50 ng/L estradiol, 12.5 mgC/L Australian NOM)
4.7. Methodology Validation

Before SPME could be used to determine log $K_{OM}$ values it was important to identify possible problems and to assess their implications. These included potential limitations of SPME such as extraction reproducibility and organic matter fouling, as well as the potential for hormone degradation.

4.7.1. Fibre Reproducibility

To assess the reproducibility of estradiol extraction by the SPME fibres several experiments were repeated in triplicate. The results for Aldrich HA are shown below in Figure 4.8. SPME extraction reproducibility was also tested for other organic matter types including alginic acid and Australian NOM. For the majority, the mass extracted by all three fibres was within the error range (5.4%) and the reproducibility of SPME extraction was observed over the studied pH range. Any significant differences in extraction such as at pH 6 may be related to experimental problems such as variation in fibre length or fibres sticking together in solution.

![Graph](image)

Figure 4.8: Fibre reproducibility studies for Aldrich HA as a function of pH. Error bars represent the total random error associated with each extraction (1mM NaHCO$_3$, 20 mM NaCl, 100 ng/L estradiol, 12.5 mgC/L Aldrich HA)
4.7.2. Hormone Degradation

As the log $K_{OM}$ experiments ran for a total of 72 hours degradation of the hormones was a possibility. In the presence of activated sludge up to 95% of estradiol can oxidise to estrone within 1 to 3 hours in aerobic conditions (Ternes et al., 1999). However, biodegradation was considerably slower in river water with degradation to estrone occurring over several days (Jürgens et al., 2002). Consequently, it is likely to be even slower in deionised water. Photodegradation may also be a problem. Jürgens et al. (2002) studied photodegradation of estradiol in river water and observed a half-life of just over 10 days or 124 hours (based on 12 hours of daylight per day). Consequently, it is possible that as much as 18% of estradiol may degrade during the experiment time (36 hours based on 12 hours of daylight per day). However, the experiments were conducted in a covered shaker which was out of direct light, therefore degradation should be less. To assess if degradation had an influence on hormone extraction several experiments were repeated with 0.5% sodium metabisulfite ($\text{Na}_2\text{S}_2\text{O}_5$) to act as a biocide, as well as with a foil covering around the flask to prevent exposure to light. Experiments were also conducted with both the biocide and foil covering. Uptake of estradiol in the absence and presence of Aldrich HA was shown in Figure 4.9. This indicated no significant difference between the control experiment and the experiments with degradation prevention. Due to the lack of biological material in the samples negligible hormone degradation was expected. Further, hormone loss to sorption to glassware was negligible (less than 1%).
Figure 4.9: Extraction of estradiol alone and in presence of Aldrich HA by PA fibre with no treatment (Control), 0.5% sodium metabisulfite biocide (Bio), foil covering (Photo) and both biocide and foil covering (Bio + Photo) (1mM NaHCO₃, 20 mM NaCl, pH 7, 100 ng/L estradiol, 12.5 mgC/L Aldrich HA)

4.7.3. Organic Matter Fouling

Fibre fouling by organic matter is a potential limitation as it can lead to altered log $K_{OM}$ values due to increased micropollutant sorption. Previous studies have reported fouling of SPME fibres by protein (Heringa et al., 2006) and humic acid (Zhang et al., 1996). In order to determine if organic matter fouling was a problem in this study the fibres were analysed using solid-state $^{13}$C cross-polarisation magic-angle spinning (CPMAS) nuclear magnetic resonance (NMR). The spectra for clean PA fibre and PA fibre exposed to 12.5 mgC/L Aldrich HA and alginic acid for 48 hours were shown Figure 4.10a-c. No difference in spectra was observed in the presence of Aldrich HA or alginic acid. Further, no fibre colour change was detected as this can indicate fouling. Therefore, no organic matter fouling of the PA fibres was detectable using solid-state $^{13}$C NMR. However, it is possible that the level of fibre contamination was below the detection limit of the instrument. Optimal detection for carbon NMR was around 1% by weight of an organic component (Apperley, Personal Communication, March 3, 2009). Therefore, if organic matter fouling occurred at concentrations lower than 0.125 mgC/L this could not be detected.
Figure 4.10: Solid-state $^{13}$C NMR spectra for a) clean PA fibre, b) PA fibre exposed to 12.5 mgC/L Aldrich HA for 48 hours and c) PA fibre exposed to 12.5 mgC/L alginic acid for 48 hours (1mM NaHCO$_3$, 20 mM NaCl, pH 7, 12.5 mgC/L organic matter)

4.8. Conclusions

By comparing estradiol extraction in the absence and presence of organic matter this suggests that SPME can be applied to quantify solute-solute interactions at environmental (low) concentrations. Therefore, SPME will be applied to determine log $K_{OM}$ values for steroidal hormones in Chapter 5. Exploration of potential methodology problems such as fibre fouling and hormone degradation has eliminated some assumed limitations and provided robustness to the log $K_{OM}$ values.
5 Quantification of Solute-Solute Interactions using Solid-Phase Microextraction

In this chapter the solid-phase microextraction (SPME) methodology developed in Chapter 4 will be applied to determine organic matter-water partition coefficients (log $K_{OM}$) for steroidal hormones at environmentally relevant concentrations. Due to gaps in current knowledge, the purpose of this chapter is to determine the influence of organic matter type, concentration and solution chemistry on partitioning.

The partitioning of estradiol to a wide range of organic matter types including natural organic matter (NOM) surrogates, polysaccharides, polyphenols and surfactants was studied. The dominant mechanism of solute-solute interactions appears to be hydrogen bonding. However, with the exception of tannic acid, no significant difference in log $K_{OM}$ values was observed for estradiol. The similarity in partitioning was attributed to the weak sorption of estradiol to the majority of studied organic matter types.

To understand how the properties of steroidal hormones influence partitioning the interaction of estrone, progesterone and testosterone with organic matter was studied. While no significant difference in testosterone partitioning was observed, log $K_{OM}$ values for estrone and progesterone were significantly influenced by both organic matter type and pH. This was due to stronger sorption to organic matter, and was related to functional group content and position within the hormones. Further, log $K_{OM}$ values for estrone decreased with increasing NaCl concentration (ionic strength). This was related to changes in organic matter conformation. Organic matter concentration also influenced sorption, with decreased partitioning with increased organic matter concentration.
5.1. Introduction

In Chapter 4 a solid-phase microextraction (SPME) technique was developed to quantify organic matter-water partition coefficients (log $K_{OM}$). Firstly, this chapter aims to demonstrate that the SPME technique developed in Chapter 4 is indeed suitable to quantify solute-solute interactions for steroidal hormones. This will be achieved by quantifying the interaction of estradiol with a wide range of organic matter types from pH 4 to 9. Further, the experimental log $K_{OM}$ values will be compared to log $K_{OM}$ values taken from the literature to assess the applicability of SPME.

Secondly, this chapter aims to determine if organic-matter water partitioning is influenced by organic matter type and concentration as well as variations in solution chemistry. Further, log $K_{OM}$ values will be determined for different hormones including estrone, progesterone and testosterone. Very few studies have considered the influence of pH or ionic strength on partitioning therefore little is known regarding the implications of solution chemistry. Consequently, the purpose of this study is to better understand the mechanisms of solute-solute interactions and the implications of the above parameters for this interaction. Log $K_{OM}$ values calculated in this chapter will be applied to quantify solute-solute interactions in stirred cell ultrafiltration in Chapter 7.

5.2. Organic Matter-Water Partitioning for Estradiol

The affinity of estradiol for organic matter was measured as a function of organic matter type (11 organics) and pH (4 to 9). The sorption isotherms, plotted as freely dissolved estradiol concentration (ng/L) versus bound estradiol concentration (ng/kg), are shown in Figure 5.1a-k. The majority of the isotherms had correlation coefficients greater than 0.95. The linearity of the sorption isotherms suggest that all sorbents act as partitioning sorbents and adsorption does not play a role (Schwarzenbach et al., 2003). This linearity is consistent with previous findings for
estradiol (Holthaus et al., 2002; Lee et al., 2003). The log $K_{OM}$ values are listed in Table 5.1.

Figure 5.1: Organic matter-water sorption isotherms for estradiol with a) Aldrich HA, b) alginic acid, c) Australian NOM, d) colloidal cellulose, e) powder cellulose, f) dextran, g) IHSS FA, h) IHSS HA, i) IHSS NOM, j) SDS and k) tannic acid (1mM NaHCO$_3$, 20 mM NaCl, 100-100000 ng/L estradiol, 12.5 mgC/L organic matter)
Table 5.1: Log $K_{OM}$ values (L/kg) with standard deviation (S.D) for estradiol from pH 4 to 9 with log $K_{OM}$ values from the literature calculated using fluorescence quenching (FQ) and solubility enhancement (SE) (Yamamoto et al., 2003)

<table>
<thead>
<tr>
<th>pH</th>
<th>± S.D</th>
<th>pH</th>
<th>± S.D</th>
<th>pH</th>
<th>± S.D</th>
<th>pH</th>
<th>± S.D</th>
<th>FQ$^1$ and SE$^2$ log $K_{OM}$ values at pH 7$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDS</td>
<td>-</td>
<td>Powder</td>
<td>-</td>
<td>3.68±0.37</td>
<td>-</td>
<td>2.87±0.74</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Cellulose</td>
<td></td>
<td>Colloidal</td>
<td></td>
<td>3.75±0.05</td>
<td>-</td>
<td>3.89±0.11</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Cellulose</td>
<td></td>
<td></td>
<td></td>
<td>3.94±0.17</td>
<td>-</td>
<td>3.82±0.08</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Alginic acid</td>
<td>3.88±0.15</td>
<td>-</td>
<td>3.96±0.22</td>
<td>-</td>
<td>3.42±0.63</td>
<td>3.75$^2$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dextran</td>
<td>-</td>
<td></td>
<td></td>
<td>3.96±0.14</td>
<td>-</td>
<td>3.77±0.35</td>
<td>2.76$^2$</td>
<td></td>
</tr>
<tr>
<td>IHSS NOM</td>
<td>4.23±0.13</td>
<td>3.95±0.13</td>
<td>3.95±0.28</td>
<td>3.71±0.84</td>
<td>3.68±0.49</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Australian NOM</td>
<td>4.16±0.13</td>
<td>3.86±0.06</td>
<td>3.98±0.21</td>
<td>3.99±0.31</td>
<td>3.84±0.72</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IHSS HA</td>
<td>4.28±0.12</td>
<td>4.09±0.05</td>
<td>3.99±0.22</td>
<td>4.04±0.39</td>
<td>3.84±0.25</td>
<td>4.92$^1$; 4.56$^2$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IHSS FA</td>
<td>4.24±0.01</td>
<td>3.65±0.37</td>
<td>4.15±0.27</td>
<td>3.78±0.36</td>
<td>-</td>
<td>4.57$^1$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aldrich HA</td>
<td>4.18±0.20</td>
<td>4.26±0.05</td>
<td>4.21±0.08</td>
<td>4.20±0.25</td>
<td>3.95±0.45</td>
<td>4.94$^1$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tannic Acid</td>
<td>5.11±0.20</td>
<td>5.29±0.07</td>
<td>4.86±0.22</td>
<td>4.51±0.35</td>
<td>4.01±0.05</td>
<td>5.28$^1$; 4.94$^2$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$Yamamoto et al. 2003

5.2.1. Influence of Organic Matter at Neutral pH

As Table 5.1 indicates there is some variability in log $K_{OM}$ values with different organic matter types. Previous studies have suggested that the properties of organic matter such as molecular weight, aromaticity and polarity can influence partitioning (Chin et al., 1997; Chiou et al., 1986). The majority of these studies focused on apolar micropollutants and it is not clear whether these properties also influence partitioning of polar micropollutants. Therefore, the relationship between experimental log $K_{OM}$ values at pH 7 and molecular weight, aromaticity and polarity are studied.
Figure 5.2 shows the relationship between organic matter molecular weight and log \( K_{OM} \) values. The molecular weight of the studied organics varied greatly from 288 to 810264 g/mol. The correlation coefficient (R) indicated a small negative trend (\( R = -0.28 \)) suggesting a decrease in log \( K_{OM} \) values with increasing molecular weight. However, as the R value is low the correlation is negligible. In contrast, Chin et al. (1997) found a strong relationship between the increasing molecular weight of humic acid and log \( K_{OM} \) values for pyrene suggesting that the additional aromatic functional groups in the larger humic acid molecules contributed to stronger sorption. However, the authors suggested that this observation was highly specific to the studied organic matter type. As a wide range of organic matter types were compared in Figure 5.2 this may explain why only a negligible correlation was observed.

![Figure 5.2: Relationship between log \( K_{OM} \) values and organic matter molecular weight (g/mol) (1mM NaHCO\(_3\) 20 mM NaCl, pH 7, 100-100000 ng/L estradiol, 12.5 mgC/L organic matter)](image)

The relationship between log \( K_{OM} \) values and specific UV absorbance (SUVA), which is an indicator of aromaticity, is shown in Figure 5.3. The R value is 0.52 which indicates a weakly positive correlation. Based on SUVA measurements, tannic acid has the greatest aromaticity and also exhibited the strongest interaction with estradiol based on the log \( K_{OM} \) values. However, the aromaticity of natural organic matter (NOM) surrogates, polysaccharides and SDS appear to have little influence on
Quantification of Solute-Solute Interactions using Solid-Phase Microextraction

organic matter-water partitioning for steroidal hormones. Gauthier et al. (1987) found increasing organic matter aromaticity led to increased interaction with pyrene, and suggested that this was due to increased polarizability of the organic matter. Increased polarizability can increase non-specific molecular interactions through induced dipole-induced dipole interactions (Schwarzenbach et al., 2003) and this may contribute to stronger partitioning for aromatic compounds. This will be discussed further in Chapter 6. In contrast, Mao et al. (2002) found aromaticity had no significant influence on sorption of phenanthrene. Therefore, similar to molecular weight, the variability may be related to the studied organic matter type and micropollutant.

![Figure 5.3: Relationship between log K_{OM} values and specific UV absorbance (SUVA) (L/mg.m) (1mM NaHCO₃ 20 mM NaCl, pH 7, 100-100000 ng/L estradiol, 12.5 mgC/L organic matter)](image)

Within the literature, several studies have observed a relationship between organic matter polarity and log K_{OM} (Kile et al., 1999), with Chiou et al. (1986) suggesting that decreased organic matter polarity led to greater organic matter-water partitioning. While polarity can be difficult to measure for complex organic matter types elemental stoichiometric ratios, specifically the ratio of oxygen to carbon (O/C), can provide an indirect method to estimate polarity (Murphy et al., 1990). Figure 5.4 shows there is no correlation between the log K_{OM} values and O/C
elemental stoichiometric ratios (R = -0.003). While the O/C elemental ratio can provide some information regarding polarity, it cannot reflect the hydrogen accepting or donating activities of the solutes (Schwarzenbach et al., 2003) which may explain the lack of correlation in Figure 5.4.

![Diagram showing log KOM values and O/C elemental stoichiometric ratio](image)

**Figure 5.4: Relationship between log K\text{OM} values and O/C elemental stoichiometric ratio (1mM NaHCO\textsubscript{3}, 20 mM NaCl, pH 7, 100-100000 ng/L estradiol, 12.5 mgC/L organic matter)**

The lack of a strong relationship between log K\text{OM} values for estradiol and organic matter molecular weight, aromaticity or polarity suggests that there is no simple correlation to characterise sorption of steroidal hormones. It is likely that the interaction of steroidal hormones with organic matter is primarily related to the functional groups present and the molecular interactions between the solutes. Based on the characteristics of estradiol and organic matter the mechanisms of interaction are expected to be hydrogen bonding and van der Waals interactions.

Estradiol contains bipolar phenolic and hydroxyl functional groups while the studied organic matter contains mainly bipolar oxygenated functional groups (Schwarzenbach et al., 2003). The NOM surrogates are composed of a wide range of functional groups, including carboxylic, phenolic and carbonyl groups (Stevenson, 1994) while the polysaccharides mainly contain hydroxyl and carboxylic moieties.
Tannic acid primarily contains phenolic moieties such as catechol and gallic acid (Kaal et al., 2005). SDS is the only organic studied which does not contain a bipolar functional group, however, it does contain a monopolar sulphate moiety.

At pH 7 two-sample t-tests indicated that there was no significant difference between the log $K_{OM}$ values in Table 5.1 for all organic matter types, with the exception of tannic acid. For NOM surrogates, polysaccharides and SDS log $K_{OM}$ values ranged from $4.21 \pm 0.08$ to $3.68 \pm 0.37$ (Table 5.1) which approximately corresponds to a factor of three. The log $K_{OM}$ value for tannic acid was significantly higher at $4.86 \pm 0.22$. Based on SPME experiments, the percentage of estradiol interacting with most organic matter types was low (5-15%) which may explain the similarity in partitioning. The interaction with tannic acid was considerably stronger (~40% sorbed to tannic acid) leading to a significantly higher log $K_{OM}$ value.

The tannic acid-water partition coefficient (Figure 5.1k) was significantly higher than the other interactions, indicating tannic acid was the strongest sorbent ($4.86 \pm 0.22$). This has been observed previously in other studies with estradiol (Yamamoto and Liljestrand, 2003; Yamamoto et al., 2003) and may be related to the large fraction of phenolic hydroxyl groups in tannic acid. A study by Jin et al. (2007) indicated that phenolic content, as opposed to total aromaticity, determines sorption of hormones with a phenolic moieties 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 neutrally charged, with the exception of dextran.

Log $K_{OM}$ values for NOM surrogates at pH 7 ranged from $4.21 \pm 0.08$ to $3.95 \pm 0.28$ (Table 5.1). There was no significant difference in the log $K_{OM}$ values. It was thought that the origin of the NOM surrogates would influence partitioning, as this can affect the structure and functional group content. For example, terrestrial humic acids such as Aldrich humic acid (HA) typically contain more phenolic and carboxylic groups compared to aquatic humics such as IHSS HA (Gauthier et al., 1987). However, a two-sample t-test indicated that there was no significant difference between log $K_{OM}$ values for Aldrich HA and IHSS HA ($4.21 \pm 0.08$ and $3.99 \pm 0.22$ 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 (Schäfer, 2001). Nevertheless, Australian and IHSS NOM had very similar log $K_{OM}$ values ($3.98 \pm 0.21$ and $3.95 \pm 0.28$ respectively) suggesting that the presence of salts in concentrated organic matter did not influence the partitioning behaviour of estradiol significantly. The influence of ionic strength will be explored further in Section 5.5.

Log $K_{OM}$ values for the polysaccharides, alginate acid, dextran and cellulose, were all similar to the NOM surrogates. Log $K_{OM}$ values ranged from $3.75 \pm 0.05$ to $3.96 \pm 0.22$ (Table 5.1). It has been suggested that organic matter lacking aromatic functional groups such as dextran will have little influence on the fate and behaviour of steroidal hormones (Jin et al., 2007). However, the similarity of polysaccharide log $K_{OM}$ values to the NOM surrogates may be due to their polarity, thus increasing the potential for hydrogen bonding. The elemental stoichiometric ratios suggest that the selected polysaccharides are more polar than both tannic acid and NOM surrogates (Figure 5.4). All of the polysaccharides contain hydroxyl moieties, and in the case of alginate acid carboxylic functional groups, enabling them to act as both hydrogen donors and acceptors. Therefore, despite lacking phenolic functional groups, polysaccharides still interact with estradiol through hydrogen bonding.

The SDS-water partition coefficient (Figure 5.1j) was lower than all other studied organic matters ($3.68 \pm 0.38$). However, this was not significant due to the high standard deviation of the log $K_{OM}$ value. SDS is an anionic surfactant and contains a hydrophilic head with a hydrophobic tail (Singh and Song, 2006). 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 (sulphate group). As the SDS concentration is below the critical micelle concentration (CMC) ($0.0082$ M at $25^\circ$C), micelle formation is not a factor in partitioning. Above the CMC aggregates of SDS molecules can form with the hydrophilic head on the outside facilitating hydrogen bonding with the solution. Further, it is unlikely that the hydrophobic tail of SDS contributes significantly to the interaction, as research by
Yamamoto et al. (2003) has indicated that hydrogen bonding is the primary mechanism of interaction between organic matter and estradiol, compared to non-specific interactions.

From this study it appears that estradiol simply does not interact strongly with organic matter, and consequently differences in partitioning to different organic matter types may be more visible with a stronger sorbing micropollutant.

5.2.2. Influence of pH

The interaction of estradiol with organic matter was studied from pH 4 to 9. Figure 5.5 indicates a significant decrease in log $K_{OM}$ values from acidic to neutral pH for most NOM surrogates and tannic acid. No significant change in log $K_{OM}$ values was observed above neutral pH for NOM surrogates or tannic acid, while no change over the entire studied pH range was seen for any polysaccharides or SDS. A decrease in log $K_{OM}$ value at alkaline pH was observed previously when studying the interaction of phenolic compounds with commercial humic acids using SPME (Hu et al., 2006; Ohlenbusch et al., 2000).

It is expected that the change in log $K_{OM}$ values from acidic to neutral conditions was related to the dissociation of organic matter, not the speciation of estradiol. Estradiol is a weak acid with an acid dissociation constant ($pK_a$) of 10.23 and therefore can be affected by pH. The fraction of neutral species ($f_{\text{neutral}}$) in the dissociating hormones can be calculated using Equation 5.1. 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 influence partitioning. Zeta potential measurements (mV) of the fibre (Figure 3.2 in Chapter 3) indicated no change in charge in the studied pH range.

\[
f_{\text{neutral}} = \frac{1}{1 + 10^{(pH-pK_a)}}
\] (5.1)
In contrast, most of the studied organic matter types are affected by pH in the investigated range. Phenolic functional groups deprotonate at alkaline pH values (pK\(_a\) of 9.9 of unsubstituted phenol), while carboxylic groups deprotonate under acidic conditions (pK\(_a\) around 4.5) (Avena et al., 1999; Sparks et al., 1997). Due to the high carboxylic functional group content in the majority of NOM surrogates studied, most are negatively charged at neutral pH which may account for decreased partitioning of estradiol to NOM surrogates from pH 4 to 7. pH changes can have implications for intramolecular bonding within the organic matter, as well as molecular shape and charge. The implications of changing pH conditions on the charge and conformation of organic matter will be discussed further in Section 5.4 where a wider pH range will be studied (4 to 12) with more steroidal hormones.

In Table 5.1 there are several log K\(_{OM}\) values missing, particularly for polysaccharides and SDS. For most organic matter types the mass of estradiol sorbed to organic matter was low (5-15%) and at some pH conditions no decrease in freely
dissolved estradiol concentration was detected, meaning the interaction could not be quantified.

5.2.3. Comparison with Other Quantification Techniques

The interaction of estradiol with organic matter has been quantified previously using fluorescence quenching and solubility enhancement. Yamamoto et al. (2003) quantified the interaction of estradiol with Suwannee River (IHSS) fulvic acid (FA) and HA, pre-filtered Aldrich HA and tannic acid using fluorescence quenching, while the interaction of estradiol with IHSS HA, alginic acid, tannic acid and dextran was quantified using solubility enhancement. The log $K_{OM}$ values calculated using SPME were compared to these two methods in Figure 5.6. SPME, fluorescence quenching and solubility enhancement are very different techniques, and therefore have different advantages and limitations. As discussed in Chapter 2, solubility enhancement can be limited by the concentration of organic matter used in the experiment, while fluorescence quenching does not consider micropollutant quenching due to molecular oxygen or degradation (Backhus et al., 2003). The limitations of SPME can be found in Section 4.2.5 of Chapter 4.

![Figure 5.6: Comparison of experimental log $K_{OM}$ values calculated using SPME with log $K_{OM}$ values calculated using fluorescence quenching and solubility enhancement (Yamamoto et al., 2003)](image-url)
In Figure 5.6, log $K_{OM}$ values calculated using fluorescence quenching were a factor of 2.6 to 8.5 times larger than those calculated using SPME. Since concentration of organic matter used in the SPME experiments was in the same concentration range (up to 10 mgC/L in the literature compared to 12.5 mgC/L in SPME experiments), this can be ruled out as a difference. Consistent with the present observation, previous studies comparing SPME and fluorescence quenching for different micropollutants such as pyrene have observed fluorescence quenching log $K_{OM}$ values a factor of 5 to 10 times higher than SPME log $K_{OM}$ values (Doll et al., 1999; Mackenzie et al., 2002). Doll et al. (1999) indicated that this was due to limitations associated with SPME, as it can disturb contaminants weakly bound to the outer shell of organic matter leading to a higher concentration in the aqueous phase, therefore underestimating partitioning. Log $K_{OM}$ values calculated using SPME and solubility enhancement were similar for IHSS HA, tannic acid and alginic acid but significantly different for dextran (Figure 5.6). The difference in the results was due to the organic carbon concentration of dextran in Yamamoto (2003), which was as high as 263 mgC/L.

This indicates that SPME is a suitable technique to quantify solute-solute interactions with steroidal hormones. However, due to weak sorption log $K_{OM}$ values for estradiol did not vary significantly with the different organic matter types or pH. Therefore, solute-solute interactions will be studied with a range of different hormones including estrone and progesterone.

5.3. Influence of Hormone Type

In this section the interaction of estrone, progesterone and testosterone with organic matter will be studied. As Section 5.2 indicated for the majority of organic matter types studied there was no significant difference in estradiol partitioning due to weak sorption. As a result a representative organic matter type from each of the NOM surrogate, polysaccharide and polyphenol groups was selected for study. These were Aldrich HA, alginic acid and tannic acid. By restricting partitioning to three organic matter types the properties of each organic can be studied to a greater extent.
compared to Section 5.2, allowing for an improved understanding of the sorption mechanism. Further, by studying several hormones containing different functional groups a better understanding of how the properties of steroidal hormones influence organic matter-water partitioning can be obtained. Log $K_{OM}$ values for all studied hormones at pH 7 are shown in Table 5.2.

### Table 5.2: Log $K_{OM}$ values determined using SPME for estradiol, estrone, progesterone and testosterone at pH 7

<table>
<thead>
<tr>
<th></th>
<th>Humic Acid ± S.D</th>
<th>Alginic Acid ± S.D</th>
<th>Tannic Acid ± S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol</td>
<td>4.21±0.08</td>
<td>3.96±0.22</td>
<td>4.86±0.22</td>
</tr>
<tr>
<td>Estrone</td>
<td>4.82±0.04</td>
<td>5.47±0.11</td>
<td>5.51±0.07</td>
</tr>
<tr>
<td>Progesterone</td>
<td>4.59±0.10</td>
<td>3.57±0.08</td>
<td>5.53±0.16</td>
</tr>
<tr>
<td>Testosterone</td>
<td>4.04±0.21</td>
<td>3.16±0.64</td>
<td>4.74±0.13</td>
</tr>
</tbody>
</table>

For all hormones partitioning was strongest for tannic acid, followed by Aldrich HA and alginic acid (Figure 5.7). However, the order of partitioning was not the same for all hormones. For tannic acid and Aldrich HA, estrone had the highest log $K_{OM}$ value followed by progesterone, while testosterone and estradiol were lower but had similar log $K_{OM}$ values. In contrast, for alginic acid log $K_{OM}$ values were highest for estrone and estradiol followed by progesterone and testosterone.

Sorption of hormones to organic matter was dependent on a number of properties of both the organic matter and the micropollutant, including acidic functional group content and charge (Yamamoto et al., 2003). At pH 7 all hormones were neutrally charged, while Aldrich HA and alginic acid were >99% dissociated and anionic, but tannic acid was primarily in a non-dissociated form (99%). In consequence, the interaction of steroidal hormones was strongest with tannic acid compared to the other organics.
Figure 5.7: Log $K_{OM}$ values for estradiol, estrone, progesterone and testosterone at pH 7 (1mM NaHCO$_3$, 20 mM NaCl, 100-100000 ng/L hormone, 12.5 mgC/L organic matter)

The hormones were all bipolar due to hydroxy substituents, except for progesterone which contained monopolar ketone groups only. Log $K_{OM}$ values for estrone and progesterone to Aldrich HA and tannic acid were 2.3 to 7.9 times greater than estradiol and testosterone respectively. This was due to the ketone functional group in the C-17 position of estrone and the C-20 position of progesterone (refer to Table 3.3 in Chapter 3). Ketone groups are strong hydrogen acceptors, and a study by Le Questel et al. (2000) demonstrated that the C-20 ketone moiety in progesterone is a triple hydrogen acceptor. The structure of estradiol and estrone is very similar as both have a phenolic hydroxyl group in the C-3 position, however, estradiol has a hydroxyl group in the C-17 position instead of a ketone group. In addition, there are similarities between testosterone and progesterone as both have a ketone group in the C-3 position, but like estradiol, testosterone also has a hydroxyl group in the C-17 position. Previous studies on steroidal hormone interactions with molecularly imprinted polymers indicated that functional groups in the C-17 position (or C-20 in case of progesterone) were more important for molecular interactions compared to C-3 functional groups (Rachkov et al., 1998; Rachkov et al., 2000). This may explain
why progesterone and estrone have a greater affinity for Aldrich HA and tannic acid compared to estradiol and testosterone.

The order of partitioning was not observed for alginic acid, with the highest partitioning observed for estrone followed by estradiol. In the case of alginic acid it appears that the presence of phenolic hydroxyl groups in the C-3 position enhances sorption. This may be related to the functional group content of the organic matter as alginic acid predominantly contains carboxylic groups while tannic acid and Aldrich HA both contain phenolic hydroxyl groups as well as carboxylic moieties in the case of Aldrich HA (Table 3.2 in Chapter 3). The relationship between log $K_{OM}$ values and octanol-water partition coefficients ($log K_{OW}$) was plotted in Figure 5.8. The $R$ values for all organics indicated weak negative relationships (-0.13 to -0.46), suggesting minimal correlation between log $K_{OM}$ and log $K_{OW}$ values. This confirms that non-specific interactions are not the dominant interaction mechanism for hormone partitioning to organic matter.

Figure 5.8: Relationship between log $K_{OM}$ and log $K_{OW}$ values plotted using a linear regression where E1 is estrone, E2 is estradiol, P is progesterone and T is testosterone (1mM NaHCO₃ 20 mM NaCl, pH 7, 100-100000 ng/L hormone, 12.5 mgC/L organic matter)
As discussed in Section 5.2.1 the mass of estradiol sorbed to the majority of organic matter types was low (5-15%) indicating weak sorption. Due to similar structural properties (hydroxyl moiety in the C-17 position) the mass of testosterone sorbed to organic matter was similar to estradiol. However, considerably more progesterone and estrone were bound to the organic matter phase, with around 80% of each bound to tannic acid. However, the mass of hormone extracted by SPME did not significantly change with the different hormones, and did not exceed 5% for any hormone therefore the assumptions of the SPME technique were still met. As estrone interacts strongly with organic matter it will be studied to determine if ionic strength and organic matter concentration influence partitioning.

5.4. Influence of pH

To understand the influence of pH on solute-solute interactions log $K_{OM}$ values were quantified from pH 4 to 12 (Table 5.3). While estradiol and estrone become negatively charged in alkaline pH conditions ($pK_a$ 10.23 and 10.34 respectively), progesterone and testosterone lack dissociable functional groups, so will remain neutral within the studied pH range. The difference in sorption of dissociating and non-dissociating hormones to organic matter has not previously been explored. However, as sorption of charged species to the fibre at high pH was negligible it was difficult to measure log $K_{OM}$ values for estradiol and estrone above pH 10.
Table 5.3: Log $K_{OM}$ values determined using SPME for estradiol, estrone, progesterone and testosterone as a function of pH (4-12)

<table>
<thead>
<tr>
<th></th>
<th>pH 4 ± S.D</th>
<th>pH 7 ± S.D</th>
<th>pH 8 ± S.D</th>
<th>pH 9 ± S.D</th>
<th>pH 10 ± S.D</th>
<th>pH 12 ± S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aldrich HA</td>
<td>4.18±0.20</td>
<td>4.21±0.08</td>
<td>4.20±0.25</td>
<td>3.95±0.45</td>
<td>3.83±0.04</td>
<td>-</td>
</tr>
<tr>
<td>Alginic Acid</td>
<td>3.88±0.15</td>
<td>3.96±0.22</td>
<td>-</td>
<td>3.42±0.63</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannic Acid</td>
<td>5.11±0.20</td>
<td>4.86±0.22</td>
<td>4.51±0.35</td>
<td>4.02±0.05</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

| Estrone |            |            |            |            |             |             |
| Aldrich HA | 5.27±0.03 | 4.82±0.04 | -          | 4.75±0.04 | 4.52±0.11   | -           |
| Alginic Acid | -        | 5.47±0.11 | 4.77±0.04 | 4.33±0.18 | -           | -           |
| Tannic Acid | 6.02±0.12 | 5.51±0.07 | 5.05±0.01 | 4.69±0.06 | -           | -           |

| Progesterone |            |            |            |            |             |             |
| Aldrich HA | 4.73±0.08  | 4.59±0.10  | -          | 4.58±0.07 | 4.48±0.02   | 4.54±0.05   |
| Alginic Acid | 3.89±0.13  | 3.57±0.08  | -          | 3.40±0.40 | 3.24±0.28   | -           |
| Tannic Acid | 5.87±0.16  | 5.53±0.16  | -          | 4.38±0.16 | 3.79±0.29   | 4.27±0.09   |

| Testosterone |            |            |            |            |             |             |
| Aldrich HA | 4.13±0.13  | 4.04±0.21  | -          | 4.17±0.19 | 4.12±0.23   | 4.45±0.22   |
| Alginic Acid | -         | 3.16±0.64  | -          | 3.67±0.37 | 3.77±0.09   | -           |
| Tannic Acid | 4.88±0.08  | 4.74±0.13  | -          | 4.14±0.40 | 3.87±0.17   | 4.40±0.07   |

In Figure 5.9 tentative dissociation of the studied organic matter types as a function of pH is plotted with experimental log $K_{OM}$ values. The purpose of this is to better understand the influence of organic matter speciation on solute-solute interactions.
Figure 5.9: Log $K_{OM}$ values for estradiol, estrone, testosterone and progesterone with organic matter dissociation (%) shown by the dotted line for a) Aldrich HA (Shin et al., 1999); b) alginic acid (Davis et al., 2003); and c) tannic acid (Kraal et al., 2006), as a function of pH (1mM NaHCO$_3$, 20 mM NaCl, 100-100000 ng/L hormone, 12.5 mgC/L organic matter).

5.4.1. Aldrich Humic Acid

The Aldrich HA-water sorption isotherms are shown in Figure 5.10. The results indicate that partitioning was significantly higher at pH 4 for progesterone and
estrone, when Aldrich HA was only 30-40% dissociated. Despite Aldrich HA being highly dissociated at pH 7 (~99%) a significant decrease in estrone partitioning was also observed at pH 9 and 10. It is likely that this was related to the dissociation of estrone. No significant difference in partitioning as a function of pH was observed for estradiol and testosterone. This was related to their weaker interaction with organic matter compared to the other hormones.

![Graph](image)

**Figure 5.10: Aldrich HA-water sorption isotherms for a) estradiol, b) estrone, c) progesterone and d) testosterone (1mM NaHCO$_3$, 20 mM NaCl, 100-100000 ng/L hormone, 12.5 mgC/L Aldrich HA)**

The Aldrich HA solid-state $^{13}$C NMR spectrum (Figure 3.6a in Chapter 3) indicated that Aldrich HA primarily contained aliphatic functional groups. This spectrum was similar to that published by Shin *et al.* (1999), and was because the Aldrich HA used in this study was not purified or fractionated. Prior to experiments Aldrich HA is often acidified as part of pre-treatment and this can dissociate certain functional groups such as carboxylic acid leading to a different $^{13}$C NMR spectrum (Stevenson, 1994). However, the total surface acidity measurements for Aldrich HA from the literature indicated it contained both carboxylic (4.80 meq/g) and phenolic hydroxyl
(2.26 meq/g) functional groups (Table 3.2 in Chapter 3) (Kim et al., 1990). Zeta potential measurements of Aldrich HA by Yan and Bai (2005) indicated an increased negative charge from pH 4 to 7 (-27 to -38 mV) due to the dissociation of the carboxylic functional groups. The charge was constant from pH 7 to 12 despite the presence of phenolic hydroxyl groups which dissociate around pH 9.9. However, based on zeta potential measurements, it appears that phenolic groups did not significantly affect the charge of Aldrich HA (Yan and Bai, 2005). Therefore, the minor difference in log $K_{OM}$ values, particularly for the non-dissociating hormones, from neutral to alkaline pH was related to Aldrich HA charge consistency from pH 7 to 12.

The decrease in hormone sorption from acidic to neutral conditions was influenced by both the speciation of Aldrich HA and conformational changes. When considering Aldrich HA as a polyelectrolyte, previous studies have suggested that the uncoiling of Aldrich HA to a linear form with increasing pH may reduce the hydrophobicity of Aldrich HA which in turn can reduce partitioning (Ra et al., 2008). An alternate view to the polyelectrolyte theory suggests that humic acid is a supramolecular structure formed by weak bonding of humic acid molecules (Piccolo, 2001). Studies have indicated that the molecular size of humic acid increased at acidic pH (around 4-5) due to the formation of hydrogen bonds between the humic acid molecules (Cozzolino and Piccolo, 2001). Previous studies have also indicated stronger sorption to higher molecular weight organics (Chin et al., 1997; Jones and Tiller, 1999) and this may also account for the increased hormone partitioning in acidic solutions. This was not observed in Figure 5.2, but may be related to the use of different organic matter types. Consequently, increased partitioning at acidic pH may be related to changes in charge and molecular weight.

At pH 10, Aldrich HA was the only organic where a log $K_{OM}$ value for the partially dissociated estradiol and estrone could be measured. This was due to the functional group content of Aldrich HA compared to alginic acid and tannic acid. While the charge of Aldrich HA is dominated by strong acidic functional groups, it also contains other functional groups, such as carbonyl and hydroxyl moieties which are
not dissociated at pH 10 (Sparks et al., 1997). Despite dissociation of phenolic functional groups and a significant decrease in partitioning from pH 9, the log $K_{OM}$ value for estrone at pH 10 was still greater than progesterone and testosterone.

Several studies have investigated the implications of pH for the sorption of various micropollutants such as polycyclic aromatic hydrocarbons (PAH) and tributyltin (Arnold et al., 1998; Schlautman and Morgan, 1993) to humic acid using techniques including equilibrium dialysis and fluorescence quenching. These studies have indicated a decrease in sorption in alkaline conditions due to deprotonation of humic acid (Schlautman and Morgan, 1993). In addition, Yamamoto and Liljestrand (2003) studied the partitioning of estradiol to colloidal Suwannee River (IHSS) HA at pH 5, 7 and 9 using fluorescence quenching. This study indicated no significant difference in partitioning as a function of pH. This differed from the findings of the present study where log $K_{OM}$ values for estradiol and IHSS HA decreased significantly from pH 4 to 7. This difference may be related to the restricted pH range used by Yamamoto and Liljestrand (2003). Due to the high carboxylic acid content of Suwannee River HA (Ritchie and Perdue, 2003) it would be significantly more dissociated at pH 5 compared to pH 4. As estradiol remains undissociated in the studied range, pH would not be expected to have significant influence on the interaction.

5.4.2. Alginic Acid

The alginic acid-water sorption isotherms for all studied hormones are shown in Figure 5.11. Log $K_{OM}$ values for alginic acid were generally lower for all hormones in the studied pH range compared to the other organic matter types. The solid-state $^{13}$C NMR spectrum for alginic acid (Figure 3.6b in Chapter 3) indicated the presence of carboxylic and ringed carbon functional groups which are common for polysaccharides. Based on titration data, the total acidity of alginic acid was predominately due to carboxylic functional groups (7.02 meq/g) (Table 3.2 in Chapter 3) (Jeon et al., 2002), and therefore it began to dissociate from pH 3. Similar to Aldrich HA, the conformation of alginic acid was affected by solution chemistry.
A study by Avaltroni et al. (2007) demonstrated that the structure of alginic acid changed with increasing pH. At acidic pH the structure is coiled and rigid due to intramolecular hydrogen bonding. As the pH increases the structure becomes more linear due to charge repulsion as a result of carboxylic dissociation, and above pH 8 depolymerisation can occur which reduces alginic acid to small, flexible fragments (Avaltroni et al., 2007).

![Graphs showing alginic acid-water sorption isotherms for estradiol, estrone, progesterone, and testosterone](image)

Figure 5.11: Alginic acid-water sorption isotherms for a) estradiol, b) estrone, c) progesterone and d) testosterone (1mM NaHCO3 20 mM NaCl, 100-100000 ng/L hormone, 12.5 mgC/L alginic acid)

From the available data, one could observe a decrease in partitioning for estradiol, estrone and progesterone as pH increased, however, based on a two-sample t-test this was not statistically significant in the case of estradiol. In contrast, log KOM values for testosterone increased as pH increased from 7 to 9. However, due to the large standard deviation associated with the log KOM value (± 0.64) at pH 7 (Table 5.3) it is likely that any difference is due to error.
The interaction of alginic acid with micropollutants has not been studied as a function of pH. Yamamoto et al. (2004; 2003) determined log $K_{OM}$ values for alginic acid for a range of estrogenic micropollutants, including estradiol at pH 7 and this is shown in Figure 5.6. The log $K_{OM}$ value for estradiol calculated using solubility enhancement was remarkably similar to results from this study with a log $K_{OM}$ value of 3.75 (Yamamoto et al., 2003) compared to 3.96.

5.4.3. Tannic Acid

Tannic acid-water sorption isotherms for all hormones are shown in Figure 5.12. The influence of pH on partitioning was strongest for tannic acid. Partitioning decreased over one order of magnitude from acidic to alkaline conditions for the majority of the hormones studied. Total surface acidity studies indicated that tannic acid contains a high content of phenolic hydroxyl moieties (9.55 meq/g) (Table 3.2 in Chapter 3) (Flores-Céspedes et al., 2006), and started to dissociate around neutral pH conditions ($pK_a$ of 8.5). The solid-state $^{13}$C NMR spectrum for tannic acid (Figure 3.6c in Chapter 3) confirmed the total acidity results, as it predominately contained polyphenolic functional groups. The strong sorption to tannic acid was due to hydrogen bonding with the high concentration of phenolic groups present in tannic acid (Jin et al., 2007; Yamamoto et al., 2003). Further, non-specific interactions may increase partitioning due to the high aromatic content of tannic acid which can lead to increased polarizability.

The strongest partitioning was observed for pH 4 when tannic acid was protonated, and this decreased slightly at pH 7, as tannic acid began to dissociate and undergo hydrolysis to gallic acid (Osawa and Walsh, 1993). For all hormones there was a significant decrease in partitioning (factor of 4 to 14) from pH 7 to pH 9 due to dissociation of tannic acid. In contrast to Aldrich HA, there was further significant decline in sorption of non-dissociating hormones from pH 10 to 12. This may be related to the presence of other functional groups, such as catechol which dissociates at pH 9.5.
Figure 5.12: Tannic acid-water sorption isotherms for a) estradiol, b) estrone, c) progesterone and d) testosterone (1mM NaHCO₃, 20 mM NaCl, 100-100000 ng/L hormone, 12.5 mgC/L tannic acid)

Similar to alginic acid, the partitioning of micropollutants to tannic acid has not been studied as a function of pH. Previously, the interaction of estradiol with tannic acid was studied at pH 7 using fluorescence quenching and solubility enhancement (Yamamoto et al., 2004; Yamamoto et al., 2003) and these results are shown in Figure 5.6.

5.5. Influence of Ionic Strength

The purpose of this section was to determine if ionic strength, represented by NaCl, influenced the interaction of estrone with Aldrich HA. Estrone was selected as a representative hormone due to its strong interaction with organic matter observed in Sections 5.3 and 5.4. Ionic strength can have implications for charge and conformation of organic matter, as well as charge and solubility of micropollutants (Ghosh and Schnitzer, 1980; Schwarzenbach et al., 2003). As wastewater effluent is
often discharged into marine environments an understanding of the influence of ionic strength on hormone partitioning is of importance. Organic matter-water sorption isotherms are shown Figure 5.13 with NaCl concentrations ranging from 0 mM to 100 mM.

![Graph showing organic matter-water sorption isotherms for estrone as a function of ionic strength.](image)

**Figure 5.13:** Organic matter-water sorption isotherms for estrone as a function of ionic strength (1 mM NaHCO₃, 0, 20, 50 and 100 mM NaCl, pH 8, 100-100,000 ng/L estrone, 12.5 mgC/L Aldrich HA)

Log $K_{OM}$ values in Figure 5.14 indicates a decrease in partitioning with increasing ionic strength from 0 mM (4.89 ± 0.02) to 100 mM (4.81 ± 0.04). Statistical tests indicated that the decrease was significant from 0 to 20 mM and from 50 to 100 mM. As ionic strength increases the structure of Aldrich HA changes from flexible and linear to coiled and rigid due to negative charge shielding (Ghosh and Schnitzer, 1980). As high ionic strength can shield the negative charge of Aldrich HA increased log $K_{OM}$ values at higher ionic strength were expected. However, a similar trend to Figure 5.14 was observed by Schlautman and Morgan (1993) for the interaction of PAHs with humic acid. It was suggested that the coiling of humic acid reduced the number of interaction sites leading to reduced partitioning. The presence of elevated ionic strength can also shield the negative charge of some micropollutants. However, as estrone was neutrally charged (pH 8) it is unlikely that the high NaCl concentrations will have any implications for estrone charge. Yamamoto and
Liljestrand (2003) studied partitioning of estradiol to colloidal organic matter (COM) at 20, 150 and 700 mM NaCl and found no difference in log $K_{OM}$ values. In contrast, Bowman et al. (2002) found increased steroidal hormone partitioning with increasing ionic strength, and attributed this to the ‘salting out’ phenomenon. However, as discussed in Section 4.2.2 in Chapter 4 salting out typically occurs at NaCl concentrations greater than 0.1 M, therefore as the highest NaCl concentration studied in this experiment was 100 mM (0.1 M) it is unlikely that salting out influenced the solubility of steroidal hormones significantly.

Figure 5.14: Log $K_{OM}$ values for estrone as a function of ionic strength at pH 8 (1mM NaHCO$_3$, 0, 20, 50 and 100 mM NaCl, pH 8, 100-100000 ng/L estrone, 12.5 mgC/L Aldrich HA)

5.6. Influence of Organic Matter Concentration

The concentration of organic matter can vary greatly in natural aquatic systems. Further, due to a reduction in atmospheric acidity the concentration of organic matter in surface waters has increased in a fourteen year period from 1990 (Monteith et al., 2007). Due to this variability, the purpose of this section was to determine the influence of organic matter concentration on organic matter-water partitioning.
The sorption isotherms in Figure 5.15 indicate a decrease in partitioning as Aldrich HA concentration increases. This decrease in partitioning in the presence of organic matter has been observed for a wide range of polar and non-polar micropollutants including steroidal hormones, PAHs and pesticides (Bowman et al., 2002; Carter and Suffet, 1982; Landrum et al., 1984) and for all phases of organic matter.

Figure 5.15: Organic matter-water sorption isotherms for estrone as a function of Aldrich HA concentration (1mM NaHCO₃, 20 mM NaCl, pH 8, 100-100000 ng/L estrone, 12.5, 25, 50 and 125 mgC/L Aldrich HA)

In the case of particulate organic matter (POM), decreased sorption as a function of organic matter concentration was observed frequently and has been termed ‘solids concentration effect’ (Bowman et al., 2002; Turner and Rawling, 2000; Voice et al., 1983). This was attributed to the transfer of the sorbent from the particulate phase to the dissolved phase during the course of the experiment (Voice et al., 1983). However, this explanation is not relevant for the dissolved organic matter (DOM) or COM. Early experiments using DOM attributed this phenomenon to likely experimental problems (Carter and Suffet, 1982). However, this decrease in partitioning has been observed using a number of different quantification techniques (e.g. equilibrium dialysis and reverse phase partitioning), and now in this experiment, therefore it is likely that this effect is indeed real.
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Figure 5.16 indicates that the log $K_{OM}$ values decreased by a factor of 5 from 12.5 mgC/L ($4.86 \pm 0.003$) to 125 mgC/L ($4.14 \pm 0.03$). For DOM, the decrease in partitioning has been attributed to conformational changes of organic matter at high concentrations (Akkanen and Kukkonen, 2003; Landrum et al., 1984). Humic acid can coil and form aggregates at high concentrations which may influence sorption. However, Ghosh and Schnitzer (1980) suggested that this change only occurs at concentrations greater than 3.5 g/L, and as all studied concentrations were considerably lower it is unlikely that the change in conformation is a factor. Therefore, the decrease in log $K_{OM}$ values may indicate that the sorption was limited by the micropollutant concentration.

![Graph of log $K_{OM}$ vs. Humic Acid Concentration](image)

Figure 5.16: Log $K_{OM}$ values for estrone as a function of Aldrich HA concentration at pH 8 (1mM NaHCO$_3$, 20 mM NaCl, 100-100000 ng/L estrone, 12.5, 25, 50 and 125 mgC/L Aldrich HA)

5.7. Conclusions

In this chapter the SPME technique developed in Chapter 4 was applied to quantify solute-solute interactions. Firstly, this study has indicated that SPME is indeed a suitable technique to determine log $K_{OM}$ values for steroidal hormones. However, due to weak sorption to organic matter estradiol partitioning was not significantly influenced by different organic matter types, with the exception of tannic acid.
Secondly, organic matter-water partitioning was studied for a wide range of steroidal hormones to determine the influence of organic matter type and solution chemistry. Within the literature most studies have focused on determining log $K_{OM}$ values for estradiol (e.g. Bowman et al., 2002; Holbrook et al., 2004; Liu et al., 2005; Yamamoto and Liljestrand, 2003; Yamamoto et al., 2003). There are limited studies on estrone (Bowman et al., 2002; Liu et al., 2005) and progesterone (López de Alda et al., 2002), but to the author’s knowledge, none for testosterone.

The strength of partitioning was influenced by hormone type. Similar to estradiol, no significant difference in partitioning with organic matter type or pH was observed for weakly sorbing testosterone. However, for the strongly sorbing hormones such as estrone and progesterone organic matter type and concentration as well as solution chemistry influenced log $K_{OM}$ values significantly. The different partitioning behaviour of the steroidal hormones appears to be related to their functional group content and position.

For most organic matter types, log $K_{OM}$ values were generally strongest in acidic solutions. Using the example of estrone and tannic acid (Figure 5.17) the fraction of hormone sorbed to the organic matter decreases with increasing pH due to dissociation of organic matter leading to a higher percentage of hormones freely dissolved in solution. Further, partitioning also decreased with increasing ionic strength and organic matter concentration as shown in Sections 5.5 and 5.6.
5.8. Recommendations for Future Studies

In this study log $K_{OM}$ values for steroidal hormones were quantified and the affect of organic matter and solution chemistry on partition were considered. However, this study has raised many questions which require further research. Firstly, a decrease in estrone partitioning with increasing organic matter was observed in this study, and has been in other previous studies (e.g. Carter and Suffet, 1982; Landrum et al., 1984). In this study it appears that the micropollutant concentration was the limiting factor. However, this phenomenon is not well understood in the literature and requires further study in the future.

Secondly, in Section 5.5 the influence of ionic strength on partitioning of neutrally charged estrone was studied. When estrone becomes negatively charged it cannot be extracted using SPME fibre, and consequently log $K_{OM}$ values cannot be quantified. However, high ionic strength can shielding the negative charge, consequently it may be possible to determine partitioning. Therefore, further study on partitioning of negatively charged micropollutants at high ionic strength is required.
Thirdly, this study predominantly used commercial organic matter surrogates, with few environmental organic matter types considered. Therefore, for an improved understanding of steroidal hormone fate in the aquatic environment partitioning to environmental organic matter types is required. Further, measuring sorption to extracellular polymeric substances (EPS) or secondary effluent may improve the understanding of steroidal hormones during wastewater treatment.
6 Theoretical Consideration of Solute-Solute Interactions

In this chapter the mechanisms of solute-solute interactions will be discussed and the concept of linear free energy relationships (LFER) will be introduced as they can be applied to model organic matter-water partition coefficients (log K<sub>OM</sub>).

Gibbs free energy and different molecular interaction mechanisms including non-specific, specific and solvophobic interactions will be discussed as these can all contribute to solute-solute interactions.

Using one-sample t-tests, experimental log K<sub>OM</sub> values from Chapter 5 will be compared to predicted log K<sub>OM</sub> values modelled using one-parameter and polyparameter LFERs. The results indicated that the majority of experimental log K<sub>OM</sub> values were significantly different than modelled log K<sub>OM</sub> values. The one-parameter LFER is unsuitable as it predicts partitioning based on a single parameter which is unable to consider the specific and non-specific interaction mechanisms associated with organic matter-water partitioning. The polyparameter LFER can consider both interaction mechanisms, but at present lacks organic matter specific system constants which limit its applicability.

From this study, it can be concluded that without specific parameters available for different types of organic matter LFERs are not suitable to predict log K<sub>OM</sub> values. Therefore, further studies are required to determine this data for a wide range of organic matter types commonly found in water and wastewater.
6.1. Introduction

An understanding of the fate and behaviour of micropollutants within aquatic systems can be improved through organic matter-water partition coefficients (log \( K_{OM} \)). However, due to the ever increasing number of micropollutants in the environment it is time consuming and expensive to measure all log \( K_{OM} \) values experimentally. Therefore, several studies have applied linear free energy relationships (LFER) to estimate log \( K_{OM} \) values (e.g. Poole and Poole, 1999; Żukowska et al., 2006). In this chapter two different LFER types will be introduced; one-parameter and polyparameter LFERs. Based on the literature, one-parameter and polyparameter LFER equations specific to polar micropollutants and organic matter will be applied to model log \( K_{OM} \) values. The purpose of this study is to determine if LFERs are suitable to quantify the interaction of steroidal hormones with organic matter. Using one-sample t-tests, experimental log \( K_{OM} \) values from Chapter 5 will be compared to log \( K_{OM} \) values modelled using both one-parameter and polyparameter LFERs to determine if the values are significantly different. Finally, the limitations of one-parameter and polyparameter LFERs will be assessed to better understand any problems associated with the application of LFERs to estimate log \( K_{OM} \) values.

6.2. Mechanisms of Interaction

6.2.1. Gibbs Free Energy

Gibbs free energy (\( G \)) is defined as the maximum amount of energy which may be obtained from a system (Smith, 2004). In the context of organic matter-water partitioning, Gibbs free energy (\( \Delta G_{OM} \)) represents the change in free energy when a micropollutant transfers from one phase to another such as from water to organic matter and is proportional to log \( K_{OM} \) (Equation 6.1) (Goss and Schwarzenbach, 2003). \( \Delta G_{OM} \) must be negative for partitioning to occur, and greater partitioning of a micropollutant from water to organic matter at equilibrium corresponds to a greater release of free energy (Ben-Naim, 1978).
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\[
\log K_{OM} \propto -\Delta G_{OM} \tag{6.1}
\]

In general, \(\Delta G_{OM}\) includes both entropy (\(\Delta S\)) and enthalpy (\(\Delta H\)) influences involved in organic matter-water partitioning as well as temperature (\(T\)) (Equation 6.2) (Schwarzenbach et al., 2003). Entropy is related to energy distribution within and between molecules and can be altered by the configuration and orientation of the molecules, as well as the mixing of phases (Carson and Watson, 2002). It is also related to cavity formation which involves breaking of intermolecular bonds (Vitha and Carr, 2006). The energy required to form a cavity in a solute is related to the cohesiveness of the solute and the required cavity size which is associated with the micropollutant size (Goss and Schwarzenbach, 2003) (Equation 6.3). Enthalpy represents the attraction of a molecule to its surroundings, be that other molecules, surfaces or cavities (Schwarzenbach et al., 2003).

\[
\Delta G_{OM} = \Delta H - T \Delta S \tag{6.2}
\]

\[
G_{cavity} = -\text{Size}G_{cohesion} \tag{6.3}
\]

When considering organic matter-water partitioning the change in \(\Delta G_{OM}\) is related to cavity formation and interaction of the micropollutant with both the water and organic matter phases (Nguyen et al., 2005). This is shown in Equation 6.4 where \(G_{\text{cavity-water}}\) and \(G_{\text{cavity-OM}}\) are the free energy involved in opening and closing cavities in both water and organic matter, while \(G_{\text{interaction-water}}\) and \(G_{\text{interaction-OM}}\) reflects the free energy required for micropollutant interaction with water and organic matter respectively (Nguyen et al., 2005).

\[
-\Delta G_{OM} = G_{\text{cavity-water}} + G_{\text{interaction-water}} - G_{\text{cavity-OM}} - G_{\text{interaction-OM}} \tag{6.4}
\]
Typically the $G_{\text{cavity}}$ terms are positive as energy is required to disrupt the surrounding molecules, while $G_{\text{interaction}}$ terms are generally negative as they represent attractive interactions meaning they release energy (Goss and Schwarzenbach, 2001). A schematic representation of organic matter-water partitioning is shown in Figure 6.1 where the organic matter phase is releasing energy as the cavity is closing ($- G_{\text{cavity-OM}}$) while energy is required to form a cavity in the water phase ($G_{\text{cavity-water}}$).

6.2.2. Non-Specific Interactions

As discussed above, organic matter-water partitioning is related to both cavity formation and micropollutant interaction with organic matter and water phases. The mechanism of interaction includes specific and non-specific interactions. Van der Waals interactions are a collective term used to represent several different types of non-specific interaction mechanisms. These include induced dipole-induced dipole, dipole-induced dipole and dipole-dipole interactions. Van der Waals interactions are the dominant form of interaction for apolar micropollutants and the strength of such
interactions are typically weaker than specific interactions and range from 0 to 10 kJ/mol (Vitha and Carr, 2006). Due to the presence of bipolar functional groups in steroidal hormones and organic matter it is unlikely non-specific interactions play an important role in partitioning, however, they will still be discussed briefly below.

Induced dipole-induced dipole interactions, also known as London dispersive energies, occur due to an uneven electron distribution in molecules which leads to the formation of a temporary dipole. This can then induce dipole formation in a second molecule (Figure 6.2). The uneven distribution is time reliant, and the intensity of the unevenness of the electron cloud is related to the polarizability of the molecule (Schwarzenbach et al., 2003). Therefore, the strength of interaction is related to the polarizabilities of the interacting molecules.

$\pi$ bonds, such as those contained in phenolic compounds, are typically more polarizable, therefore induced dipole-induced dipole interactions may be an important mechanism of interaction for phenolic rich solutes (Huang et al., 2005). Consequently, such interactions may contribute to the strong interaction observed between tannic acid and steroidal hormones in Chapter 5.

![Figure 6.2](image-url)  

**Figure 6.2:** Induced dipole-induced dipole interactions between temporary dipoles with the dotted circle representing continuously moving electron clouds (Adapted from Vitha and Carr, 2006)

Dipole-induced dipole interactions (Debye energies) represent the energy of the interaction between the permanent dipole of one molecule with an induced dipole of a neighbouring molecule (Vitha and Carr, 2006). A schematic diagram of this process is shown below in Figure 6.3. Permanent dipoles are caused by a difference in electronegativity between atoms within the molecule and can induce an uneven electron distribution in the second molecule creating a temporary dipole. The
strength of dipole-induced dipole interactions is related to both the dipole moment of the first molecule and the polarizability of the second compound (Schwarzenbach et al., 2003). The dipole moment (debye units) represents the magnitude of electronegativity, multiplied by the distance between the atoms.

Figure 6.3: Dipole-induced dipole interactions where the permanent dipole on the left induces a temporary dipole in the neighbouring molecule (Adapted from Vitha and Carr, 2006)

Dipole-dipole interactions (Keesom energies) can form between molecules with permanent dipoles as shown in Figure 6.4. The strength of the interaction depends on the dipole moment of both molecules and their orientation (Schwarzenbach et al., 2003).

Figure 6.4: Dipole-dipole interactions occurring between two permanent dipoles

6.2.3. Specific Interactions

Hydrogen bonding is a specific interaction, and is related to functional groups content as well as molecular orientation (Vitha and Carr, 2006). Hydrogen bonding was introduced previously in Chapters 2. The schematic diagram in Figure 6.5 indicates that specific interactions can only occur with complementary polar molecules, either hydrogen donors or acceptors. Therefore, polar molecules may not be able to interact via hydrogen bonding if they are both only hydrogen acceptors for example. The strength of hydrogen bonding can vary from 1 to 40 kJ/mol (Vitha and Carr, 2006).
In the case of hormone-organic matter interactions, hydrogen bonding will influence both cavity formation and the interaction between solutes (Goss and Schwarzenbach, 2003). This is because both hormones and organic matter are bipolar with the exception of progesterone and SDS which are both monopolar. As a result, predictions regarding the strength of interaction can be difficult to determine, as hydrogen bonding can have opposing effects for partitioning. For example, increased cavity energy will lead to a decrease in partitioning as the micropollutant is more likely to remain in solution, while increased interaction between the solutes will increase partitioning (Goss and Schwarzenbach, 2003).

6.2.4. Solvophobic Interactions

Solvophobic interactions are an entropy driven process and involve the aggregation of apolar molecules (Rodnikova, 2007). Solvophobic interactions may have implications for cavity formation, as Poole and Poole (1999) indicated water has a solvophobic tendency to expel micropollutants from solution to organic matter. This is due to water wanting to retain its initial structure, and therefore it pushes out any micropollutants which may disturb the structure forcing them to aggregate (Rodnikova, 2007). However, as the studied micropollutants are polar it is unlikely
that solvophobic interactions will play a significant role in organic matter-water partitioning.

6.3. Theoretical Concepts of LFERs

As discussed in Chapter 2 there are several methods available to experimentally quantify solute-solute interactions, and in Chapter 5 solid-phase microextraction (SPME) was applied to quantify log $K_{OM}$ values for four steroidal hormones. Considering that there are countless different micropollutants present in the environment it difficult to determine log $K_{OM}$ values for a large number of micropollutants by experimental methods. Therefore, several models have been developed to estimate the interaction, such as LFERs. Some models such as one-parameter LFERs only considers a single property of the micropollutants such as solubility, while polyparameter LFERs take into account both specific and non-specific interactions as well as cavity formation. In this section both one-parameter and polyparameter LFERs will be described in order to better understand the theoretical concepts of both models.

6.3.1. One-Parameter LFERs

The simplest form of LFER is the one-parameter LFER. This can predict organic matter-water partitioning through double logarithmic correlations using a single physio-chemical property of a micropollutant such as water solubility or hydrophobicity (Gawlik et al., 1997). This implies that the transfer energies ($\Delta G_{OM}$) of organic matter-water partitioning and the comparison (e.g. octanol-water partitioning) are linearly related (Goss and Schwarzenbach, 2001). However, most LFER models do not consider this before the relationship is applied.

The most common one-parameter LFER correlation is octanol-water partition coefficients (log $K_{OW}$), which represents hydrophobicity. Within the literature the majority of one-parameter LFERs have been developed for hydrophobic, apolar compounds (e.g. Chiou et al., 1983; Kopinke et al., 1995). In theory, one-parameter
LFERs are only applicable when developed for a specific compound class and phase (Goss and Schwarzenbach, 2001). Consequently, this section will focus on one-parameter LFERs developed specifically for polar micropollutants. These include partitioning of phenols to dissolved organic matter (DOM) (Ohlenbusch and Frimmel, 2001) and partitioning of pharmaceuticals to sewage sludge (Carballa et al., 2008). Further, a general model for sorption of polar micropollutants to a wide range of soils was developed (Nguyen et al., 2005). These three one-parameter LFER models from the literature will be applied to determine if they are suitable to predict sorption of steroidal hormones to DOM. The equations used include Equation 6.5 (Carballa et al., 2008), Equation 6.6 (Nguyen et al., 2005) and Equation 6.7 (Ohlenbusch and Frimmel, 2001), and the modelled log \( K_{OM} \) values will be discussed further in Section 6.4.1. The units of the log \( K_{OM} \) values are in L/kg. However, as the log \( K_{OW} \) values are dimensionless the constants in Equations 6.5, 6.6 and 6.7 are therefore assumed to have units of L/kg due to the linear relationship between log \( K_{OM} \) and log \( K_{OW} \) values (MacKay, 2001).

\[
\log K_{OM} = 0.74 \log K_{OW} + 0.15 \tag{6.5}
\]

\[
\log K_{OM} = 0.73 \log K_{OW} + 0.52 \tag{6.6}
\]

\[
\log K_{OM} = 0.314 \log K_{OW} + 1.595 \tag{6.7}
\]

Another one-parameter LFER is molecular connectivity indices which are a type of quantitative structure-activity relationship (QSAR). Molecular connectivity indices are based on the topology of the molecular structure of a micropollutant and are calculated by considering the non-hydrogen part of the structure (Sabljic, 1984). To be applied to polar micropollutants Meylan et al. (1992) developed statistically derived fragment correction values for different polar functional groups. The correction values were then subtracted from the calculated log \( K_{OM} \) value depending on the polar functional group content of the micropollutant. Molecular connectivity
indices with fragment correction can be calculated using PeKocWIN in EPI Suite version 3.20 (US EPA, 2007) and only require the micropollutant molecular structure. The predicted log \( K_{OM} \) values for the studied hormones will be discussed in Section 6.4.1.

Water solubility has also been used to predict log \( K_{OM} \) values previously, as solubility can influence micropollutant movement through soil as well as sorption (Gawlik et al., 1997). However, log \( K_{OM} \) values based on water solubility will not considered in this study as there are no equations available in the literature which consider polar micropollutants.

6.3.2. Polyparameter LFERs

As one-parameter LFERs cannot consider molecular interactions, polyparameter LFERs were developed to take into account specific and non-specific interactions associated with organic matter-water partitioning. The polyparameter LFER is shown in Equation 6.8 where:

\[
\log K_{OM} = eE + sS + aA + bB + vV + c
\]  

\( e \) was the difference in capacity for organic matter and water to interact through polarization

\( E \) was excess molar refraction (cm\(^3\)/10)

\( s \) was the difference in capacity for organic matter and water to interact through dipole-dipole interactions

\( S \) was dipolarity/polarizability

\( a \) was difference in hydrogen bond acidity of organic matter and water

\( A \) was overall hydrogen bond acidity (hydrogen donor)

\( b \) was difference in hydrogen bond basicity of organic matter and water

\( B \) was overall hydrogen bond basicity (hydrogen acceptor)

\( V \) was McGowan’s characteristic volume (cm\(^3\) mol/100)
was ease of cavity formation  
\(c\) was solute specific free energy constant

The capital letters (e.g. \(E\)) are solute descriptors and are specific to the micropollutants. The solute descriptors for the studied hormones are shown in Table 6.1. \(E\) represents polarizability and accounts for interactions involving induced dipoles, specifically dipole-induced dipole and induced dipole-induced dipole interactions (Goss, 2006). \(S\) represents dipolarity and polarizability and contains information regarding permanent dipole interactions, namely dipole-dipole interactions (Nguyen et al., 2005). \(A\) and \(B\) represent the contribution of hydrogen bonding, while \(V\) indicates cavity formation. \(E\) and \(V\) were calculated from molecular structure, and consequently did not vary within the literature. However, \(S\), \(A\) and \(B\) can be either calculated experimentally, using techniques such as high performance liquid chromatography retention (Zissimos et al., 2002), or theoretically through a group contribution method (Platts et al., 2000; Zhao et al., 2002). Consequently \(S\), \(A\), and \(B\) can vary significantly. Until recently, different notation was used for solute descriptors in Equation 6.8, with \(R_2\) representing \(E\), \(\pi^H_2\) representing \(S\), \(\sum\alpha H_2\) representing \(A\), \(\sum\beta H_2\) or \(\sum\beta_0^2\) representing \(B\) and \(\nu^X\) representing \(V\). Within the literature, the term linear solvation energy relationship (LSER) is also frequently used, but these differs from polyparameter LFERs as they typically lack the \(S\) parameter which indicates dipolarity and polarizability (Goss and Schwarzenbach, 2001).

Table 6.1: Solute descriptors from the literature for estradiol, estrone, progesterone and testosterone

<table>
<thead>
<tr>
<th></th>
<th>(E)</th>
<th>(S)</th>
<th>(A)</th>
<th>(B)</th>
<th>(V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol</td>
<td>1.80(^a)(^b)</td>
<td>3.30(^c), 1.77(^b)</td>
<td>0.88(^a), 0.86(^b)</td>
<td>0.95(^c), 1.10(^b)</td>
<td>2.20(^a)(^b)</td>
</tr>
<tr>
<td>Estrone</td>
<td>1.73(^a)</td>
<td>3.10(^d)</td>
<td>0.56(^c)</td>
<td>0.91(^b)</td>
<td>2.16(^a)</td>
</tr>
<tr>
<td>Progesterone</td>
<td>1.45(^a)(^b), 1.58(^c)</td>
<td>2.49(^a)(^b), 3.29(^c)</td>
<td>0(^a)(^b)(^c)</td>
<td>1.14(^a)(^b), 1.16(^c)</td>
<td>2.62(^a)(^b)(^c)</td>
</tr>
<tr>
<td>Testosterone</td>
<td>1.54(^a)(^b), 1.61(^c)</td>
<td>2.59(^a)(^b), 2.32(^c)</td>
<td>0.32(^a)(^b), 0.35(^c)</td>
<td>1.19(^a)(^b), 1.13(^c)</td>
<td>2.38(^a)(^b)(^c)</td>
</tr>
</tbody>
</table>

\(^{a}\)Kiriden and Poole, 1998; \(^{b}\)Zissimos et al. 2002; \(^{c}\)Zhao et al. 2002
The system constants, represented by lower case symbols (e.g. $e$), are related to properties of both the aqueous and organic matter phases (Goss, 2005). Using multiple linear regressions the system constants can be determined by applying Equation 6.8 to experimental log $K_{OM}$ values (Niederer et al., 2006). It is suggested that as few as 20 to 30 experimental log $K_{OM}$ values are required to calculate system constants, provided a wide range of micropollutants are studied (Goss and Schwarzenbach, 2001). Consequently, the dimensions of the system constants are in L/kg as they are determined from experimental log $K_{OM}$ values. It is better to use experimental log $K_{OM}$ values based on a single organic matter type. However, most studies use a range of log $K_{OM}$ values from the literature and consequently many different types of organic matter with different carbon contents are used.

Polyparameter LFER equations have been developed to estimate partitioning of nonionic micropollutants between water, air and solid phases. However, very few polyparameter LFER have been developed to estimate partitioning of micropollutants to organic matter. Two studies that have developed polyparameter LFER equations for soil organic matter are Nguyen et al. (2005) (Equation 6.9) and Poole and Poole (1999) (Equation 6.10). Despite being developed for soil organic matter the models consider partitioning as the dominant mechanism of interaction. Equation 6.10 originally used the older notation for Equation 6.8 as described above, however, it was adapted to the currently used solute parameters. There is no $s$ parameter in Equation 6.10. This is because the model was designed for wet soil, and water and wet soil have similar dipolar and polarizable properties. Therefore, no difference in capacity to interact through dipole-dipole interactions was expected (Poole and Poole, 1999). Both equations are based on experimental log $K_{OM}$ values for a range of literature sources, and consequently Poole and Poole (1999) have been criticised for using a data set that was not properly reviewed (Nguyen et al., 2005).

$$
\begin{align*}
\log K_{OM} &= 1.10(\pm 0.10)E - 0.72(\pm 0.14)S + 0.15(\pm 0.15)A \\
&\quad - 1.98(\pm 0.14)B + 2.28(\pm 0.14)V + 0.14(\pm 0.10) \\
\end{align*}
$$

(6.9)
The sign of the solute parameter in polyparameter LFER equations indicates the likelihood of micropollutant interactions occurring with water or organic matter. Negative parameters imply a preference for the micropollutant to remain in the water phase, while positive parameters indicate that the micropollutant favours organic matter (Żukowska et al., 2006). Based on Equations 6.9 and 6.10, cavity formation ($V$) and excess molar refractivity ($E$) increases micropollutant sorption to organic matter. In contrast, hydrogen bond basicity (hydrogen acceptor) ($B$) and dipolarity and polarizability ($S$), in the case of Equation 6.9, promote micropollutant interaction with the aqueous phase. Equations 6.9 and 6.10 differ in relation to the influence of hydrogen bond acidity (hydrogen donor) ($A$) on organic matter-water partitioning, however, the $a$ system constant in Equations 6.9 and 6.10 is lower than most other constants. The influence of solute parameters on sorption is outlined in Figure 6.6. The double arrow for $S$ and $A$ suggests that the micropollutant can either go to the aqueous phase or to the organic matter. This is due to the aqueous and solid phases having similar dipolarity and polarizability ($S$) and hydrogen bond acidity ($A$) properties (Breivik and Wania, 2003).

![Image](image_url)

Figure 6.6: The influence of polyparameter LFER solute parameters on organic matter-water partitioning (Adapted from Breivik and Wania, 2003)
6.4. LFER Predicted log $K_{OM}$ Values for Steroidal Hormones

Using one-parameter and polyparameter LFER equations, log $K_{OM}$ values were calculated for estradiol, estrone, progesterone and testosterone. These were compared to experimental log $K_{OM}$ values at pH 7 based on Table 5.2 in Chapter 5 and includes log $K_{OM}$ values for three different types of organic matter, Aldrich humic acid (HA), alginic acid and tannic acid. In order to assess if experimental log $K_{OM}$ values ($\chi$) was significantly different to LFER modelled log $K_{OM}$ values ($\mu$) a one-sample t-test was applied (Equation 6.11) where $n$ was the sample size and $s^2$ was standard deviation of $\chi$. If the one-sample t-test statistic $|t|$ exceeded the t critical value (determine from t-distribution table) the experimental log $K_{OM}$ value and the modelled log $K_{OM}$ value was significantly different. However, if $|t|$ was less than or equal to the critical value the difference between the two values was not considered significant. This was used to assess both one-parameter and polyparameter LFERs.

$$|t| = \left( \frac{\chi - \mu}{s^2} \right) \sqrt{n}$$

(6.11)

6.4.1. One-Parameter LFERs

Using the equations determined by Carballa et al. (2008), Nguyen et al. (2005) and Ohlenbusch and Frimmel (2001) log $K_{OM}$ values based on log $K_{OW}$ one-parameter LFERs were calculated for steroidal hormones. Within the literature there are a number of different log $K_{OW}$ values calculated from both experimental and modelling techniques, and some of the more common values are listed in Table 6.2. As only Hansch et al. (1995) determined log $K_{OW}$ values experimentally these will be used to model log $K_{OM}$ values using Equations 6.5, 6.6 and 6.7.
Table 6.2: Literature and modelled log K_{OW} values for estradiol, estrone, progesterone and testosterone

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol</td>
<td>4.01</td>
<td>4.01</td>
<td>3.94</td>
<td>4.13</td>
</tr>
<tr>
<td>Estrone</td>
<td>3.13</td>
<td>4.54</td>
<td>3.43</td>
<td>3.69</td>
</tr>
<tr>
<td>Progesterone</td>
<td>3.87</td>
<td>4.63</td>
<td>3.67</td>
<td>4.04</td>
</tr>
<tr>
<td>Testosterone</td>
<td>3.32</td>
<td>3.84</td>
<td>3.27</td>
<td>3.48</td>
</tr>
</tbody>
</table>

*US EPA, †Advanced Chemistry Development Inc.

The modelled log K_{OM} values for all studied hormones are shown in Table 6.3 as well as experimental log K_{OM} values from Chapter 5. Using Equation 6.6 (Nguyen et al., 2005), which was based on soil organic matter, the predicted log K_{OM} values were closest to the experimental log K_{OM} values from Chapter 5. This may be because it was calculated using a wide range of polar micropolllutants with different sorption behaviour. However, the one-sample t-tests indicated that similar log K_{OM} values were only observed for alginic acid and only for weaker sorbing hormones such as testosterone and estradiol. For Aldrich HA and tannic acid one-parameter LFER log K_{OM} values were up to 3 orders of magnitude less than the experimental log K_{OM} values.

Table 6.3: One-parameter LFER predicted log K_{OM} values for estradiol, estrone, progesterone and testosterone based on log K_{OW} values

<table>
<thead>
<tr>
<th></th>
<th>Estradiol</th>
<th>Estrone</th>
<th>Progesterone</th>
<th>Testosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp log K_{OM}*</td>
<td>HA</td>
<td>AA</td>
<td>TA</td>
<td>HA</td>
</tr>
<tr>
<td>log K_{OM}^{a} (Eq 6.5)</td>
<td>3.12</td>
<td>2.47</td>
<td>3.01</td>
<td>2.61</td>
</tr>
<tr>
<td>log K_{OM}^{b} (Eq. 6.6)</td>
<td>3.45</td>
<td>2.80</td>
<td>3.35</td>
<td>2.94</td>
</tr>
<tr>
<td>log K_{OM}^{c} (Eq. 6.7)</td>
<td>2.60</td>
<td>2.32</td>
<td>2.56</td>
<td>2.38</td>
</tr>
</tbody>
</table>

*Calculated in Chapter 5, HA is Aldrich humic acid, AA is alginic acid, TA is tannic acid

^{a}Carballa et al. 2008; ^{b}Nguyen et al. 2005; ^{c}Ohlenbusch and Frimmel 2001

It was expected that Equation 6.7 (Ohlenbusch and Frimmel, 2001) would be the most applicable as it was determined for DOM therefore similar sorption
mechanisms were expected. Equations 6.5 and 6.6 were developed for sludge and soil respectively therefore the adsorption may contribute to interaction. However, Equation 6.7 differed the most from experimental log $K_{OM}$ values, with most estimated values at least one order of magnitude less. Ohlenbusch and Frimmel (2001) only used phenolic micropollutants such as pentachlorophenol to determine Equation 6.7 which may limit its applicability to steroidal hormones.

This study demonstrated one-parameter LFERs based on log $K_{OW}$ values are not suitable to predict log $K_{OM}$ values even when developed for polar micropollutants. As one-parameter LFER only consider a single parameter they cannot represent the different molecular interactions which contribute to sorption (Goss and Schwarzenbach, 2001). This is particularly important for polar compounds, as they can interact with organic matter through both specific and non-specific interactions.

As one-parameter LFERs based a single physico-chemical parameter was not successful for steroidal hormones Log $K_{OM}$ value predictions based on molecular structure were considered. Log $K_{OM}$ values were calculated by molecular connectivity indices using PcKocWIN (US EPA, 2007) (Table 6.4). Based on one-sample t-tests, modelled log $K_{OM}$ values were similar to experimental log $K_{OM}$ values for estradiol with Aldrich HA and alginic acid, and for testosterone with alginic acid. Further, the order of partitioning observed in Chapter 5 was not indicated in Table 6.4 with a low log $K_{OM}$ value for progesterone indicating weaker sorption compared to estradiol.

While molecular connectivity indices can considered the molecular structure, predicted log $K_{OM}$ values for steroidal hormones is limited due to unequal fragment correction of polar groups. PcKocWIN applies fragment correction to hydroxyl and ketone functional groups, but not to phenolic hydroxyl groups as it does not consider them to be polar functional groups despite being strong hydrogen donors and acceptors (Niederer et al., 2006). Consequently, the phenolic hydroxyl group in the C-3 position of estradiol and estrone were not corrected for leading to higher predicted log $K_{OM}$ values compared to progesterone and testosterone. Further,
molecular connectivity indices do not consider organic matter properties, with experimental log \( K_{OM} \) values for tannic acid being over an order of magnitude greater than modelled log \( K_{OM} \) values for many hormones (Table 6.4).

**Table 6.4: One-parameter LFER predicted log \( K_{OM} \) values for estradiol, estrone, progesterone and testosterone based on hormone molecular structure using molecular connectivity indices (PcKocWIN (US EPA, 2007))**

<table>
<thead>
<tr>
<th></th>
<th>Estradiol</th>
<th>Estrone</th>
<th>Progesterone</th>
<th>Testosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA</td>
<td>4.21</td>
<td>4.86</td>
<td>5.47</td>
<td>4.59</td>
</tr>
<tr>
<td>AA</td>
<td>3.96</td>
<td>4.82</td>
<td>5.51</td>
<td>4.59</td>
</tr>
<tr>
<td>TA</td>
<td>4.82</td>
<td>5.51</td>
<td>5.53</td>
<td>4.74</td>
</tr>
</tbody>
</table>

*Calculated in Chapter 5, HA is Aldrich humic acid, AA is alginic acid, TA is tannic acid

This indicates that at present neither studied one-parameter LFER was suitable to predict log \( K_{OM} \) values for steroidal hormones to a range of different organic matters. This was because such models cannot consider the specific and non-specific interactions which lead to partitioning. Due to inherent limitations such as the use of a single parameter and the lack of organic matter properties in the model one-parameter LFER should not be applied to predict partitioning of steroidal hormones.

### 6.4.2. Polyparameter LFERs

Using Equations 6.9 and 6.10 taken from Nguyen *et al.* (2005) and Poole and Poole (1999) log \( K_{OM} \) values were modelled using soil organic matter polyparameter LFERs (Table 6.5). The hormone specific solute parameters were selected from previous studies (Kiridena and Poole, 1998; Zhao *et al.*, 2002; Zissimos *et al.*, 2002) and are shown in Table 6.1.
Theoretical Consideration of Solute-Solute Interactions

Table 6.5: Predicted log $K_{OM}$ values for estradiol, estrone, progesterone and testosterone using polyparameter LFERs developed by Nguyen et al. (2005) and Poole and Poole (1999)

<table>
<thead>
<tr>
<th></th>
<th>Estradiol</th>
<th>Estrone</th>
<th>Progesterone†</th>
<th>Testosterone†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp log $K_{OM}^{*}$</td>
<td>HA</td>
<td>AA</td>
<td>TA</td>
<td>HA</td>
</tr>
<tr>
<td></td>
<td>4.21</td>
<td>3.96</td>
<td>4.86</td>
<td>4.82</td>
</tr>
<tr>
<td>log $K_{OM}^{a}$ (Eq 6.9)</td>
<td>3.01$^c$, 3.53$^d$</td>
<td>3.01$^c$</td>
<td>3.09$^d$, 3.78$^e$</td>
<td>3.09$^d$, 3.48$^e$</td>
</tr>
<tr>
<td>log $K_{OM}^{b}$ (Eq 6.10)</td>
<td>3.37$^c$, 3.71$^d$</td>
<td>3.76$^c$</td>
<td>4.17$^d$, 4.22$^e$</td>
<td>3.53$^d$, 3.70$^e$</td>
</tr>
</tbody>
</table>

*Calculated in Chapter 5, †For progesterone and testosterone solute parameters from Kiridena and Poole (1998) were omitted as they were the same as Zissimos et al. (2002)

HA is Aldrich humic acid, AA is alginic acid, TA is tannic acid

$^a$Nguyen et al. 2005; $^b$Poole and Poole 1999; $^c$Kiridena and Poole, 1998; $^d$Zissimos et al. 2002; $^e$Zhao et al. 2002

Using one-sample t-tests, experimental log $K_{OM}$ values for the interaction of estradiol and testosterone with alginic acid were found to be similar to polyparameter LFER modelled log $K_{OM}$ values. However, all predicted log $K_{OM}$ values for progesterone and estrone, and for estradiol and testosterone in the presence of Aldrich HA and tannic acid were significantly different to the experimental log $K_{OM}$ values. In some cases the experimental log $K_{OM}$ values were over two orders of magnitude greater.

With the exception of estrone, log $K_{OM}$ values modelled using Equation 6.10 followed the same order of partitioning as was observed in Chapter 5, with stronger sorption of progesterone to organic matter compared to testosterone and estradiol. In contrast, there was very little difference in partitioning for all hormones for Equation 6.9 particularly when using solute parameters calculated by Kiridena and Poole (1998) (which were the same as Zissimos et al. (2002) for progesterone and testosterone). These parameters were calculated experimentally using thin-layer chromatography. The difference in partitioning between the two equations may be related to the assumed carbon fraction in the organic matter used in the models. Equation 6.9 (Nguyen et al., 2005) was based on experimental log $K_{OM}$ values with 0.1 to 9.4% organic carbon, while Equation 6.10 (Poole and Poole, 1999) assumed
58% organic carbon. The organic matter used to determine experimental log $K_{OM}$ values in Chapter 5 contained between 36 and 56% organic carbon. This may explain why Equation 6.10 was a better fit than Equation 6.9.

This study indicated that polyparameter LFER modelled log $K_{OM}$ values could be used to a limited extent to predict the partitioning of weakly sorbing steroidal hormones such as testosterone to alginate acid. However, for strongly sorbing hormones such as progesterone and estrone neither Equations 6.9 nor 6.10 could predict log $K_{OM}$ values which were similar to the experimental log $K_{OM}$ values in Chapter 5. This suggests that the currently available polyparameter LFERs for soil organic matter are not suitable to predict the interaction of steroidal hormones with DOM. As the solute parameters are available for all hormones this suggests that system constants specific to the studied DOM are required. Without such information, polyparameter LFERs cannot be used to successfully predict partitioning.

### 6.5. Limitations of LFERs

#### 6.5.1. One-Parameter LFERs

As Section 6.4.1. indicated one-parameter LFERs are not suitable to predict log $K_{OM}$ values for steroid hormones. As well as only considering a single physico-chemical or structural parameter, the one-parameter LFER fails to consider the sorbent properties. According to Karickhoff (1984), sorption is relatively independent of the sorbent type when organic carbon content is indexed. However, experimental log $K_{OM}$ values in Chapter 5 for progesterone and estrone indicated that sorption varied significantly with different types of organic matter even when the organic carbon concentration was identical.

In addition to the general problems associated with one-parameter LFERs there are specific issues related to the log $K_{OW}$ values. Within the literature there is considerable variability in log $K_{OW}$ values for the same micropollutant, as can be
observed in Table 6.2. This may be related to experimental uncertainty, as well as differences in methods used by chemical modelling software, and can lead to significant differences in log $K_{OW}$ values. For example, published log $K_{OW}$ values for estrone range from 3.13 to 4.54 (Hansch et al., 1995; Nghiem et al., 2004), which is over one order of magnitude difference. Therefore, significantly different log $K_{OM}$ values can be predicted by using different log $K_{OW}$ values. The same problem exists for water solubility where values for the same micropollutant can vary significantly due to different techniques and experimental conditions such as temperature. For example, within the literature the solubility of estradiol can vary from 1.51 to 13 mg/L (Lai et al., 2000; Shareef et al., 2006).

6.5.2. Polyparameter LFERs

While polyparameter LFERs are able to identify different molecular interactions, there are still several limitations which prevent better prediction of log $K_{OM}$ values. Firstly, due to the system constants, polyparameter LFERs are specific to a particular organic matter type, with Niederer et al. (2007) demonstrating considerable variability in system constants for different types of natural organic matter (NOM) surrogates. While it may be possible to find solute parameters for commonly used organic matter types, such as Aldrich HA, there are only few studies which have considered the partitioning of micropollutants to tannic acid and alginic acid (e.g. Yamamoto and Liljestrand, 2003; Yamamoto et al., 2003) let alone specific humic or fulvic acids. Therefore, without additional data, or conducting experiments with a wider range of micropollutants, it is not possible to accurately estimate log $K_{OM}$ values for alginic acid or tannic acid using polyparameter LFERs. In the supporting information (SI-8) of Niederer et al. (2007) a polyparameter LFER for Aldrich HA-water partitioning, based on Aldrich HA-air partitioning models, was developed. However, this equation uses the $L$ solute parameter which is the hexadecane-air partition coefficient and is used to represent van der Waals interactions. This replaces the $E$ solute parameter in Equation 6.8. The $L$ parameter is not readily available for hormones as they are not volatile, therefore this equation cannot be used at present.
Secondly, at present polyparameter LFERs are only suitable to predict the partitioning of neutral micropollutants (Goss and Schwarzenbach, 2001). Some one-parameter LFER studies such as Carballa et al. (2008) have attempted to correct for dissociation of micropollutants by using pH dependent octanol-water partition coefficients ($D_{OW}$). $D_{OW}$ can be calculated using Equation 6.11 with fraction of neutral species ($f_{neutral}$) at the studied pH. Further, the influence of organic matter dissociation was not considered by either one-parameter or polyparameter LFER. Chapter 5 indicated that organic matter dissociation influences partitioning significantly despite micropollutants remaining neutrally charged, therefore the charge of both the micropollutant and the organic matter needs to be considered by LFERs in the future.

$$D_{OW} = K_{OW} f_{neutral} \quad (6.11)$$

6.6. Conclusions

One-parameter and polyparameter LFERs were evaluated and applied to estimate log $K_{OM}$ values for steroidal hormones. Based on a one-sample t-test, the majority of experimental log $K_{OM}$ values were significantly different to the predicted one-parameter and polyparameter LFER log $K_{OM}$ values. This was especially the case for strongly sorbing solutes such as progesterone and tannic acid. As a number of different molecular interactions are involved in solute-solute interactions, one-parameter LFERs cannot be applied to model log $K_{OM}$ values. However, as polyparameter LFERs includes parameters which represent hydrogen bonding, cavity formation and van der Waals forces they should be suitable to predict log $K_{OM}$ values. From observations in Chapter 5 and other studies it is clear that the type of organic matter can have a significant influence on partitioning. Therefore, before polyparameter LFERs can be applied successfully knowledge of system constants for a wider range of organic matter types is required. Niederer et al. (2007) has done this for a range of NOM surrogates for organic matter-air partitioning studies, however, this is required for other organics commonly found in water and wastewater,
including non-humic organic matter. Therefore, while there is great potential for polyparameter LFERs in the future, at present it is not suitable to model organic matter-water partitioning without organic matter specific system constants.

6.7. Recommendations for Future Studies

This study has indicated that at present it is not possible to apply polyparameter LFERs developed in the literature to predict the interaction between DOM and steroidal hormones. Therefore, polyparameter LFERs need to be developed for specific DOM types. Firstly, this requires experimental log $K_{OM}$ values to be calculated for a diverse range of micropollutants. This would need to be repeated for different organic matter types. Secondly, due to variations in quantification techniques as discussed in Chapter 2 it is important that the same technique is used. This one of the limitations of polyparameter LFERs calculated from the literature as a number of different techniques can be used. Using the experimental log $K_{OM}$ values it would be possible to determine system constant via multiple regressions which could then be used to predict log $K_{OM}$ values using polyparameter LFERs. Consequently, much work is required before polyparameter LFERs can be successfully applied.
7 Implications of Solute-Solute Interactions in Stirred Cell Ultrafiltration

In this chapter the removal of steroidal hormones in the presence of organic matter will be studied using stirred cell ultrafiltration. The purpose of this is to elucidate the mechanisms of hormone removal including membrane sorption, solute-foulant interactions and solute-solute interactions. Previous studies generally focus on membrane sorption and solute-foulant interactions, with solute-solute interactions often not considered as they are difficult to quantify. This study applies organic matter-water partition coefficients (\( \log K_{OM} \)) calculated in Chapter 5 to quantify solute-solute interactions in ultrafiltration (UF).

Estrone retention in the presence of Aldrich humic acid (HA), alginic acid and tannic acid was studied as a function of membrane molecular weight cut-off (MWCO). Increased estrone retention over the entire studied membrane MWCO range was observed only in the presence of Aldrich HA. Due to limited membrane sorption and solute-foulant interactions, the primary mechanism of interaction was solute-solute interactions. This was confirmed by \( \log K_{OM} \) values.

Estrone retention was found to increase with increasing organic matter concentrations, suggesting organic matter was the limiting solute in this interaction. The main mechanism of hormone retention was solute-solute interactions, as well as solute-foulant interactions at large MWCO membranes.

The influence of solution chemistry on the retention of estrone and progesterone was studied. Generally, solute-solute interactions were the dominant removal mechanism, however, sorption to the membrane was also important for the removal of progesterone. Solid-phase microextraction (SPME) calculated \( \log K_{OM} \) values were unsuitable to predict hormone retention based on solute-solute interactions above the acid dissociation constant (\( pK_a \)) of a micropollutant. This is because SPME cannot extract negatively charged species.
Further, increasing hormone concentrations had a negligible influence on retention, which confirms that steroidal hormones are not the limiting solute in this interaction. Consequently, this study indicated that solute-solute interactions in membrane filtration can be quantified using experimental log $K_{OM}$ values. However, this was more successful at high concentrations, suggesting that environmental organic matter concentrations (12.5 mgC/L) may be too low to see the effects accurately.
7.1. Introduction

Natural organic matter (NOM) can influence the retention of micropollutants in membrane filtration. The purpose of this study is to elucidate the mechanisms of hormone retention in the presence of organic matter using ultrafiltration (UF) by considering membrane sorption, solute-foulant interactions and solute-solute interactions. In Chapters 4 and 5 a solid-phase microextraction (SPME) technique was developed to quantify the interaction of steroidal hormones with organic matter and in this chapter this technique will be evaluated for its suitability to quantify the contribution of solute-solute interactions towards hormone retention.

Micropollutant retention as a function of organic matter type and concentration as well as solution chemistry will be studied to determine how these factors affect the mechanisms of micropollutant removal. The influence of hormone type will be considered by comparing the retention of estradiol, estrone, progesterone and testosterone in the presence of organic matter as Chapter 5 indicated different sorption behaviour for the studied steroidal hormones. Finally hormone concentrations ranging from 100 ng/L to 1 mg/L will be studied to better understand the limiting factors in solute-solute interactions.

As discussed in Chapter 2, the majority of hormone removal studies focus on nanofiltration (NF), however, UF was selected for this study. Due to the small molecular weight of micropollutants (e.g. 270 to 314 g/mol for studied hormones) UF is unable to retain hormones by size exclusion, therefore the presence of organic matter can play an important role for hormone removal by UF. Hydrophilic regenerated cellulose UF membranes were selected for this study to minimise micropollutant sorption and organic matter fouling, and therefore allow solute-solute interactions to be observed. Estrone was selected as a representative micropollutant as it is commonly found in surface water (Kolpin et al., 2002) and studies have demonstrated that estrone can induce behavioural changes in some fish at concentrations as low as 3 ng/L (Murphy et al., 2001). Further, as demonstrated in
Chapter 5, estrone interacts strongly with organic matter and this may improve the likelihood of quantifying solute-solute interactions for UF.

7.2 Organic Matter Type

7.2.1. Solute Retention

In Chapter 5 the studied organic matter properties including functional group content and charge influenced the strength of solute-solute interactions, and consequently this may have implications for estrone retention. The influence of organic matter type on estrone retention at pH 8 is shown in Figure 7.1. Over the studied membrane molecular weight cut-off (MWCO) range estrone retention without organic matter ranged from 10 to 28%. Estrone retention increased significantly in the presence of Aldrich humic acid (HA) with an 11-32% increase in retention over the MWCO range. Increased retention of micropollutants in the presence of Aldrich HA was observed previously for NF (Hu et al., 2007; Jin et al., 2007). Increased estrone retention for the 100 kDa membrane in the presence of alginic acid was observed, while increased estrone retention with tannic acid was seen only for the 1 kDa membrane. With the exception of the above examples, the presence of alginic acid and tannic acid did not have a significant influence on estrone retention. Further, for estrone alone and in the presence of organic matter there was greater estrone retention by smaller MWCO membranes compared to larger MWCO membranes.
The retention of organic matter as a function of membrane MWCO is shown in Figure 7.2a. For all organic matter types high retention was observed for 1 to 10 kDa membranes, but this decreased for tannic acid and Aldrich HA for larger MWCO membranes. Aldrich HA has a molecular weight ranging from 600-600000 g/mol (molecular diameter of 1.2-13.9 nm), and consequently high organic retention was observed for 1 to 10 kDa membranes (molecular diameter of 1.6 to 5.4 nm). Schäfer et al. (2002) studied NOM retention using a similar range of MWCO membranes and found reduced NOM retention for 10 kDa membranes. This was related to organic matter molecular weight as the studied NOM had a molecular weight of 1381 g/mol (molecular diameter of 1.9 nm) and therefore was not significantly retained by the 10 kDa membrane. The high retention of tannic acid above the 1 kDa membrane was unexpected as tannic acid has a small molecular weight of 1701 g/mol (molecular diameter of 2.1 nm). Consequently, it is possible aggregation of the small tannic acid molecules occurred leading to greater retention. The studied membrane range did not have a significant influence on alginic acid retention with around 90% retention observed for all membrane MWCOs. Alginic acid has a molecular weight of around 12-80 kg/mol (molecular diameter of 5.9-16.2 nm) which is similar the pore size of
the 100 kDa membrane (18.2 nm). The molecular diameters and membrane pore diameters were calculated using Equation 3.1 in Chapter 3. This equation does not consider the molecular shape of the organic molecules and therefore it can only be used as an estimate.

![Graph showing organic matter retention and SUVA in permeate as a function of MWCO](image)

Figure 7.2: a) Organic matter retention and b) SUVA in permeate as a function of MWCO (1 mM NaHCO₃, 20 mM NaCl, pH 8, 12.5 mgC/L Aldrich HA, alginic acid or tannic acid)

Specific UV absorbance (SUVA) in the permeate is shown in Figure 7.2b as this represents the aromaticity of organic matter (Weishaar et al., 2003). For Aldrich HA the aromaticity in the permeate increases with increasing membrane MWCO, suggesting that more aromatic fractions were retained by the 1 to 10 kDa membranes compared to larger MWCO membranes. For tannic acid SUVA in the permeate
increased for the 1 to 3 kDa membranes then remained constant for larger MWCO membranes. As tannic acid has a smaller molecular weight, higher MWCO membranes did not have a significant impact on the retention of aromatic fractions. Similar results to the Aldrich HA SUVA values have been observed by Schäfer et al. (2002) and it was suggested that larger molecules are typically more aromatic and therefore can be better retained by smaller MWCO membranes. Further, Chin et al. (1994) also demonstrated a correlation between aromaticity and increasing molecular weight for humic acid. Previous studies have indicated that steroidal hormones have a strong affinity for aromatic compounds (Jin et al., 2007; Yamamoto et al., 2003), therefore increased retention of aromatic compounds by small MWCO membranes may have implications for solute-solute interactions. Further, no SUVA values were calculated for alginic acid as it lacks aromatic functional groups and hence UV absorbance.

The error associated with organic matter retention and SUVA (Figure 7.2) was considerably larger than estrone retention (Figure 7.1). This was due to the different analysis techniques. The error associated with the TOC analyser was 14.4%, while liquid scintillation counting, which is a more reliable technique, had an average error of 2.0%. Error calculation will be discussed further in Appendix 3.

7.2.2. Membrane Sorption

The influence of membrane sorption was quantified using Equation 3.16 in Chapter 3 to determine if sorption significantly contributed to estrone retention. Figure 7.3 indicated that the sorption of estrone to the membrane did not vary significantly in the presence of organic matter. This differs from Hu et al. (2007) who found increased sorption of estrone with Aldrich HA using polyamide and cellulose acetate NF membranes. This may be related to the use of hydrophilic membranes in the present study, as well as the low hormone concentration. Jermann et al. (2009) compared estradiol sorption to hydrophobic and hydrophilic UF membranes in the presence of organic matter and found no difference in sorption with organic matter for hydrophilic membranes. For all organics, greater sorption to the membrane was
observed for 1 kDa membranes compared to 100 kDa membranes (Figure 7.3) suggesting that longer experimental times led to greater sorption. However, sorption of estrone to the membranes was still low (0.04-0.11ng/cm² or 5-10%). Further, sorption of estrone to the glassware during overnight stirring and to the stainless steel stirred cell was negligible.

Figure 7.3: Estrone sorption to the membrane (ng/cm²) as a function of MWCO (1 mM NaHCO₃, 20 mM NaCl, pH 8, 100 ng/L estrone, 12.5 mgC/L Aldrich HA, alginic acid or tannic acid)

The sorption of organic matter to the membrane is shown in Figure 7.4 as this can have implications for fouling and membrane modification. Generally, sorption of organic matter to the membrane was minimal, which was due to the use of the hydrophilic membrane. Greater sorption of tannic acid (0.01-0.02 mg/cm² or 9-17%) compared to the other studied organics was observed for most MWCO membranes, and this may account for the high tannic acid retention in Figure 7.2a. Further, 30% (0.04 mg/cm²) sorption of alginic acid by the 100 kDa membrane was observed which corresponds to greater retention of estrone in the presence of alginic acid (Figure 7.1). However, increased alginic acid sorption did not increase estrone sorption to the membrane (Figure 7.3), therefore the mechanism of estrone removal by UF was either solute-foulant or solute-solute interactions, and this will be discussed further below.
7.2.3. Solute-Foulant Interaction

Flux ratio, which is an indicator of membrane fouling, was shown in Figure 7.5. For all studied organic matter types some fouling was observed for 30 and 100 kDa membranes, with the most significant being for alginic acid. No fouling was observed for estrone in the absence of organic matter. Jermann et al. (2009) suggested organic matter fouling was the dominant cause of micropollutant retention in UF in the presence of Aldrich HA and alginic acid. While it is likely that solute-foulant interactions contribute significantly to the retention of estrone with alginic acid for the 100 kDa membrane, no flux decline was observed for 1 to 10 kDa membranes. The pure water flux of the 30 and 100 kDa membranes was significantly larger than the 1 to 10 kDa membranes (281-348 vs 21-80 L/m².h). High flux can increase concentration polarisation, which is the concentration of solutes at the membrane surface, and lead to fouling for the larger MWCO membranes (Aoustin et al., 2001). However, based on minimal membrane sorption and solute-foulant interactions increased estrone retention in presence of Aldrich HA by the 1 to 10 kDa membranes and tannic acid by the 1 kDa membrane was predominantly due to
solute-solute interactions. Further, for Figure 7.5 the flux ratio was lower at the beginning of each experiment. This was caused by pressurising the cell. This is an experimental artefact of the stirred cells and was observed for all flux ratio graphs. In Figure 7.5a flux ratio varied slightly for some membranes. This was not due to membrane fouling by estrone or increased membrane hydrophilicity, but instead was caused by slight variations in pressure due to the use of an analogue pressure gauge.

![Graph showing flux ratio as a function of MWCO for different solutes](image)

Figure 7.5: Flux ratio as a function of MWCO for a) estrone in background electrolyte b) estrone with Aldrich HA, c) estrone with alginic acid and d) estrone with tannic acid (1 mM NaHCO₃ 20 mM NaCl, pH 8, 100 ng/L estrone, 12.5 mgC/L Aldrich HA, alginic acid or tannic acid, pressure from 0.5 to 5 bar)

7.2.4. Solute-Solute Interactions

Given the negligible membrane sorption and solute-foulant interactions observed in Figures 7.3 and 7.5 and the strong interaction of estrone with organic matter shown...
in Chapter 5 it was likely that solute-solute interactions were the main estrone removal mechanism. Predicted hormone retention based solute-solute interactions (R_{p\%}) are shown in Table 7.1. These were estimated by considering organic matter retention (R_{OM\%}) and the mass of hormone sorbed to organic matter (m_{OM}) based on organic matter-water partition coefficients (log K_{OM}) (refer to Section 3.9.6 in Chapter 3 for more detail). For all organic matter types R_{p\%} was greater than experimental hormone retention (R_{\%}), with the difference being most significant for alginic acid and tannic acid. When comparing R_{p\%} and R_{\%} it is important to consider the associated error. The error associated with R_{\%} ranged from 5 to 12\%, while R_{OM\%} ranged from 15 to 18\% depending on MWCO. The error calculation methods are outlined in Appendix 3. Considering the error margin, there was some similarity between R_{p\%} and R_{\%} in the presence of Aldrich HA for the 1, 5 and 100 kDa membranes.
Based on this study it appears that solute-solute interactions are the dominant removal mechanism as membrane sorption and solute-foulant interactions are generally negligible. However, despite the strong sorption of estrone to all studied organic matter types indicated in Chapter 5, the influence of solute-solute interactions could only be predicted for Aldrich HA at certain MWCO membranes. Therefore, the influence of organic matter concentration and solution chemistry will be studied using Aldrich HA.
7.3. Influence of Organic Matter Concentration

7.3.1. Solute Retention

In this section the retention of estrone with increasing organic matter concentration was studied. In Chapter 5 organic matter concentration was observed to influence organic matter-water partitioning, consequently it is likely that organic matter concentration will alter estrone retention by UF. Figure 7.6 indicates that estrone retention increased significantly with increasing organic matter concentration. This was observed for both 10 and 100 kDa membranes.

High organic matter concentrations led to reduced activity of estrone during liquid scintillation counting due to colour quenching. Therefore, separate calibration curves at different organic matter concentrations were required to determine estrone mass and this will be discussed further in Appendix 2.

Agbekodo et al. (1996) observed greater pesticide retention by NF at higher organic matter concentrations and this was attributed to increased micropollutant sorption on the membrane with increasing organic matter concentration. Similarly, Dalton et al. (2005) found increased micropollutant retention at high organic matter to micropollutant ratios, and suggested that organic matter sorption sites were the limiting factor for micropollutant retention by membrane filtration. In contrast, Berg et al. (1997) found no difference in triazine retention by NF with increasing organic matter concentration, and attributed this to the type of organic matter compared to Agbekodo et al. (1996). This suggests that micropollutant retention in the presence of organic matter is variable and is dependent on organic matter type, as well as concentration.
Figure 7.6: Estrone retention for 10 and 100 kDa MWCO membranes as a function of Aldrich HA concentration (1 mM NaHCO₃, 20 mM NaCl, pH 8, 100 ng/L estrone, Aldrich HA concentrations of 0, 12.5, 25, 50 and 125 mgC/L)

Aldrich HA retention with increasing organic matter concentration is shown in Figure 7.7. For 10 kDa membranes Aldrich HA retention did not change significantly with increasing concentration, however, increased retention was observed for 100 kDa membranes at 125 mgC/L. Schäfer et al. (2002) found no difference in retention for NOM concentrations ranging from 15 to 60 mgC/L by 1 to 30 kDa membranes which is confirmed with an insignificant change in organic matter retention by both membranes from 12.5 to 50 mgC/L in Figure 7.7.
Figure 7.7: Aldrich HA retention for 10 and 100 kDa MWCO membranes as a function of Aldrich HA concentration (estimated using a UV-Visible spectrophotometer) (1 mM NaHCO₃ 20 mM NaCl, pH 8, Aldrich HA concentrations of 0, 12.5, 25, 50 and 125 mgC/L)

As the TOC analyser was mainly used for the analysis of low organic carbon concentration samples, the maximum allowable organic matter concentration was set to 10 mgC/L and analysis of samples containing higher concentrations was possible through dilution. For the 125 mgC/L experiment the UV-visible spectrophotometer was applied to estimate organic matter retention. Generally, UV-visible spectrophotometers are used to quantify absorbance and cannot be used to measure the concentration of organic matter directly. Therefore, the concentration was estimated using a calibration curve, which is discussed further in Appendix 2. With the exception of this example, the TOC analyser was used to measure organic carbon concentration for the rest of this chapter.

7.3.2. Membrane Sorption

Estrone sorption as a function of Aldrich HA concentration is shown in Figure 7.8. Unlike Agbekodo et al. (1996), organic matter concentration did not increase estrone sorption to the membrane. In fact, sorption decreased slightly with increasing Aldrich
Implications of Solute-Solute Interactions in Stirred Cell Ultrafiltration

HA concentration. It is expected that the minimal sorption was related to the hydrophilic nature of the membrane. Consequently, membrane sorption does not contribute to the removal of estrone with increasing organic matter concentrations.

![Diagram showing estrone sorption for 10 and 100 kDa MWCO membranes (ng/cm²) as a function of Aldrich HA concentration (1 mM NaHCO₃, 20 mM NaCl, pH 8, 100 ng/L estrone, Aldrich HA concentrations of 0, 12.5, 25, 50 and 125 mgC/L)]

Figure 7.8: Estrone sorption for 10 and 100 kDa MWCO membranes (ng/cm²) as a function of Aldrich HA concentration (1 mM NaHCO₃, 20 mM NaCl, pH 8, 100 ng/L estrone, Aldrich HA concentrations of 0, 12.5, 25, 50 and 125 mgC/L)

7.3.3. Solute-Foulant Interactions

In Figure 7.9a no significant decline in flux was observed for the 10 kDa membranes, while up to 60% decline in flux occurred for the 100 kDa membranes (Figure 7.9b). Aoustin et al. (2001) found similar fouling of 100 kDa regenerated cellulose membranes in the presence of NOM and suggested that fouling was due to large UV-absorbing molecules present in Aldrich HA which can coagulate and lead to pore blocking. Consequently, solute-foulant interactions may be an important mechanism for estrone retention by 100 kDa membranes. Fouling or coagulation is also expected to contribute to the increased Aldrich HA retention by the 100 kDa membrane at 125 mgC/L as observed in Figure 7.7.
Figure 7.9: Flux ratio as a function of Aldrich HA concentration for a) 10 kDa and b) 100 kDa MWCO membranes (1 mM NaHCO₃, 20 mM NaCl, pH 8, 100 ng/L estrone, Aldrich HA concentrations of 0, 12.5, 25, 50 and 125 mgC/L, pressure from 0.5 to 5 bar)

7.3.4. Solute-Solute Interactions

Due to minimal hormone sorption and negligible flux decline in the case of the 10 kDa membranes, it was expected that increased hormone retention in the presence of organic matter was related to solute-solute interactions. For 100 kDa membranes R% and Rₚ% were similar at all studied organic matter concentrations, while for 10 kDa membranes R% and Rₚ% were only similar at high organic matter concentrations (25-125 mgC/L) (Table 7.2). This suggests that solute-solute interactions are an important removal mechanism for 10 and 100 kDa membranes. The similarity between R% and Rₚ% at high organic matter concentrations suggests that the organic matter concentration used in Section 8.2 (12.5 mgC/L) was too low to see the influence of solute-solute interactions accurately. Therefore, organic matter concentration is the limiting factor of the studied solute-solute interactions.
Table 7.2: Comparison of experimental hormone retention ($R\%\_\text{e}$) with predicted hormone retention ($R\%\_\text{p}$) determined using log $K_{OM}$ values (L/kg) as a function of organic matter concentration (pH 8, 40 ng initial estrone mass ($m_{\text{TOT}}$))

<table>
<thead>
<tr>
<th>log $K_{OM}$ (L/kg) ± S.D</th>
<th>Predicted mass sorbed to OM ($m_{\text{OM}}$) (ng)*</th>
<th>Predicted hormone retention ($R%_\text{p}$)*</th>
<th>Experimental hormone retention ($R%_\text{e}$)*</th>
<th>Experimental OM retention ($R%_\text{OM}$)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.5 mgC/L</td>
<td>4.86±0.003</td>
<td>18.3±1.0</td>
<td>43±7</td>
<td>27±2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(46% sorbed)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 kDa</td>
<td>18±8</td>
<td></td>
<td>95±8</td>
<td></td>
</tr>
<tr>
<td>100 kDa</td>
<td>20±4</td>
<td></td>
<td>44±5</td>
<td></td>
</tr>
<tr>
<td>25 mgC/L</td>
<td>4.63±0.01</td>
<td>20.0±1.1</td>
<td>48±8</td>
<td>38±3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(50% sorbed)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 kDa</td>
<td>23±4</td>
<td></td>
<td>95±8</td>
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</tr>
<tr>
<td>100 kDa</td>
<td>28±3</td>
<td></td>
<td>46±6</td>
<td></td>
</tr>
<tr>
<td>50 mgC/L</td>
<td>4.39±0.01</td>
<td>21.3±1.2</td>
<td>51±9</td>
<td>35±3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(53% sorbed)</td>
<td></td>
<td></td>
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<tr>
<td>10 kDa</td>
<td>27±5</td>
<td></td>
<td>96±8</td>
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<td>100 kDa</td>
<td>27±3</td>
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<td>50±6</td>
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</tr>
<tr>
<td>125 mgC/L</td>
<td>4.13±0.03</td>
<td>24.7±1.3</td>
<td>59±10</td>
<td>53±4</td>
</tr>
<tr>
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<td></td>
<td>(62% sorbed)</td>
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<td></td>
</tr>
<tr>
<td>10 kDa</td>
<td>37±7</td>
<td></td>
<td>96±8</td>
<td></td>
</tr>
<tr>
<td>100 kDa</td>
<td>38±5</td>
<td></td>
<td>60±7</td>
<td></td>
</tr>
</tbody>
</table>

*error was calculated using error propagation (Appendix 3)

7.4. Influence of pH

7.4.1 Solute Retention

The pH can have implications for the properties of the micropollutant, organic matter and membrane including charge and confirmation. Therefore, in this section the influence of pH on hormone retention was studied as it was expected that this would alter the hormone removal mechanisms. Estrone and progesterone removal in the presence of Aldrich HA was studied as a function of pH by 10 and 100 kDa membranes (Figure 7.10). Progesterone was selected for study as it remains neutrally charged as it does not contain dissociable functional groups within the studied pH range. In contrast, estrone has an acid dissociation constant ($pK_a$) of 10.34 (Kwon et al., 2006) and therefore it is negatively charged at high pH.
For 10 kDa membranes a slight increase in estrone retention in the presence of Aldrich HA was observed in alkaline solutions (Figure 7.10a). When considering the error associated with retention, no change was observed for progesterone. While progesterone remained neutrally charged, estrone was 31 and 97% dissociated at pH 10 and 12 respectively. In contrast, for 100 kDa membranes greater retention of both estrone and progesterone was observed at pH 4, but retention remained constant from pH 7 to 12 (Figure 7.10b and d). Further, for both 10 and 100 kDa membranes more progesterone was retained compared to estrone in both the presence and absence of Aldrich HA. This will be explored further in Section 7.4.2.

The influence of pH on micropollutant retention in the presence of organic matter has been studied previously using both NF and UF. Hu et al. (2007) found increased
hormone retention at low pH by NF and attributed this to increased hormone and humic acid sorption, as well as decreased flux leading to solute-fouulant interactions. Nghiem and Hawkes (2007) studied pharmaceutical removal by humic acid fouled NF membranes as a function of pH and found retention increased above the micropollutants’ pKₐ value due to electrostatic repulsion. Conversely, Schäfer et al. (2006) found decreased retention of bisphenol A above its pKₐ value due to decreased sorption to the UF membrane. Consequently, the different mechanisms of removal can have a significant influence for micropollutant retention as a function of pH.

Organic matter retention and SUVA in the permeate is shown in Figure 7.11. For both 10 and 100 kDa membranes no significant difference in Aldrich HA retention as a function of pH was observed (Figure 7.11a). In Figure 7.11b there was no difference in SUVA in the permeate for 100 kDa membranes, however, SUVA decreased with increasing pH for 10 kDa membranes. This suggests greater retention of large aromatic compounds at high pH. This corresponds to the changing conformation of Aldrich HA which is more linear at high pH (Ghosh and Schnitzer, 1980). The effective diameter of Aldrich HA at pH 3, 8 and 12 is shown in Figure 7.12 and indicates increased molecular size in alkaline pH conditions. Further, the aromatic fraction of Aldrich HA mainly contains phenolic moieties which are ringed carbon molecules. Previous studies have demonstrated that such groups lack flexibility (von der Lieth et al., 1996) and therefore this may contribute to their retention. For 100 kDa membranes no change in SUVA was observed and it was likely that this was due to the large MWCO of the membrane which led to lower retention of organic matter.
Figure 7.11: a) Organic matter retention and b) SUVA in permeate for Aldrich HA as a function of pH for 10 and 100 kDa MWCO membranes (1 mM NaHCO₃, 20 mM NaCl, 12.5 mgC/L Aldrich HA)
Further, pH also has implications for the membrane charge. Schäfer et al. (2002) measured zeta potential for 1 to 100 kDa regenerated cellulose membranes, and found that the negative charge increased with increasing pH. Therefore, the charge of the membrane may have implications for the removal mechanisms of steroidal hormones.

A limitation of the air pressurised stirred cell configuration was that it was not possible to adjust the pH during the experiment. The NaHCO₃ buffer, which was selected as a representative of natural waters, was relatively weak and experiments indicated that some acidic and alkaline pH values varied considerably for smaller MWCO membranes. This will be discussed further in Appendix 1. However, the pH was relatively stable for the 10-100 kDa membranes.

7.4.2. Membrane Sorption

pH had little influence on hormone sorption to the membrane (Figure 7.13). A slight decrease in estrone sorption at alkaline pH for the 10 kDa membrane was observed in Figure 7.13a. This may be related to hormone dissociation and the negative charge of
the membrane. While organic matter did not influence estrone sorption, decreased sorption of progesterone in the presence of Aldrich HA for 10 kDa membranes was observed (Figure 7.13c). Similar results for estradiol were observed by McCallum et al. (2008) and Yoon et al. (2004) and it was suggested that decreased sorption was due to competition with organic matter for membrane sorption sites. Progesterone adsorbs to the membrane significantly more than estrone leading to greater removal of progesterone in Figure 7.10.

![Figure 7.13](image)

**Figure 7.13: Hormone sorption to the membrane (ng/cm²) as a function of pH for a) estrone by 10 kDa membranes, b) estrone by 100 kDa membranes, c) progesterone by 10 kDa membranes and d) progesterone by 100 kDa membranes (1 mM NaHCO₃ 20 mM NaCl, 100 ng/L estrone and progesterone, 12.5 mgC/L Aldrich HA)**

Greater sorption of Aldrich HA was observed at pH 4 (Figure 7.14). At pH 4 Aldrich HA is expected to be rigid and coiled due to the lack of charge repulsion between the carboxylic functional groups (Ghosh and Schnitzer, 1980). This is confirmed by Figure 7.12 which shows negligible charge (measured by zeta potential) at low pH.
Consequently, Aldrich HA can aggregate and deposit on the membrane and it is likely that this led to greater hormone retention by the 100 kDa membranes at acidic pH. Further, several other studies have also observed greater organic matter sorption at acidic pH conditions (e.g. Jones and O'Melia, 2000; Jucker and Clark, 1994; Küchler and Miekeley, 1994).

![Figure 7.14: Aldrich HA sorption to the membrane (mg/cm²) as a function of pH for 10 and 100 kDa MWCO membranes (1 mM NaHCO₃ 20 mM NaCl, 12.5 mgC/L Aldrich HA)](image)

7.4.3. Solute-Foulant Interactions

The influence of solute-foulant interactions as a function of pH is shown in Figure 7.15 for 10 and 100 kDa membranes. pH had no influence on fouling for 10 kDa membranes with no change in flux ratio observed at any studied pH solution. For 100 kDa membranes flux decline was observed for all studied pH values, with the most fouling at pH 4. This has been observed previously by Hong and Elimelech (1997) for NF. As Aldrich HA can aggregate at acidic pH this can reduce permeability through the membrane and lead to increased fouling (Devitt and Wiesner, 1998). Therefore, based on Figure 7.10c and d it was likely Aldrich HA fouling contributed to the increased hormone retention observed for 100 kDa membranes at pH 4. This was further confirmed by increased organic matter deposition at pH 4 in Figure 7.14.
Figure 7.15: Flux ratio as a function of pH for a) 10 kDa MWCO and b) 100 kDa MWCO membranes (1 mM NaHCO₃, 20 mM NaCl, 100 ng/L estrone and progesterone, 12.5 mgC/L Aldrich HA, pressure from 0.5 to 5 bar)

7.4.4. Solute-Solute Interactions

The influence of solute-solute interaction for hormone retention was assessed for estrone and progesterone using experimental log K_{OM} values. In Table 7.3 R_{p%} was considerably greater than R_{%} for pH 4 to 9, but then deceased until retention could not be predicted at pH 12. The overestimated R_{p%} values may be related to the low concentration of organic matter (12.5 mgC/L) as discussed in Section 7.3.4. In contrast, R_{%} does not decrease at alkaline pH, and even increased from pH 10 to 12 for the 10 kDa membranes due to increased retention of aromatic organic matter fractions (Figure 7.11b). As sorption of estrone to the membrane did not change significantly with increasing pH (Figure 7.13) and there was no change in flux ratio (Figure 7.15) it was unlikely that the estrone retention mechanisms were sorption or solute-foulant interactions. Despite being negatively charged above pH 10, estrone still contains a ketone group in the C-17 position which is a hydrogen acceptor and can therefore interact with Aldrich HA through hydrogen bonding. Therefore, solute-solute interactions are expected to be the dominant removal mechanism at alkaline pH, however, they cannot be quantified using SPME. The decreased log K_{OM} values
at high pH were due to the reduced capacity of the fibre to extract negatively charged micropollutants, which is a significant limitation of SPME.

**Table 7.3: Comparison of experimental hormone retention (R\%b) with predicted hormone retention (RP\%) determined using log KOM values (L/kg) for estrone as a function of pH (40 ng initial estrone mass (mTOT))**

<table>
<thead>
<tr>
<th>pH</th>
<th>Log KOM (L/kg) ± S.D</th>
<th>Predicted mass sorbed to OM (mOM) (ng)*</th>
<th>Predicted hormone retention (RP%)*</th>
<th>Experimental hormone retention (R%)*</th>
<th>Experimental OM retention (ROM%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>5.27±0.03</td>
<td>27.0±1.5</td>
<td>64±11</td>
<td>26±2</td>
<td>95±16</td>
</tr>
<tr>
<td></td>
<td>10 kDa</td>
<td>(68% sorbed)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>100 kDa</td>
<td></td>
<td>48±9</td>
<td>22±3</td>
<td>71±13</td>
</tr>
<tr>
<td>7</td>
<td>4.82±0.04</td>
<td>17.4±0.9</td>
<td>41±7</td>
<td>26±2</td>
<td>95±16</td>
</tr>
<tr>
<td></td>
<td>10 kDa</td>
<td>(43% sorbed)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>100 kDa</td>
<td></td>
<td>25±5</td>
<td>15±2</td>
<td>58±11</td>
</tr>
<tr>
<td>8</td>
<td>4.86±0.03</td>
<td>18.3±1.0</td>
<td>42±7</td>
<td>25±2</td>
<td>93±15</td>
</tr>
<tr>
<td></td>
<td>10 kDa</td>
<td>(46% sorbed)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>100 kDa</td>
<td></td>
<td>27±5</td>
<td>15±2</td>
<td>59±11</td>
</tr>
<tr>
<td>9</td>
<td>4.75±0.04</td>
<td>15.9±0.9</td>
<td>36±6</td>
<td>24±2</td>
<td>92±15</td>
</tr>
<tr>
<td></td>
<td>10 kDa</td>
<td>(40% sorbed)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>100 kDa</td>
<td></td>
<td>23±5</td>
<td>17±2</td>
<td>59±11</td>
</tr>
<tr>
<td>10</td>
<td>4.52±0.11</td>
<td>11.5±0.6</td>
<td>25±4</td>
<td>35±3</td>
<td>88±14</td>
</tr>
<tr>
<td></td>
<td>10 kDa</td>
<td>(29% sorbed)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>100 kDa</td>
<td></td>
<td>18±3</td>
<td>18±2</td>
<td>62±12</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>37±3</td>
<td>94±15</td>
</tr>
<tr>
<td></td>
<td>10 kDa</td>
<td></td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>100 kDa</td>
<td></td>
<td>-</td>
<td>19±2</td>
<td>65±12</td>
</tr>
</tbody>
</table>

*error was calculated using error propagation (Appendix 3)

Unlike estrone, progesterone does not contain dissociable functional groups and there was no significant decrease in log KOM values with increasing pH. In Table 7.4 RP\% was lower than the R\%. This suggests that other mechanisms as well as solute-solute interactions contribute to the retention of progesterone. Based on Figure 7.13c and d, sorption of progesterone to the membrane is an important removal mechanism. Further, solute-foulant interactions may also contribute to progesterone retention by the 100 kDa membranes in acidic solutions.
Table 7.4: Comparison of experimental hormone retention ($R\%$) with predicted hormone retention ($R_{P\%}$) determined using log $K_{OM}$ values (L/kg) for progesterone as a function of pH (40 ng initial estrone mass ($m_{TOT}$))

<table>
<thead>
<tr>
<th>Progesterone</th>
<th>log $K_{OM}$ (L/kg) ± S.D</th>
<th>Predicted mass sorbed to OM ($m_{OM}$) (ng)*</th>
<th>Predicted hormone retention ($R_{P%}$)*</th>
<th>Experimental hormone retention ($R%$)*</th>
<th>Experimental OM retention ($R_{OM%}$)*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>pH 4</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 kDa</td>
<td>4.73±0.08</td>
<td>15.6±0.8</td>
<td>37±6</td>
<td>46±4</td>
<td>95±16</td>
</tr>
<tr>
<td>100 kDa</td>
<td></td>
<td></td>
<td>28±5</td>
<td>40±5</td>
<td>71±13</td>
</tr>
<tr>
<td><strong>pH 7</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 kDa</td>
<td>4.59±0.10</td>
<td>12.6±0.7</td>
<td>30±5</td>
<td>56±5</td>
<td>95±16</td>
</tr>
<tr>
<td>100 kDa</td>
<td></td>
<td></td>
<td>18±4</td>
<td>31±4</td>
<td>58±11</td>
</tr>
<tr>
<td><strong>pH 8</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 kDa</td>
<td></td>
<td></td>
<td>-</td>
<td>58±5</td>
<td>93±15</td>
</tr>
<tr>
<td>100 kDa</td>
<td></td>
<td></td>
<td>-</td>
<td>35±4</td>
<td>59±11</td>
</tr>
<tr>
<td><strong>pH 9</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 kDa</td>
<td>4.58±0.07</td>
<td>12.3±0.7</td>
<td>28±5</td>
<td>60±5</td>
<td>92±15</td>
</tr>
<tr>
<td>100 kDa</td>
<td></td>
<td></td>
<td>18±4</td>
<td>35±4</td>
<td>59±11</td>
</tr>
<tr>
<td><strong>pH 10</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 kDa</td>
<td>4.48±0.02</td>
<td>10.6±0.6</td>
<td>23±4</td>
<td>59±5</td>
<td>88±14</td>
</tr>
<tr>
<td>100 kDa</td>
<td></td>
<td></td>
<td>17±3</td>
<td>35±4</td>
<td>62±12</td>
</tr>
<tr>
<td><strong>pH 12</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 kDa</td>
<td>4.54±0.05</td>
<td>11.7±0.6</td>
<td>27±5</td>
<td>63±5</td>
<td>94±15</td>
</tr>
<tr>
<td>100 kDa</td>
<td></td>
<td></td>
<td>19±4</td>
<td>33±4</td>
<td>65±12</td>
</tr>
</tbody>
</table>

*error was calculated using error propagation (Appendix 3)

Therefore, membrane sorption, organic matter deposition and solute-solute interactions all contributed to the removal of estrone and progesterone in the presence of organic matter as a function of pH. Hormone sorption to the membrane appeared to be independent of pH, while organic matter deposition only influenced hormone removal in acidic pH solutions for 100 kDa membranes. Further, this study has highlighted a limitation of SPME as experimental log $K_{OM}$ values calculated by this technique cannot be used to predict retention above the pK$_a$ of the micropollutant.
7.5. Influence of Ionic Strength

7.5.1. Solute Retention

In this section hormone retention as a function of ionic strength was studied. The ionic strength of a solution can have implications for properties of the organic matter, micropollutant and membrane as elevated ionic strength can shield negative charges. In Figure 7.16 estrone retention decreased with ionic strength (NaCl) at pH 8. Devitt et al. (1998) observed decreased retention of atrazine in the presence of organic matter with increasing ionic strength and attributed this to the reversibility of atrazine-organic matter interactions at high ionic strengths. As estrone is neutrally charged at pH 8 ionic strength is unlikely to have an influence on charge. Indeed Nghiem and Schäfer (2002) found ionic strength did not influence estrone retention by NF in the absence of organic matter at neutral pH. Conversely, high ionic strength is expected to influence the retention of negatively charged estrone through negative charge shielding. However, this was not studied here.

![Figure 7.16: Estrone retention as a function of ionic strength for 1, 10 and 100 kDa MWCO membranes (1 mM NaHCO₃, 0, 20, 50 and 100 mM NaCl, pH 8, 100 ng/L estrone, 12.5 mgC/L Aldrich HA)](image_url)
Consequently, it was likely that the decreased retention of estrone was related to the influence of ionic strength on Aldrich HA or the membrane. In Figure 7.17a there was little difference in Aldrich HA retention with increasing ionic strength for 1 and 10 kDa membranes, while retention decreased significantly from 0 to 20 mM NaCl concentration for 100 kDa membranes. The difference in Aldrich HA retention for different MWCO membranes was related to the charge and conformation changes of Aldrich HA with increasing ionic strength. As discussed in Chapter 5, Aldrich HA is in a linear form at low ionic strength due to charge repulsion, while increasing ionic strength can partially shield the negative charge leading to coiling (Ghosh and Schnitzer, 1980). Consequently, Jucker and Clark (1994) suggested that humic acid has a larger average size at low ionic strength compared to high ionic strength. This is confirmed by Figure 7.18 which indicated that the effective diameter of Aldrich HA decreased from 0 to 20 mM (3754 to 1670 nm). However, at 100 mM the effective diameter increased to 2869 nm and it is likely that this was due to Aldrich HA aggregation due to the reduced negative charge (Figure 7.18). Aldrich HA retention by 100 kDa membranes reflects the changes in effective diameter, while 1 and 10 kDa membranes exhibit high retention of Aldrich HA due to steric effects, therefore the change in size with ionic strength does not influence retention.

In Figure 7.17b SUVA in the permeate increased significantly with increasing ionic strength for 1 and 10 kDa membranes indicating greater retention of aromatic Aldrich HA fractions at low ionic strength. This suggests that for the 1 and 10 kDa membranes the coiling of Aldrich HA due to increasing ionic strength led to less retention of aromatic fractions. This is similar to the observation in Figure 7.11b. No significant difference in SUVA was observed for the 100 kDa membranes suggesting that ionic strength has little influence on the retention of aromatic molecules due to the large MWCO of the membrane. Further, ionic strength had no influence on SUVA in the feed samples.
Figure 7.17: a) Aldrich HA retention and b) SUVA in the permeate as a function of ionic strength for 1, 10 and 100 kDa MWCO membranes (1 mM NaHCO₃, 0, 20, 50 and 100 mM NaCl, pH 8, 12.5 mgC/L Aldrich HA)
Figure 7.18: Effective diameter and zeta potential of Aldrich HA as a function of ionic strength (1 mM NaHCO₃, 0, 20, 50 and 100 mM NaCl, 12.5 mgC/L Aldrich HA)

7.5.2. Membrane Sorption

No significant difference in sorption of estrone to the membrane with increasing ionic strength was observed in Figure 7.19. Greater sorption to 1 kDa membranes compared to 100 kDa membranes was observed, but this could be attributed to the longer experimental times, as discussed in Section 7.2.2.

In Figure 7.20 negligible Aldrich HA sorption to the membrane was found at 0 mM NaCl concentration for all studied membranes. At low ionic strength Aldrich HA is charged and therefore unlikely to sorb to the membrane. As ionic strength increased greater sorption was observed for all membranes, until 100 mM when negligible sorption was measured for 1 and 10 kDa membranes. Aldrich HA sorption increased in the case of 100 kDa membranes. At high ionic strength the negative charge of the UF membrane is shielded and Aldrich HA is coiled, with Figure 7.18 suggesting aggregation occurs. Bacchin et al. (1996) found increased deposition of colloids at high ionic strength for 300 kDa membranes. Therefore, it is likely that colloidal Aldrich HA is depositing on to the membrane at 100 mM NaCl. However, for 1 and
Implications of Solute-Solute Interactions in Stirred Cell Ultrafiltration

10 kDa membranes the low flux (21 to 80 L/m².h) may prevent deposition of colloidal Aldrich HA to the membrane in the presence of 100 mM NaCl.

Figure 7.19: Estrone sorption to the membrane (ng/cm²) as a function of ionic strength for 1, 10 and 100 kDa MWCO membranes (1 mM NaHCO₃, 0, 20, 50 and 100 mM NaCl, pH 8, 100 ng/L estrone, 12.5 mgC/L Aldrich HA)

Figure 7.20: Aldrich HA sorption to the membrane (mg/cm²) as a function of ionic strength for 1, 10 and 100 kDa MWCO membranes (1 mM NaHCO₃, 0, 20, 50 and 100 mM NaCl, pH 8, 12.5 mgC/L Aldrich HA)
7.5.3. Solute-Foulant Interactions

Similar to the other studied solution conditions, no flux decline was observed for 1 and 10 kDa membrane. Flux decline was only observed for 100 kDa membranes and this was most significant at the 100 mM NaCl concentration (Figure 7.21). This is most likely due to Aldrich HA aggregation and deposition at high ionic strength. Increased flux decline at high ionic strength was previously observed by Yuan and Zydney (2000) for a 30 kDa membrane in the presence of humic acid. This trend has also been observed previously in NF (e.g. Braghetta et al., 1997; Hong and Elimelech, 1997) and was attributed to increased hydraulic resistance due to organic matter deposition and fouling.

![Figure 7.21: Flux ratio as a function of ionic strength for a) 1 kDa membranes, b) 10 kDa membranes and c) 100 kDa membranes (1 mM NaHCO₃, 0, 20, 50 and 100 mM NaCl, pH 8, 100 ng/L estrone, 12.5 mgC/L Aldrich HA, pressure from 0.5 to 5 bar)
7.5.4. Solute-Solute Interactions

As ionic strength had little impact on membrane sorption and only influenced solute-foulant interactions at 100 kDa membranes it is likely that the dominant removal mechanism was solute-solute interactions. Log $K_{OM}$ values in Table 7.5 indicates a decrease in partitioning with increasing ionic strength, which corresponds to the decrease in estrone retention observed in Figure 7.16 suggesting the importance of solute-solute interactions. This was confirmed when comparing $R_{p\%}$ and $R_{s\%}$ which were mostly similar when considering the error associated with retention calculation. Therefore, the dominant mechanism of hormone retention as a function of ionic strength was solute-solute interactions.

Table 7.5: Comparison of experimental hormone retention ($R_{s\%}$) with predicted hormone retention ($R_{p\%}$) determined using log $K_{OM}$ values (L/kg) as a function of ionic strength (pH 8, 40 ng initial estrone mass ($m_{TOT}$))

<table>
<thead>
<tr>
<th>Ionic Strength</th>
<th>Log $K_{OM}$ (L/kg) ± S.D</th>
<th>Predicted mass sorbed to OM ($m_{OM}$) (ng)*</th>
<th>Predicted hormone retention ($R_{p%}$)*</th>
<th>Experimental hormone retention ($R_{s%}$)*</th>
<th>Experimental OM retention ($R_{OM%}$)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mM</td>
<td>4.89±0.02 19.1±1.0</td>
<td>44±7 37±2 92±14</td>
<td>44±8 31±3 91±15</td>
<td>36±7 22±3 76±14</td>
<td></td>
</tr>
<tr>
<td>1 kDa</td>
<td>44±7 48% sorbed</td>
<td>1 kDa</td>
<td>43±7 39±2 93±14</td>
<td>41±7 25±2 90±15</td>
<td>21±4 16±2 47±9</td>
</tr>
<tr>
<td>10 kDa</td>
<td>44±8 31±3 91±15</td>
<td>10 kDa</td>
<td>42±7 33±2 89±14</td>
<td>42±7 24±2 89±15</td>
<td>26±5 16±2 56±10</td>
</tr>
<tr>
<td>100 kDa</td>
<td>36±7 22±3 76±14</td>
<td>100 kDa</td>
<td>38±6 32±2 89±14</td>
<td>38±7 26±2 88±15</td>
<td>26±5 12±2 60±11</td>
</tr>
<tr>
<td>20 mM</td>
<td>4.86±0.003 18.3±1.0</td>
<td>20 kDa</td>
<td>43±7 39±2 93±14</td>
<td>41±7 25±2 90±15</td>
<td>21±4 16±2 47±9</td>
</tr>
<tr>
<td>1 kDa</td>
<td>44±7 46% sorbed</td>
<td>1 kDa</td>
<td>42±7 33±2 89±14</td>
<td>42±7 24±2 89±15</td>
<td>26±5 16±2 56±10</td>
</tr>
<tr>
<td>10 kDa</td>
<td>42±7 24±2 89±15</td>
<td>10 kDa</td>
<td>38±6 32±2 89±14</td>
<td>38±7 26±2 88±15</td>
<td>26±5 12±2 60±11</td>
</tr>
<tr>
<td>100 kDa</td>
<td>38±6 32±2 89±14</td>
<td>100 kDa</td>
<td>38±6 32±2 89±14</td>
<td>38±7 26±2 88±15</td>
<td>26±5 12±2 60±11</td>
</tr>
<tr>
<td>50 mM</td>
<td>4.88±0.01 18.7±1.0</td>
<td>50 mM</td>
<td>42±7 33±2 89±14</td>
<td>42±7 24±2 89±15</td>
<td>26±5 16±2 56±10</td>
</tr>
<tr>
<td>1 kDa</td>
<td>42±7 47% sorbed</td>
<td>1 kDa</td>
<td>38±6 32±2 89±14</td>
<td>38±7 26±2 88±15</td>
<td>26±5 12±2 60±11</td>
</tr>
<tr>
<td>10 kDa</td>
<td>42±7 24±2 89±15</td>
<td>10 kDa</td>
<td>38±6 32±2 89±14</td>
<td>38±7 26±2 88±15</td>
<td>26±5 12±2 60±11</td>
</tr>
<tr>
<td>100 kDa</td>
<td>38±6 32±2 89±14</td>
<td>100 kDa</td>
<td>38±6 32±2 89±14</td>
<td>38±7 26±2 88±15</td>
<td>26±5 12±2 60±11</td>
</tr>
<tr>
<td>100 mM</td>
<td>4.81±0.04 17.2±0.9</td>
<td>100 mM</td>
<td>38±6 32±2 89±14</td>
<td>38±7 26±2 88±15</td>
<td>26±5 12±2 60±11</td>
</tr>
<tr>
<td>1 kDa</td>
<td>38±6 43% sorbed</td>
<td>1 kDa</td>
<td>38±6 32±2 89±14</td>
<td>38±7 26±2 88±15</td>
<td>26±5 12±2 60±11</td>
</tr>
<tr>
<td>10 kDa</td>
<td>38±7 26±2 88±15</td>
<td>10 kDa</td>
<td>38±6 32±2 89±14</td>
<td>38±7 26±2 88±15</td>
<td>26±5 12±2 60±11</td>
</tr>
<tr>
<td>100 kDa</td>
<td>38±7 26±2 88±15</td>
<td>100 kDa</td>
<td>38±6 32±2 89±14</td>
<td>38±7 26±2 88±15</td>
<td>26±5 12±2 60±11</td>
</tr>
</tbody>
</table>

*error was calculated using error propagation (Appendix 3)
7.6. Influence of Hormone Type and Concentration

7.6.1. Hormone Type

Retention and membrane sorption of estradiol, estrone, progesterone and testosterone are shown in Figures 7.22 and 7.23. Based on the different properties of the studied hormones, it was expected that their retention and sorption to the membrane would differ. In Figure 7.22 the greatest retention was observed for progesterone followed by estrone. Based on SPME experiments, this was expected due to strong solute-solute interactions. A significant increase in retention in the presence of Aldrich HA was observed for testosterone. This was unexpected as SPME experiments suggested testosterone sorbed weakly to organic matter. A similar order of retention by UF in the presence of organic matter was observed previously in the literature by Yoon et al. (2007). Further, Koyuncu et al. (2008) found significantly higher retention of progesterone (98%) in the absence of organic matter by a hydrophilic NF membrane compared to estrone (80%), estradiol (64%) and testosterone (62%). However, retention was above 95% for all hormones in the presence of organic matter.

Membrane sorption was low for all hormones with the exception of progesterone (Figure 7.23). This was also observed in Section 7.4.2. Previous studies have attributed the strong sorption of progesterone to its hydrophobicity (log \( K_{OW} \)) (Ng and Elimelech, 2004; Yoon et al., 2006). However, log \( K_{OW} \) values within the literature vary considerably (see Table 6.2 in Chapter 6), and most experimental and modelled log \( K_{OW} \) values indicate estradiol is more hydrophobic than progesterone. Therefore, the strong sorption may be related to the presence of strongly hydrogen accepting ketone functional groups in the C-3 and C-20 positions of progesterone. The strength of hydrogen accepting and donating functional groups for all hormones is shown below in Table 7.6. As regenerated cellulose contains hydroxyl groups hydrogen bonding is possible. Further, Table 7.6 also indicates that testosterone contains strong hydrogen accepting functional groups, which may account for significant retention in the presence of organic matter observed in Figure 7.22.
Figure 7.22: Hormone retention as a function of MWCO membranes for a) estradiol, b) estrone, c) progesterone and d) testosterone (1 mM NaHCO₃ 20 mM NaCl, pH 8, 100 ng/L hormone, 12.5 mgC/L Aldrich HA)

Table 7.6: Strength of hydrogen accepting and donating functional groups in steroidal hormones (Adapted from Gancia et al., 2001)

<table>
<thead>
<tr>
<th></th>
<th>Estradiol</th>
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<th>Progesterone</th>
<th>Testosterone</th>
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<tbody>
<tr>
<td>H-Acceptor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-3</td>
<td>0.3</td>
<td>0.3</td>
<td>1.7</td>
<td>1.7</td>
</tr>
<tr>
<td>C-17*</td>
<td>1.36</td>
<td>1.61</td>
<td>1.52</td>
<td>1.36</td>
</tr>
<tr>
<td>Total</td>
<td>1.66</td>
<td>1.91</td>
<td>3.22</td>
<td>3.06</td>
</tr>
<tr>
<td>H-Donor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-3</td>
<td>1.89</td>
<td>1.89</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C-17*</td>
<td>0.91</td>
<td>0</td>
<td>0</td>
<td>0.91</td>
</tr>
<tr>
<td>Total</td>
<td>2.8</td>
<td>1.89</td>
<td>0</td>
<td>0.91</td>
</tr>
</tbody>
</table>

*C-20 position for progesterone
Figure 7.23: Hormone sorption to the membrane (ng/cm²) as a function of MWCO membranes for a) estradiol, b) estrone, c) progesterone and d) testosterone (1 mM NaHCO₃ 20 mM NaCl, pH 8, 100 ng/L hormone, 12.5 mgC/L Aldrich HA)

7.6.2. Hormone Concentration

To determine if hormone concentration influenced retention mechanisms the removal of estrone at concentrations ranging from 100 ng/L to 1 mg/L were studied using 1 and 10 kDa membranes. Figure 7.24 indicates that there was no significant difference in retention with increasing hormone concentration. This was observed in both the presence and absence of Aldrich HA and for both 1 and 10 kDa membranes. This suggests that estrone is not the limiting factor in the studied solute-solute interactions, confirming the results in Section 7.3 where increased hormone retention was observed with increased organic matter concentration.
Figure 7.24: Estrone retention as a function of estrone concentration for a) 1 kDa membranes and b) 10 kDa membranes (1 mM NaHCO₃ 20 mM NaCl, pH 8, estrone concentrations of 0.1, 1, 10, 100, 1000 µg/L, 12.5 mgC/L Aldrich HA)

7.7. Conclusions

Micropollutant retention mechanisms in the presence of organic matter include membrane sorption, solute-foulant interactions and solute-solute interactions. Solute-solute interactions are typically overlooked due to quantification difficulties. This study indicates that in principle it is possible to quantify such interactions using experimental log K_{OM} values calculated using solid-phase microextraction (SPME). This was most effective at high organic matter concentrations such as in Table 7.2. At low organic matter concentrations (12.5 mgC/L), log K_{OM} values generally
overestimate solute-solute interactions, suggesting that the concentration was too low to see the effects accurately.

The retention of steroidal hormones was studied as a function of organic matter type and concentration as well as solution chemistry. By quantifying the different removal mechanisms, the dominant mechanism was solute-solute interactions as membrane sorption and solute-foulant interactions were generally negligible. Further, hormone retention as a function of solution chemistry was strongly influenced by solute-solute interactions, with trends in hormone retention with increasing ionic strength mirrored in the log $K_{OM}$ values. However, solute-foulant interactions appear to be important for hormone retention by 100 kDa membranes and membrane sorption also contributed to the removal of progesterone.

This study also exposed a significant limitation of SPME calculated log $K_{OM}$ values. Due to limited capacity to extract negatively charged species, SPME could not be used to predict retention based on solute-solute interactions above the $pK_a$ of the micropollutant. Further, as discussed above the studied organic matter concentration may be too low to accurately determine the influence of solute-solute interactions. This demonstrates the difficulty associated with studying solute-solute interactions at environmental concentrations.

Finally, this study also identified the limiting solute in the studied solute-solute interaction. Retention increased with increasing organic matter concentration, while no difference was observed with increasing estrone concentration, indicating that organic matter is the limiting factor. Consequently, it is organic matter concentration rather than the hormone concentration which will determine the strength of solute-solute interactions and therefore influence micropollutant fate in the aquatic environment. This indicates the necessity of studying solute-solute interactions at environmentally realistic organic matter concentrations.
7.8. Recommendations for Future Studies

This study raised some important issues which require further consideration. Firstly, the pH was relatively unstable within the stirred cells, meaning that hormone removal as a function of pH could only be studied with 10 and 100 kDa membranes. This is a limitation of the stirred cell configuration therefore it may be necessary to use a different type of UF membrane system which allows for pH adjustment such as cross flow filtration.

Another approach may be to use a different buffer instead of NaHCO₃, which is questionable as NaHCO₃ represents natural aquatic systems. One option is a phosphate buffer which is a stronger buffer and covers a wider pH range (Beynon and Easterby, 1996). However, a phosphate buffer is not representative of natural water systems and is not suitable in the presence of certain inorganic ions such as calcium. Alternatively, by pressurising the cell with inert nitrogen instead of air pH stability problems can be prevented. This is because nitrogen does not exchange with carbonate like the carbon dioxide in lab air does. This is discussed further in Appendix 1.

Another issue raised in this study was limitations associated with SPME. Many micropollutants exist in an ionic form at neutral pH (e.g. ibuprofen) therefore log K_{OM} values calculated using the methodology in Chapter 4 cannot be used to determine micropollutant removal due to solute-solute interactions. Therefore, a different SPME methodology needs to be developed for ionic micropollutants at environmental levels. This may involve longer fibre-water equilibrium times. Further, as the stirred cells are pressurised, it may be useful to quantify log K_{OM} values using SPME at a range of pressures to determine the influence of pressure on solute-solute interactions.
8 Steroidal Hormone Removal by an Ion Exchange-Ultrafiltration (IX-UF) Hybrid Process

In this chapter the removal of steroidal hormones using an ion exchange-ultrafiltration (IX-UF) hybrid process will be studied. A commonly used IX resin, magnetic ion exchange (MIEX®), was applied due to its effective removal of natural organic matter (NOM) from water. The purpose of this study is to assess hormone removal mechanisms by IX-UF and to determine the influence of membrane molecular weight cut-off (MWCO), organic matter concentration and hormone type on removal. Further, removal will be studied at pH 8 and 11 as estradiol becomes negatively charged above pH 10 and therefore can interact with MIEX® through ion exchange.

Removal of estradiol by MIEX® and UF were studied separately to determine baseline removal and were 21-31% and 30-65% respectively depending on pH and membrane MWCO. Using the IX-UF hybrid process approximately 90% estradiol removal was observed by 5 to 100 kDa membranes, indicating that this process was more effective than MIEX® or UF separately. Removal was lower for 1 and 3 kDa membranes (62-75%). It was suggested that high estradiol removal by 5 to 100 kDa membranes was due to deposition of MIEX® on the membrane leading to fouling.

The presence of high concentrations of NOM decreased estradiol removal by IX-UF at both pH 8 and 11. MIEX® preferentially sorbs NOM over estradiol. This was due to the negative charge of NOM at both studied pH values and the considerably higher concentration and molecular weight of NOM compared to estradiol.

The removal of estradiol, estrone, progesterone and testosterone by the IX-UF hybrid process was studied to determine the influence of hormone type. Due to significant fouling for 10 and 100 kDa membranes no difference in removal was observed for the different hormones. However, greater removal of progesterone was observed by the 1 kDa membrane due to the presence of strong hydrogen accepting
functional groups in progesterone leading to greater interaction with both the MIEX® and the UF membrane. Therefore, this study indicates that combining MIEX® with UF can significantly improve removal of steroidal hormones, and thus has implications for improved water treatment.
8.1. Introduction

In this chapter an ion exchange-ultrafiltration (IX-UF) hybrid process will be developed to improve the removal of steroidal hormones from water. Magnetic ion exchange (MIEX®) was selected as the studied IX resin and its characteristics and removal mechanisms will be discussed in Section 8.2. MIEX® was selected for three primary reasons. Firstly, it was designed for the removal of natural organic matter (NOM) from water and consequently this may influence removal of steroidal hormones through solute-solute interactions. Secondly, previous studies have indicated that micropollutants can be removed to some extent by MIEX® (e.g. Humbert et al., 2005; Schäfer et al., 2001). Thirdly, MIEX® can be retrofitted to existing water treatment plants for NOM removal therefore it can also have implications for micropollutant removal.

The purpose of this study is to determine if steroidal hormones can be removed from water using an IX-UF hybrid process. This will be achieved by considering removal as a function of membrane molecular weight cut-off (MWCO), organic matter concentration and hormone type. In the majority of studies estradiol was selected as a representative hormone as it is the most potent natural steroidal hormone and improved removal is critical. Further, estradiol contains dissociable functional groups and it is mainly negatively charged above pH 10.23. Organic matter concentration was studied to determine if solute-solute interactions influence hormone removal by IX-UF. Previous studies have indicated that the presence of NOM reduces micropollutant removal by MIEX® due to competition for resin sorption sites (Choi et al., 2007). However, as Chapter 5 indicated estradiol can interact with NOM through hydrogen bonding and this may lead to greater removal by IX-UF at high organic matter concentration.

Previously, all IX-UF hybrid processes have focused on NOM removal (e.g. Humbert et al., 2007; Son et al., 2005), rather than the removal of micropollutants. Therefore the aim of this study is to understand and enhance hormone removal using an IX-UF hybrid process.
8.2. Principles of MIEX®

8.2.1. Characteristics

MIEX® is an anionic exchange resin which consists of a magnetic core with a polymer shell (Lee et al., 2003). The polymer, polyacrylate, is macroporous and contains quaternary amide functional groups, which assist with dissolved organic matter (DOM) removal through ion exchange (Johnson and Singer, 2004). MIEX® differs from traditional IX resins due to its small size and magnetic properties. These properties were implemented to maximise DOC removal and resin reuse. The resin beads are expected to have a mean diameter of 150-180 µm which is approximately 2 to 5 times smaller than other IX resins (Kitis et al., 2007). However, Figure 3.5 in Chapter 3 shows smaller MIEX® fragments, indicating that resin break-up can occur. Due to the small size, MIEX® has a greater surface area to volume ratio compared to other resins, meaning there are more exchange sites which increases uptake kinetics, therefore more DOM can be removed (Boyer and Singer, 2005; Singer and Bilyk, 2002). Further, the magnetic core enables fast agglomeration and settling of resin particles, and this leads to high (up to 99.9%) resin recovery rates (Fearing et al., 2004).

8.2.2. Contaminant Removal Mechanisms

Uptake and desorption are the mechanisms required for ion exchange (Figure 8.1). The DOM is removed by MIEX® through ion exchange with the chloride ions at the active sites on the surface. Quaternary amide moieties act as DOM chloride exchange sites (Wert et al., 2005). The presence of negatively charged carboxylic groups in DOM enables removal as they are attracted to the active sites (Wert et al., 2005). The resin is not limited to the removal of DOM. MIEX® also has the ability to remove other anions including nitrate, sulphate, bromide, chromium and arsenic (Humbert et al., 2005; Jha et al., 2006). The removal efficiency of these compounds will depend on anion competition for exchange sites.
The resin is regenerated through desorption (Figure 8.1), which is a reversal of the uptake process. Desorption is achieved with the assistance of a 10% w/w NaCl brine. Through a reverse of the ion exchange process, the DOM sorbed to the quaternary amide active sites are substituted for chloride ions, and the exchanged DOM goes to the brine (Kitis et al., 2007). This process occurs due to the high chloride concentration, as the chloride ions have a strong affinity for the exchange sites and force the DOM from the resin (Slunjski et al., 2000). Depending on water quality 2% w/w NaOH can be added to assist regeneration, as the increase in pH increases the solubility of DOM (Slunjski et al., 2000). Conventional IX resins can become fouled by DOM, and thus cannot be reused effectively. Therefore, the ability to regenerate is advantageous.

Figure 8.1: MIEX® uptake and desorption chemistry with NR\textsuperscript{+}\textsubscript{3} representing the quaternary amide exchange sites (Adapted from Slunjski et al., 2000)

In addition, uncharged micropollutants can be removed by absorption to the MIEX\textsuperscript{©} polyacrylate coating through specific and non-specific sorption mechanisms (Tan and Kilduff, 2007). The removal of atrazine and isoproturon using MIEX\textsuperscript{©} was studied by Humbert et al. (2008; 2005). The results demonstrated only 7% of atrazine and 5% of isoproturon could be removed using MIEX\textsuperscript{©} after a contact time of 30 minutes (Humbert et al., 2005). By increasing the contact time to 24 hours slightly higher (approximately 12%) removal was observed (Humbert et al., 2008). The poor performance was attributed to the non-ionic nature of the pesticides. Further, Mastrup and Schäfer (2001) and Schäfer et al. (2001) studied estrone removal by MIEX\textsuperscript{©} and found removal was influenced by pH, temperature and ionic
strength. This study found approximately 40% removal of estrone by MIEX® from pH 3 to 10 when estrone was neutrally charged, while above pH 10 when estrone was in an ionic form removal increased to approximately 65%. This suggests that while ion exchange interactions are important for estrone removal by MIEX®, estrone must still interact with MIEX® through either specific (e.g. hydrogen bonding) or non-specific (e.g. van der Waals forces) interactions as 30% is still removed below pH 11. The proposed interaction mechanisms for estradiol are shown in Figure 8.2.

![Figure 8.2: The primary mechanisms of estradiol removal by MIEX® including a) specific and non-specific sorption and b) ion exchange adapted from Li and SenGupta (2004). The resin structure was adapted from Hubicka and Kolodynska (2001). Bipolar (*) and hydrogen acceptor (#) groups capable of hydrogen bonding are indicated.](image)

8.3. MIEX® Sorption Kinetics

In order to determine a suitable contact time for the IX-UF hybrid process equilibrium between the studied steroidal hormones and MIEX® was determined. In all experiments a MIEX® concentration of 10 mL/L was used as previous studies have suggested that this concentration is optimal for NOM and inorganic anion
removal (Humbert et al., 2005; Kitis et al., 2007). The methodology used was outlined in Section 3.9.4 of Chapter 3. The results indicated that after 1 hour all hormones were at equilibrium (Figure 8.3), consequently a contact time of 1 hour was used for all experiments. Industrial applications of MIEX® for water treatment typically apply contact times of 10 to 30 minutes (Morran et al., 2004; Slunjski et al., 2000), however, other studies have suggested that a contact time of 1 hour is more suitable for optimum contaminant removal (Fearing et al., 2004).

Figure 8.3: Sorption (%) of a) estradiol; b) estrone; c) progesterone; and d) testosterone to MIEX® at pH 8 and 11 (1 mM NaHCO₃, 20 mM NaCl, 100 ng/L hormone concentration, 10 mL/L MIEX®)

Figure 8.3 also indicates greater removal of estradiol and estrone at pH 11 when they were negatively charged compared to pH 8 when they were neutral. This is because estradiol and estrone could interact with MIEX® through ion exchange at pH 11. No difference was observed for progesterone and testosterone at pH 11 as they lacked
dissociable functional groups. The charge of both the micropollutant and MIEX® can have an influence on sorption. Robberson et al. (2006) compared the sorption of a pharmaceutical, nalidixic acid, to neutral and anion-exchange polymers in pH solutions adjusted above and below the acid dissociation constant (pKₐ) of nalidixic acid, which was 5.9. This study indicated that greater sorption of charged nalidixic acid to the anion-exchange polymer occurred due to ion exchange, while the neutrally charged nalidixic acid sorbed strongly to the neutral polymer. These findings confirmed the results of our study as a greater mass of negatively charged estradiol and estrone was sorbed to MIEX® at pH 11. Further confirming the influence of both the charge of the micropollutant and polymer, Section 4.5 in Chapter 4 indicated up to one order of magnitude less sorption of negatively charged estradiol to neutral polyacrylate fibre due to reduced hydrogen bonding ability.

8.4. Removal of Estradiol by IX-UF

8.4.1. Estradiol Removal

The removal of estradiol by IX-UF was studied as a function of membrane MWCO (1 to 100 kDa). The purpose of this section was to assess the removal mechanisms of estradiol by the IX-UF process and to determine the influence of membrane MWCO on estradiol removal. However, before removal by IX-UF could be considered, it was important to assess removal of estradiol by MIEX® and UF separately.

Figure 8.3a indicates that MIEX® could remove around 30% of estradiol from solution at pH 8, while approximately 65% of estradiol could be removed at pH 11. At pH 11 estradiol was 85% dissociated, therefore it could interact with MIEX® through ion exchange (Figure 8.2), leading to greater removal compared to pH 8 when estradiol was neutrally charged and could only absorb to the polyacrylate skeleton of MIEX® through specific and non-specific interactions. The significant removal of estradiol at pH 8 suggests that uptake was due to hydrogen bonding with polyacrylate. Both estradiol and polyacrylate contain functional groups capable of
hydrogen bonding (Figure 8.2), therefore this removal mechanism is indeed possible. Schäfer et al. (2001) observed similar removal results for estrone at pH 8 and 11.

Estradiol retention by 1 to 100 kDa hydrophilic UF membranes at pH 8 was studied in Section 7.6.1 of Chapter 7. In the absence of organic matter estradiol retention was low and varied from 21-31% with decreasing membrane MWCO. The low removal was due to the small molecular weight of estradiol (278 g/mol), the absence of organic matter and the use of a hydrophilic membrane which reduced estradiol sorption.

Estradiol removal by IX-UF as a function of membrane MWCO is shown in Figure 8.4. Removal of estradiol was lowest for 1 kDa membranes with 66-68% removal. However, estradiol removal increased to approximately 90% for 5 to 100 kDa membranes. Despite the different removal mechanisms, pH had a minimal influence on removal. Compared to MIEX® and UF alone the IX-UF hybrid process could remove more estradiol from solution (Figure 8.5). In industrial applications the contact time and MIEX® dose can depend on the water quality, with the doses used typically ranging from 2 to 20 mL/L (Morran et al., 2004) with a contact time of approximately 10 to 30 minutes, as mentioned in Section 8.2. Therefore, due to the longer contact time used in the present study (1 hour), slightly lower estradiol removal by industrial MIEX® treatment processes could be expected.
Figure 8.4: Estradiol removal (%) by IX-UF as function of MWCO at pH 8 and 11 (1 mM NaHCO₃, 20 mM NaCl, 100 ng/L estradiol, 10 mL/L MIEX®)

Figure 8.5: Summary of estradiol removal efficiency by MIEX® alone, UF alone and IX-UF hybrid process

The high estradiol removal by 5 to 100 kDa membranes was an unexpected result. Estradiol was smaller than the average 1 kDa membrane pore diameter (0.8 nm versus 1.6 nm calculated using by Equation 3.1 in Chapter 3) and the regenerated cellulose active layer was hydrophilic (contact angle 26° (Pieracci et al., 1999)), consequently adsorption to the membrane was not significant (see Figure 7.23 in Chapter 7). Further, no difference in removal was observed for the different pH conditions, therefore increased estradiol removal by large MWCO membranes using IX-UF may be related to solute-foulant interactions.
8.4.2. Solute-Foulant Interactions

Flux ratio \((J/J_0)\) as a function of membrane MWCO is shown in Figure 8.6. Flux decline was most significant for the 100 kDa membrane with up to 50% decrease in flux observed. Fouling was also observed for 10 and 30 kDa membranes, however, this was generally less significant. In the absence of MIEX® no flux decline was observed. Son et al. (2005) and Humbert et al. (2007) also observed a significant flux decline during the removal of NOM using MIEX® and a 100 kDa membrane. Reduced membrane flux in the presence of particulate matter can generally be attributed to either accumulation of particles within the pores or deposition of particles on the membrane surface to form a cake layer (Wiesner and Aptel, 1996). Kim et al. (1993) studied fouling of UF membranes with silver particles and found pore blocking predominantly occurred when the pore diameter of the membrane was within the same order of magnitude as the particle diameter. Figure 8.7 indicates that the majority of MIEX® fragments had a diameter less than 20 µm which is considerably larger than the pore diameter of the 100 kDa membrane (18.2 nm calculated by Equation 3.1 in Chapter 3). Therefore, it was unlikely that pore blocking was a significant cause of flux decline.

![Figure 8.6: Flux ratio \((J/J_0)\) as a function of MWCO during the removal of estradiol by IX-UF at a) pH 8; and b) pH 11 (1 mM NaHCO₃, 20 mM NaCl, 100 ng/L estradiol, 10 mL/L MIEX®)
To better understand membrane fouling by the IX-UF hybrid process hydraulic membrane resistance was considered. Hydraulic resistance, $R_M$ (m), represents the intrinsic resistance of a clean membrane and can be calculated using Equation 8.1 which is based on Darcy’s Law where:

$$R_M = \frac{\Delta P}{\eta J}$$

(8.1)

$\Delta P$ was the pressure (bar)

$\eta$ was the dynamic viscosity (Pa.s)

$J$ was flux (L/m²·h)

To assess fouling, the total membrane resistance ($R_T$) after the experiment was determined also using Equation 8.1. As $R_T$ can be considered the sum of $R_M$ and the hydraulic resistance due to fouling ($R_F$) (Waite et al., 1999), $R_F$ could be determined using Equation 8.2.
Membrane resistance is shown in Table 8.1 for all studied MWCO membranes at pH 8 and 11. Membrane hydraulic resistance due to fouling, indicated by $R_F$, was observed for 5 to 100 kDa membranes at both studied pH values, while negative $R_F$ values were observed for 1 and 3 kDa membranes suggesting fouling did not occur. Bacchin \textit{et al.} (1996) suggested that $R_F$ is proportional to particle deposition on the membrane, which indicates that MIEX® deposited on the surface of 5 to 100 kDa membranes leading to high removal (90%) of estradiol.

Table 8.1: The clean membrane hydraulic resistance ($R_M$), total membrane hydraulic resistance ($R_T$) and fouling hydraulic resistance ($R_F$) in the presence of 10 mL/L MIEX® as a function of MWCO

<table>
<thead>
<tr>
<th></th>
<th>pH 8</th>
<th></th>
<th>pH 11</th>
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<tbody>
<tr>
<td></td>
<td>$R_M$</td>
<td>$R_F$</td>
<td>$R_M$</td>
</tr>
<tr>
<td>1 kDa</td>
<td>8229.2</td>
<td>7924.1</td>
<td>-305.1</td>
</tr>
<tr>
<td>3 kDa</td>
<td>5714.0</td>
<td>5560.7</td>
<td>-153.3</td>
</tr>
<tr>
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<td>39.1</td>
</tr>
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</tr>
<tr>
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<td>36.0</td>
</tr>
<tr>
<td>100 kDa</td>
<td>41.9</td>
<td>80.6</td>
<td>38.7</td>
</tr>
</tbody>
</table>

The mass of MIEX® deposited on the membrane can be estimated by considering the specific resistance of the deposit on the membrane, $\alpha$ (m/kg). The Carman–Kozeny equation (Equation 8.3) can be applied to determine $\alpha$ where:

$$\alpha = \frac{180(1 - \varepsilon)}{\rho_p d_p^2 \varepsilon^3}$$  \hspace{1cm} (8.3)

$\varepsilon$ was the porosity (-)

$\rho_p$ was MIEX® density (kg/m$^3$)

$d_p$ was the average MIEX® particle diameter (m)
The porosity of the deposit was calculated by assuming an average MIEX® particle diameter of 42 µm and was 0.26. Therefore, \( \alpha \) was \( 4.5 \times 10^{11} \) m/kg. With the specific resistance known it was possible to calculate the mass of MIEX® deposited on the membrane using Equation 8.4 where \( M_P \) was mass of MIEX® deposited (kg) and \( A \) was membrane area (m²). The mass deposited is shown in Table 8.2. The total mass of MIEX® in the experiment was 3.6 g. Table 8.2 indicates a greater mass deposited on the membrane at pH 8 compared to 11. The influence of pH on fouling will be discussed further in Section 8.4.3. For the 10 kDa membrane at pH 8 the predicted mass deposited (11.1 mg) was greater than the total MIEX® mass. This error can be related to the assumptions applied to calculate \( M_P \) including average particle size, particle packing and density of solution containing MIEX®. Consequently, these values should only be considered as an estimate of MIEX® membrane deposition.

\[
M_P = \frac{AR_F}{\alpha} \tag{8.4}
\]

Table 8.2: Mass of MIEX® deposited (MP) on the membrane in the presence of 10 mL/L MIEX® as a function of MWCO

<table>
<thead>
<tr>
<th></th>
<th>pH 8</th>
<th></th>
<th>pH 11</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( M_P ) (kg)</td>
<td>( M_P ) (g)</td>
<td>( M_P ) (kg)</td>
</tr>
<tr>
<td>5 kDa</td>
<td>( 3.4 \times 10^{-2} )</td>
<td>3.4</td>
<td>( 7.0 \times 10^{-3} )</td>
</tr>
<tr>
<td>10 kDa</td>
<td>( 1.1 \times 10^{-2} )</td>
<td>11.1</td>
<td>( 2.5 \times 10^{-3} )</td>
</tr>
<tr>
<td>30 kDa</td>
<td>( 3.0 \times 10^{-3} )</td>
<td>3.0</td>
<td>( 1.6 \times 10^{-3} )</td>
</tr>
<tr>
<td>100 kDa</td>
<td>( 3.3 \times 10^{-3} )</td>
<td>3.3</td>
<td>( 8.0 \times 10^{-4} )</td>
</tr>
</tbody>
</table>

To determine if pore blocking also contributed to fouling focused ion beam scanning electron microscopy (FIB-SEM) coupled with energy dispersive X-ray (EDX) was applied. An FIB-SEM cross-sectioned image of the 100 kDa membrane is shown in Figure 8.8a. The 100 kDa membrane was selected as it was the most likely to be influenced by pore blocking. Using EDX the elemental composition of the membrane surface deposit (Spectrum 1) and membrane material (Spectra 2 and 3) was analysed. MIEX® has an iron (Fe) oxide core, therefore its presence can be detected using EDX. Fe was only detected in Spectrum 1 (Figure 8.8b), but not in Spectra 2 and 3 (Figure 8.8c and d) suggesting that MIEX® did not penetrate into the
membrane, but deposited on the surface. The thickness of the deposits on the 100 kDa membrane ranged from 25 to 37 µm. Consequently, it can be concluded that fouling was mainly due to cake layer formation.

Figure 8.8: a) A cross-section of a fouled 100 kDa membrane imaged using FIB-SEM. Elemental analysis of the membrane surface and supporting material was conducted using EDX and is shown in a) Spectrum 1, b) Spectrum 2 and c) Spectrum 3 (1 mM NaHCO₃ 20 mM NaCl, pH 8, 10 mL/L MIEX®)
8.4.3. Influence of pH on Solute-Foulant Interactions

While the pH appeared to have little influence on estradiol removal by the IX-UF hybrid process, flux decline was more significant at pH 8 for 10, 30 and 100 kDa membranes compared to pH 11 (Figure 8.6). At the studied pH values the charge of all membranes used was negative, with the charge varying between -7.5 and -17.3 mV at pH 8 (Schäfer, 2001). As pH increased the zeta potential (mV) of the regenerate cellulose membranes becomes more negative (Schäfer et al., 2002), therefore at pH 11 the membranes were expected to have a greater negative charge compared to pH 8. The charge of the MIEX® polymer was positive due to the quaternary amide functional group, and was not significantly affected by pH. Zeta potential measurements indicated the charge was 29.4 mV at pH 8 and decreased to 25.8 mV at pH 11, however, this difference was not significant (Figure 8.9). The core of MIEX® was composed of iron oxide, and previous studies have indicated that the charge and aggregation properties of iron oxide particles can be influenced by pH (Schäfer et al., 2000; Waite et al., 1999). In fact, Waite et al. (1999) studied the impact of iron oxide aggregation on UF as a function of pH and found that at high pH flux decline was reduced due to rapid aggregation of the iron oxide particles. This was attributed to a decrease in zeta potential with increasing pH. Therefore, reduced flux decline at high pH may be related to the changing membrane charge, as well as the altered iron oxide properties.

![Figure 8.9: Zeta potential (mV) of MIEX® at pH 8 and 11 (1 mM NaHCO₃, 20 mM NaCl, 10 mL/L MIEX*)](image_url)

*Figure 8.9: Zeta potential (mV) of MIEX® at pH 8 and 11 (1 mM NaHCO₃, 20 mM NaCl, 10 mL/L MIEX*)*
The concentration of estradiol as a function of permeate volume is shown in Figure 8.10a and b for all MWCO membranes. At pH 11 the concentration of estradiol in the permeate exceeded the average MIEX® feed concentration (39 ng/L) after approximately 100-150 mL for both the 1 and 3 kDa membranes. The MIEX® feed concentration was considerably lower than the feed concentration prior to MIEX® addition (100 ng/L) as estradiol can interact with MIEX® through ion exchange at pH 11. However, for the 1 and 3 kDa membrane experiments the pH was not stable and decreased from pH 11 to approximately pH 8-9. The pH was stable for 5 to 100 kDa membranes. The pH instability for 1 and 3 kDa membranes may be related to the higher pressure (5 bar) and longer experimental time, as well as the weak buffer used, NaHCO₃. This will be discussed further in Appendix 1. As the pH decreased estradiol gained a hydrogen ion and ceased to be negatively charged, therefore it desorbed from MIEX®, leading to a higher concentration of estradiol in the permeate compared to the MIEX® feed. Despite desorption from MIEX®, estradiol removal was not negative, as this was calculated based on the feed concentration prior to MIEX® addition (Equations 3.12 and 3.13 in Chapter 3). However, estradiol removal by IX-UF for the 1 and 3 kDa membranes at pH 11 was underestimated as the pH change rendered the ion exchange removal mechanism ineffective.
Figure 8.10: Estradiol concentration (ng/L) in permeate as a function of volume for all MWCO membranes at a) pH 8; and b) pH 11. The estradiol concentration prior to MIEX® addition was 100 ng/L (1 mM NaHCO₃, 20 mM NaCl, 10 mL/L MIEX®).

This study has suggested that the IX-UF hybrid process can be used to remove up to 90% of estradiol from solution by 5 to 100 kDa membranes due to MIEX® deposition. However, due to limited MIEX® deposition for the 1 and 3 kDa membranes estradiol removal was reduced (62-75%). Furthermore, instability at pH 11 may limit estradiol removal by 1 and 3 kDa membranes as the ion exchange mechanism becomes ineffective as the pH decreases.
8.5. Removal of Estradiol in the Presence of NOM by IX-UF

In this section the removal of estradiol in the presence of NOM by the IX-UF hybrid process was studied. As MIEX® was developed for removing DOM from drinking water a NOM isolated from surface water was selected instead of the commercial organic matter types used in Chapter 7. The characteristics of the studied NOM are shown in Table 3.1 in Chapter 3. Chapter 5 indicated that estradiol sorbs to NOM surrogates through hydrogen bonding, therefore greater removal of estradiol by IX-UF may be observed in the presence of organic matter. Further, Chapter 7 suggested that organic matter concentration is the limiting solute in the studied solute-solute interactions, therefore NOM concentration from 12.5 to 125 mgC/L was studied as this is expected to influence estradiol removal by IX-UF.

Figure 8.11 indicated that estradiol removal gradually decreased as the NOM concentration increased. This was observed for both pH 8 and 11. The decrease in estradiol removal as a function of NOM concentration suggests that MIEX® preferentially sorbs NOM over estradiol. NOM contains many negatively charged functional groups and therefore is in an anionic form at both pH 8 and 11. Therefore, it appears that solute-solute interactions do not increase estradiol removal. This may be due to the weak sorption of estradiol to NOM observed in Chapter 5.
NOM removal by MIEX® and UF alone at 12.5 mgC/L concentration was studied. Approximately 32-45% of NOM could be removed by MIEX® alone, while only 26% could be removed using UF alone. The low removal by the 10 kDa membrane was due to the molecular weight of the studied NOM (1381 g/mol) which has a molecular diameter approximately 3 times smaller than the pore diameter of the membrane (1.9 nm vs. 5.4 nm calculated by Equation 3.1 in Chapter 3). Similar retention was observed by Schäfer et al. (2002). When MIEX® and a 10 kDa membrane were combined approximately 70% of NOM could be retained. Further, greater removal of UV absorbing compounds (indicated by specific UV absorbance (SUVA)) by IX-UF (87%) compared to UF (11%) alone was also observed. The greater removal by IX-UF was due to the presence of MIEX® as previous studies have indicated it has a strong affinity for UV absorbing organic matter fractions (Boyer and Singer, 2008; Humbert et al., 2005).

Estradiol only interacts with MIEX® via ion exchange at pH 11. At pH 11 estradiol removal without NOM was 94%, however, this decreased to approximately 64% removal in the presence of 125 mgC/L NOM. The ion exchange sites on MIEX®
become limited at such high NOM concentrations, and therefore it appeared that NOM out-competed estradiol. This was due to the considerably higher concentration of NOM (12.5-125 mgC/L) compared to estradiol (100 ng/L).

At pH 8, estradiol interacted with MIEX® through absorption mechanisms including hydrogen bonding and van der Waals interactions. Despite the different mechanisms of interaction, the presence of NOM led to a decrease in removal, from 92% removal of estradiol alone to 66% removal with 125 mgC/L NOM at pH 8. NOM is still negatively charged at pH 8 and can be removed by MIEX® by ion exchange. MIEX® has a macroporous structure and the larger NOM molecules (1381 g/mol compared to 272 g/mol for estradiol) may hinder estradiol from entering the MIEX® pores (Humbert et al., 2008), and consequently reduce estradiol sorption to the resin.

Increasing organic matter concentration also has implications for flux ratio. Figure 8.12 indicates that the addition of NOM reduced flux decline. Similar changes in fouling were observed by Waite et al. (1999) who found reduced flux decline by iron oxide particles in the presence of fulvic acid. It was suggested that the presence of organic matter can alter particle aggregation subsequently influencing fouling. As the NOM concentration increased to 125 mgC/L flux decline also increased slightly. This was most likely due to deposition of NOM on the membrane at high organic matter concentrations.
Figure 8.12: Flux ratio \( (J/J_0) \) as a function of organic matter concentration during the removal of estradiol by IX-UF at a) pH 8; and b) pH 11 (1 mM NaHCO₃, 20 mM NaCl, 100 ng/L estradiol, NOM concentrations 0, 12.5, 25, 50, 125 mg/L)

8.6. Influence of Hormone Type on Removal by IX-UF

As discussed in Chapter 1, many natural hormones were detected in wastewater effluent, as well as in surface waters. While estrone, progesterone and testosterone share many similarities with estradiol, they contain different functional groups (refer to Table 3.3 in Chapter 3) which has implications for their dissociation, hydrophobicity and hydrogen bonding ability. This can therefore alter their fate and behaviour within the aquatic environment. To understand how hormone properties influence removal by the IX-UF hybrid process removal was studied with 1, 10 and 100 kDa membranes. Both Figure 8.13a and b indicated significant removal (~90%) of all hormones by 10 and 100 kDa membranes. Similar to discussed above, this may be related to the deposition of MIEX® in a cake layer on the surface of the membrane which increased removal of the hormones. For 1 kDa membranes hormone removal was significantly lower and ranged from to 42-74%. This trend was observed for both pH 8 and 11.
Due to high hormone removal by 10 and 100 kDa membranes hormone type did not have a significant influence on removal. In contrast, for 1 kDa membranes greater removal of progesterone was observed at both pH 8 and 11 compared to the other hormones, while removal was lowest for testosterone. For 1 kDa membranes the influence of solute-foulant interactions was minimal (Figure 8.6) and removal was related to sorption to the membrane and MIEX®. Progesterone contains strong hydrogen accepting ketone functional groups, with Le Questel *et al.* (2000) suggesting that the ketone group in the C-20 position is a triple hydrogen acceptor. Further, progesterone sorbed strongly to regenerate cellulose membranes (see Figure 7.13 in Chapter 7).
In contrast, negligible sorption of testosterone to regenerated cellulose was observed in Figure 7.23 in Chapter 7. Less than 20% of testosterone was removed by MIEX® alone (Figure 8.3). As discussed in Chapter 5, functional groups in the C-17 position play a major role in molecular interactions such as hydrogen bonding compared to the C-3 groups (Rachkov et al., 1998). Due to bipolarity, the hydroxyl group present in the C-17 position of testosterone was more polar than the monopolar ketone moiety present in estrone and progesterone (C-20). As a result testosterone was more likely to remain in the aqueous phase as opposed to partitioning to MIEX® or the membrane.

Similar to estradiol, estrone was negatively charged at pH 11 (pKₐ of 10.34) therefore it could interact with the resin through ion exchange. However, like estradiol there was no significant difference in estrone removal at pH 8 and 11. Again, it was likely that this was due to changing pH conditions during the 1 kDa membrane experiment which led to a final pH between 8 and 9. Progesterone and testosterone do not dissociate, therefore can only interact with MIEX® through specific and non-specific absorption. As a result there was little difference in removal of progesterone and testosterone as a function of pH. Consequently, it appears that the ion exchange sorption mechanism had little influence on micropollutant removal by IX-UF hybrid processes.

8.7. Conclusion

Improved removal of steroidal hormone removal from wastewater effluent is of great importance. The results from this study of the application of an IX-UF hybrid process are promising with approximately 90% hormone removal by 5 to 100 kDa membranes and 10 mL/L MIEX® in 350 mL of solution. The high removal was attributed to deposition of MIEX® on the membrane.

The presence of high NOM concentrations led to significantly reduced estradiol removal. Due to the negative charge of NOM at both pH 8 and 11 it was likely that MIEX® preferentially sorbed NOM over estradiol. This was also observed at pH 8.
when estradiol was neutral and interacted with MIEX® through specific and non-specific sorption. Therefore, it is likely that the greater concentration and molecular weight of NOM compared to estradiol reduced sorption.

Further, the type and position of polar functional groups present in the hormones influenced removal by IX-UF, with the greatest removal of progesterone being due to strong hydrogen accepting functional groups in the C-20 position. This was only observed for 1 kDa membranes, as significant fouling for 10 and 100 kDa membranes meant that there was no difference in removal as a function of hormone type.

8.8. Recommendations for Future Studies

This study indicated that significant removal of steroidal hormones using an IX-UF hybrid system is possible, however, further investigations are required in terms of treatment of brines containing micropollutants and desorption. Following the IX-UF hybrid process, MIEX® can contain a high concentration of micropollutants. As discussed in Section 8.2.2 the resin can be reused for future experiments by recharging with NaCl brine. Regeneration efficiency is typically high, with Jha et al. (2006) observing 75-94% regeneration of MIEX® for inorganic anions such as arsenic. As the brine now contains a high concentration of micropollutants disposal can be difficult and can have significant environmental implications if an unsuitable disposal method is applied. Therefore, further research is required on the treatment and disposal of such brines. Further, the influent water quality may have implications for removal efficiency, with potentially reduced micropollutant removal in solutions with high ionic strength due to desorption. Therefore, more research is required on the implications of water quality on hormone removal by IX-UF hybrid processes.
9 Implications of Solute-Solute Interactions in the Wider Research Environment

In this chapter hormone sorption to solid-phase microextraction (SPME) fibres, ultrafiltration (UF) membranes and magnetic ion exchange (MIEX®) resin will be compared and the mechanisms of sorption will be considered. Further, the influence of solute-solute interactions on the fate of steroidal hormones during wastewater treatment and in the aquatic environment will be considered.

The results indicate that all hormones sorbed strongly to SPME fibre compared to UF membranes and MIEX®, with sorption 1 to 2 orders of magnitude greater. This was due to the polyacrylate coating of the SPME fibre which enhances micropollutant partitioning. The sorption of estrone to UF regenerated cellulose membranes was compared to other membrane polymers from the literature. The membrane-water partition coefficients \( K_{MW} \) calculated from the sorption isotherms were similar for regenerated cellulose and polyamide, suggesting that the membrane properties such as hydrogen bond ability influenced sorption, as well as pore size.

The removal of steroidal hormones during wastewater treatment is variable, with a number of removal pathways identified including sorption to biomass, degradation and volatilisation. Using mass balance calculations and fugacity models the fate of estradiol and estrone during wastewater treatment can be predicted. However, the predicted removal results are inconsistent suggesting that parameters of the individual treatment plants such as sludge concentration and retention time as well as influent properties will influence hormone removal. Finally, the influence of organic matter on removal pathways was considered, and it was suggested that sorption to dissolved organic matter (DOM) during treatment can increase hormone concentration in the effluent by reducing degradation and decreasing sorption to sludge.
Finally, the implications of solute-solute interactions on hormone bioavailability in the aquatic environment will be discussed. Studies have found that the presence of DOM can decrease the bioavailability of micropollutants, consequently reduced bioaccumulation of strongly sorbing hormones such as estrone in aquatic organisms is expected, and has been predicted using fugacity-based food web modelling.

Therefore, this chapter indicates the importance of solute-solute interactions during wastewater treatment, as well as in the aquatic environment, indicating why it is necessary to consider these interactions in the wider research context.
9.1. Introduction

Throughout this project solute-solute interactions have been considered at a laboratory scale with a focus on quantification techniques. The purpose of this chapter is to emphasise the importance of solute-solute interactions in the wider research environment by considering hormone fate during wastewater treatment. Wastewater was considered over drinking water treatment due to the abundant concentration of micropollutants and organic matter, consequently solute-solute interactions are expected to influence hormone removal pathways. Further, the consequences of solute-solute interactions in the natural environment, specifically bioavailability and bioaccumulation, will be considered. During this project the sorption of steroidal hormones to a number of different materials including solid-phase microextraction (SPME) fibre, ultrafiltration (UF) membranes and magnetic ion exchange (MIEX®) resin were studied. Therefore, sorption will be compared by considering polymer type and pH.

9.2. Steroidal Hormone Removal

9.2.1. Hormone Sorption Comparison

Within this project different polymers were applied to remove hormones from solution including polyacrylate (PA) and regenerated cellulose. Therefore, the difference in sorption ability of PA fibre used for SPME, regenerated cellulose UF membranes and MIEX®, which has a PA skeleton, was compared (Figure 9.1). Sorption was compared in units of mass of hormone (ng) sorbed to the polymer volume (cm³). The MIEX® beads varied in diameter from approximately 0.5 to 200 μm, therefore an average diameter, which was 42 μm (determined from Figure 8.7 in Chapter 8), was used for the volume calculations. In all experiments the hormone concentration was 100 ng/L, however, the mass of hormone in solution for 10 ng in 100 mL for SPME and MIEX® experiments, and 40 ng in 400 mL for the UF experiments.
Figure 9.1: Sorption of estradiol, estrone, progesterone and testosterone to PA fibre, ultrafiltration membrane and MIEX® (1 mM NaHCO₃, 20 mM NaCl, pH 8, 100 ng/L hormone)

With the exception of testosterone, hormone sorption by PA fibre was approximately 2 orders of magnitude greater than sorption by UF membranes or MIEX®. Testosterone sorption was around 1 order of magnitude different for PA fibre. The strongest sorption by PA fibre was observed for estrone and progesterone and was due to strong hydrogen bonding with the fibre. Based on the chemical structure of both PA and regenerated cellulose hydrogen bonding with steroidal hormones is possible (Figure 9.2). PA can contain a monopolar ketone and bipolar amine groups, while regenerated cellulose contains bipolar hydroxyl groups. Both the amide and ketone groups are stronger hydrogen acceptors than hydroxyl groups allowing for greater sorption. However, it was likely that the greater sorption of hormone by the PA fibre was related to its absorbent coating. The coating has a volume of 0.77 µL and this was used to calculate sorption in Figure 9.1. As described in Chapter 4, the PA coating of SPME fibre acts a liquid allowing micropollutants to partition into the polymer instead of adsorbing to the surface and this enables greater sorption of steroidal hormones (Lord and Pawliszyn, 2000).
As PA fibre and MIEX® were both composed of a PA polymer it was assumed that hormone sorption would be similar. However, as Figure 9.1 indicates this was not the case. Therefore, the sorption of steroidal hormones by MIEX® and PA fibre at pH 8 and 11 were compared (Table 9.1). For estradiol and estrone sorption to the PA fibre decreases slightly at pH 11, while sorption to MIEX® increases. This is due to the changing charge of estradiol and estrone as discussed in Section 8.3 of Chapter 8. No significant change was observed for progesterone and testosterone as they lack dissociable functional groups. Further, the PA polymer used for MIEX® contains approximately 25% iron oxide (National Industrial Chemicals Notification and Assessment Scheme, 1997) therefore it is less pure than the PA fibre, and this may have reduced sorption. In addition, it is possible unknown additives were included in PA fibre which may enhance sorption.

**Figure 9.2: Hydrogen bonding of estrone with a) PA resin and b) regenerated cellulose (PA structure adapted from Hubicka and Kolodynska (2001)). Bipolar (*) and hydrogen acceptor (#) groups capable of hydrogen bonding are indicated.**
Table 9.1: Sorption of steroidal hormones by PA fibre and MIEX® at pH 8 and 11 (1 mM NaHCO₃ 20 mM NaCl, 100 ng/L estrone)

<table>
<thead>
<tr>
<th></th>
<th>PA Fibre (ng/cm³)</th>
<th>MIEX® (ng/cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol pH 8</td>
<td>510.05</td>
<td>5.54</td>
</tr>
<tr>
<td>Estradiol pH 11</td>
<td>134.46</td>
<td>12.23</td>
</tr>
<tr>
<td>Estrone pH 8</td>
<td>710.29</td>
<td>3.37</td>
</tr>
<tr>
<td>Estrone pH 11</td>
<td>75.47</td>
<td>10.44</td>
</tr>
<tr>
<td>Progesterone pH 8</td>
<td>559.79</td>
<td>7.77</td>
</tr>
<tr>
<td>Progesterone pH 11</td>
<td>549.83</td>
<td>4.37</td>
</tr>
<tr>
<td>Testosterone pH 8</td>
<td>74.03</td>
<td>2.87</td>
</tr>
<tr>
<td>Testosterone pH 11</td>
<td>65.71</td>
<td>1.88</td>
</tr>
</tbody>
</table>

9.2.2. Sorption to Other Membrane Polymers

Estrone sorption isotherms for three different types of membrane polymers, regenerated cellulose, polyamide and polyamide-urea, are shown in Figure 9.3 in ng/cm². Estrone sorption was similar for regenerated cellulose (PLAC (1 kDa)) and polyamide (TFC-SR2), but sorption slightly was lower for polyamide-urea (X-20). Despite the different polymers, all active layers of all membranes were hydrophilic and had contact angles ranging from 26 to 33°C (Nghiem, 2005; Pieracci et al., 1999). However, the support layer of TFC-SR2 is polysulphone which is hydrophobic. Further, the charge of all membranes was negative at pH 8 (Nghiem, 2005; Schäfer et al., 2002). Nghiem (2005) also indicated that estrone can hydrogen bond with polyamide through ketone and amine functional groups. Membrane-water partition coefficients, $K_M$ (L/cm²) were calculated using Equation 9.1 where $C_W$ was freely dissolved micropollutant concentration (ng/L) and $C_S$ was the amount sorbed specific to surface area (ng/cm²).

$$K_M = \frac{C_S}{C_W}$$ (9.1)

$K_M$ values for PLAC and TFC-SR2 were similar with $8.5\times10^{-4}$ and $7.8\times10^{-4}$ L/cm², while less estrone partitioned to X-20 with a $K_M$ value of $3.5\times10^{-4}$ L/cm². Nghiem and Schäfer (2002) suggested lower adsorption of estrone to X-20 compared to TFC-
SR2 was due to lack of diffusion into the membrane. The pore size of X-20 is smaller than the estrone molecule, thus estrone can only adsorb to the surface but not penetrate into the membrane pores. As TFC-SR2 is a loose NF membrane it has a similar pore diameter to the 1 kDa PLAC membrane (1.3 nm vs. 1.6 nm). While sorption to the support layer is possible, Nghiem and Schäfer (2002) suggested that the majority of sorption occurs to the active layer. As both PLAC and TFC-SR2 can interact with estrone through hydrogen bonding this may contribute to similar sorption.

Figure 9.3: Estrone sorption isotherms for regenerate cellulose (PLAC), polyamide (TFC-SR2) (Nghiem and Schäfer, 2002) and polyamide-urea (X-20) (Nghiem and Schäfer, 2002) membranes (1 mM NaHCO₃ 20 mM NaCl)

Therefore, of all the different polymers studied in this project, PA SPME fibre exhibits the greatest sorption of steroidal hormones. This was related to its polymer coating which allowed for greater partitioning. Further, for all polymers hydrogen bonding facilitated hormone sorption.
9.3. Fate of Steroidal Hormones during Wastewater Treatment

9.3.1. Hormone Removal

In this section the fate of steroidal hormones during wastewater treatment will be considered, with particular emphasis on the implications of solute-solute interactions. Currently, the dominant type of wastewater treatment is conventional treatment. This typically applies primary clarification, followed by biological treatment such as activated sludge to degrade organic matter and micropollutants. This can also be followed by tertiary treatment such as filtration or nutrient removal. The removal of steroidal hormones during conventional wastewater treatment is variable with removal between 59-100% for estradiol and 14-85% for estrone (Baronti et al., 2000; Braga et al., 2005b; Esperanza et al., 2007; Johnson et al., 2000). Further, the initial influent hormone concentration can increase during treatment (Anderson et al. 2003; Joss et al. 2004). This is because estradiol and estrone are typically excreted from the human body in the form of inactive glucuronide or sulphonide conjugates (Larsson et al., 1999). During wastewater treatment the glucuronide or sulphonide conjugates are metabolised into the active unconjugated form by bacteria such as E. coli (Ternes et al., 1999) (Figure 9.1). Testosterone and progesterone can also be excreted as glucuronide and sulphonic conjugates (Loose-Mitchell and Stancel, 2001) and therefore may be cleaved into their active forms during wastewater treatment.
While the majority of wastewater treatment plants currently use conventional treatment, other forms of treatment processes have been studied within the literature. For example, the application of membrane bioreactors for wastewater treatment is increasing due to high organic matter removal and smaller space requirements (Judd, 2008; Koh et al., 2008; Marrot et al., 2004). Previous studies have observed between 49-98% removal of estradiol and 80-96% removal of estrone from wastewater using this technology (Hu et al., 2007; Joss et al., 2004). Advanced membrane processes such as forward osmosis and membrane distillation (Cartinella et al., 2006) and advanced oxidation processes such as ozonation (Huber et al., 2005; Ikehata et al., 2006) have also been applied to remove micropollutants from wastewater. However, the majority of studies are at the laboratory or pilot plant scale and therefore will not be considered further. As conventional treatment is the most common form of wastewater treatment today it will be the focus of this study on the fate of hormones.
9.3.2. Environmental Fate Modelling

To determine the fate of micropollutants within conventional wastewater treatment processes it is necessary to consider the different removal pathways, which include biological degradation, sorption to sludge/biomass, volatilisation and discharge in the effluent (Carballa *et al.*, 2007; Rogers, 1996). Fugacity based models can be applied to predict the concentration of a micropollutant in a particular phase by considering the different removal pathways (Mastrup *et al.*, 2005). The concept of fugacity was introduced in Chapter 2 and represents the capacity of a micropollutant to escape from a particular phase. Over the past 10 to 20 years fugacity models have been used to predict the fate of a number of micropollutants in wastewater treatment including pharmaceuticals, alkylphenols and phthalates (Clark *et al.*, 1995; Khan and Ongerth, 2004; Mastrup *et al.*, 2005; Tan *et al.*, 2007). Further, other studies have applied mass balances using experimental data to assess micropollutant fate (Carballa *et al.*, 2007; Heidler and Halden, 2008).

Predicted hormone removal to different pathways during conventional wastewater treatment is shown in Table 9.2. Volatilisation was not considered as steroidal hormones are non-volatile and typically have low Henry’s constants. The majority of the results applied experimental mass balance to predict hormone fate within wastewater treatment. This was calculated by quantifying the hormone concentration in the influent, effluent and sludge. Carballa *et al.* (2007) applied both experimental data and solid-water distribution coefficients (K_D) to predict the fate of steroidal hormone using a mass balance approach. The application of K_D values or organic matter-water partition coefficients (K_OM) prevents analytical difficulties associated with the sludge phase and allows for rapid prediction of hormone fate provided K_D or K_OM values exist for the studied micropollutant and for the specific particles and organics involved (Carballa *et al.*, 2007).
Table 9.2: Fate of estradiol and estrone within wastewater treatment predicted using mass balance and fugacity based modelling

<table>
<thead>
<tr>
<th>Micropollutant</th>
<th>% Effluent</th>
<th>% Sludge</th>
<th>% Biodegradation</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol + Estrone</td>
<td>&lt;2.00</td>
<td>11.00</td>
<td>87.00</td>
<td>a</td>
</tr>
<tr>
<td>Estradiol + Estrone</td>
<td>12.00</td>
<td>4.00</td>
<td>84.00</td>
<td>b</td>
</tr>
<tr>
<td>Estradiol + Estrone</td>
<td>62.90</td>
<td>57.60</td>
<td>-21.00</td>
<td>c</td>
</tr>
<tr>
<td>Estradiol + Estrone*</td>
<td>126.90</td>
<td>5.70</td>
<td>-33.00</td>
<td>c</td>
</tr>
<tr>
<td>Estradiol</td>
<td>33.05</td>
<td>23.23</td>
<td>43.72</td>
<td>d†</td>
</tr>
<tr>
<td>Estrone</td>
<td>67.08</td>
<td>5.82</td>
<td>27.10</td>
<td>d†</td>
</tr>
</tbody>
</table>

*Calculated based on solid-water distribution coefficient (K_D); †Fugacity model

aAnderson et al. (2003); bBraga et al. (2005b); cCarballa et al. (2007); dUS EPA (2007)

To the author’s knowledge no fugacity models exist specifically for natural steroidal hormone fate during wastewater treatment. Instead, the fate of estradiol and estrone was modelled using STPWIN (US EPA, 2007). STPWIN is based on the fugacity model developed by Clark et al. (1995) and predicts hormone removal to the different pathways using biodegradation half-lives predicted using BIOWIN (US EPA, 2007) for the primary clarifier, aeration and settling tank. The model also considers physico-chemical properties of the studied micropollutant including molecular weight, vapour pressure, degradation and K_D value which is determined using the octanol-water partition coefficient (log K_{OW}) with one-parameter linear free energy relationships (LFER) (refer to Chapter 6). The use of log K_{OW} values to predict K_D values is a significant limitation of this model. Steroidal hormones are polar and can interact through hydrogen bonding, thus hydrophobicity has little influence on sorption. Further, the use of biological half-lives is a limitation of the model as biodegradation can vary depending on the type of microorganisms present, temperature and presence of other solutes (Clark et al., 1995). Consequently, predictions using SPTWIN can only provide a rough estimate of hormone fate.

Table 9.2 indicates the fate of micropollutants within wastewater treatment is variable and consequently difficult to predict. Further, the influence of hormone cleavage can be observed in the mass balance predictions by Carballa et al. (2007) with a high percentage of estradiol and estrone in the effluent and negative biodegradation. The significant difference in prediction is related to a number of
factors associated with wastewater treatment such as retention time, activated sludge concentration, microorganism population and influent composition. Further, the influence of organic matter-water partitioning is rarely considered, which is a major problem as this interaction can potentially influence hormone sorption to sludge and degradation significantly and this will be discussed in Section 9.3.3.

9.3.3. Influence of Solute-Solute Interactions

During conventional wastewater treatment only 60 to 70% of dissolved organic matter (DOM) is removed as it contains significant non-biodegradable fractions (Escalas et al., 2003; Katsoyiannis and Samara, 2007). While some hormones sorbed to DOM can be removed, the remaining DOM can also have implications for hormone degradation and sludge sorption removal pathways.

Degradation of steroidal hormones during conventional wastewater treatment is expected to be rapid, with batch experiments using activated sludge indicating that estradiol is 95% degraded within 3 hours (Ternes et al., 1999). However, it is likely this is overestimated for real systems containing natural organic matter (NOM), as a study by Joss et al. (2004) indicated reduced biodegradation with organics present in the influent. In fact, Johnson and Sumpter (2001) suggested that estradiol sorption to organic matter may reduce degradation to some extent. This was confirmed by Ren et al. (2007) who found that increased organic carbon concentration decreased hormone degradation by activated sludge. Further, Huang and Sedlak (2001) suggested that at the low hormone concentrations, which are typically found in wastewater influent, removal by biodegradation is low and consequently sorption to biomass and organic matter are more important for hormone removal.

In the activate sludge phase of conventional treatment the organic carbon concentration of the suspended solids is approximately 2400 mgC/L (Heidler and Halden, 2008), consequently estrogenic sorption to sludge is likely to be an important removal pathway. Several studies have quantified log KD values for sludge for estradiol and estrone, and these are shown in Table 9.3. Log KD values were
around 1 to 3 orders of magnitude lower than log $K_{OM}$ values calculated in Chapter 5. As sludge can be considered a solid the hormone sorption mechanism is adsorption, which is typically a weaker mechanism than partitioning (refer to Chapter 2). Therefore, the different sorption mechanisms may account for the low $K_D$ values for sludge. Further, as steroidal hormones appear to have a greater affinity for NOM it is likely that adsorption to sludge will be reduced in the presence of DOM. Indeed, Katsoyiannis and Samara (2007) found decreased sorption to sludge in the presence of DOM for a range of apolar micropollutants. Further, Matsui et al. (1998) found that the presence of humic acid led to a higher concentration of apolar pyrene and polar 1-aminopyrene in the aqueous phase due to sorption to DOM, leading to greater discharge into the environment.

Table 9.3: Literature log $K_D$ values (L/kg) for estradiol and estrone to activated sludge

<table>
<thead>
<tr>
<th></th>
<th>Carabella et al. 2008</th>
<th>Anderson et al. 2005</th>
<th>Clara et al. 2004</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol</td>
<td>2.30-2.83</td>
<td>2.68</td>
<td>2.84</td>
</tr>
<tr>
<td>Estrone</td>
<td>2.18-2.77</td>
<td>2.60</td>
<td>-</td>
</tr>
</tbody>
</table>

Further, it is also likely that the source and hence characteristics of the wastewater will influence solute-solute interactions. For example, wastewater from the paper and pulp industry typically contains a high concentration of tannins (Ali and Sreekrishnan, 2001). As Chapter 5 indicated, steroidal hormones interact strongly with tannic acid. Consequently, reduced sorption to sludge in the presence of tannin-rich water may lead to a greater hormone concentration in the aqueous phase.

Using the example of conventional wastewater treatment solute-solute interactions can reduce hormone degradation and sorption to sludge, which can consequently increase the hormone concentration discharged in the effluent. Therefore, knowledge of solute-solute interactions is essential, and more work is required using organic matter types commonly found in wastewater such as effluent organic matter.
9.4. Fate of Steroidal Hormones within the Aquatic Environment

In this section the influence of solute-solute interactions on steroidal hormone fate within the aquatic environment will be considered. Section 9.3 indicated the removal efficiency of hormones by conventional treatment is variable and incomplete, consequently hormones are expected to be discharged in wastewater effluent into receiving waters. Further, waste products from wastewater recycling processes such as RO concentrate discharged into streams or the ocean may also contribute as a source of micropollutants in the environment (Al-Rifai et al., 2007; Watkinson et al., 2007). RO concentrate typically contains a high concentration of micropollutants and this is often discharged into the environment after minimal treatment. Previous studies have indicated that low levels of steroidal hormones can have significant implications for the growth and development of aquatic organisms (e.g. Jobling et al., 2002; Routledge et al., 1998), therefore knowledge of hormone bioavailability within the aqueous environment is important.

Akkanen and Kukkonen (2003) indicated that micropollutant sorption to DOM and organic matter coated particles can lead to decreased micropollutant bioavailability and consequently reduced uptake by aquatic organisms. Micropollutant uptake or bioaccumulation by organisms can be predicted using a fugacity-based food web (Campfens and Mackay, 1997). Lai et al. (2002) applied this model to predict the fate of steroidal estrogens in a river system. This model indicated greater bioaccumulation of estradiol in plankton and fish, while greater bioaccumulation of estrone was observed for benthic organisms who live in the lowest level of a water body. Experimental log KOM values in Chapter 5 can be used to explain this result. Due to lower sorption to organic matter, estradiol will be more bioavailable than estrone. Therefore, organisms which live in the water column such as fish and plankton can absorb estradiol through respiration leading to greater bioaccumulation compared to estrone. Conversely, estrone is more likely to sorb to organic matter and sediment and this can lead to greater bioaccumulation in benthic organisms which live in sediment. Consequently, bioavailability and bioaccumulation of steroidal
hormones in aquatic organisms can be influenced by the presence of organic matter, as well as the strength of solute-solute interactions.

Further, the solution chemistry of the aquatic environment can influence the fate of steroidal hormones. Braga et al. (2005a) found high concentrations of steroidal hormones including estradiol and estrone in ocean sediment adjacent to and 7 km from a primary treatment ocean outfall. As the hormones can sorb to sediment it was suggested that the high ionic strength of seawater led to aggregation of sediment and consequently deposition. In Chapter 7 greater deposition of Aldrich HA was also observed at high ionic strengths, which was indicated by flux decline and deposition on the 100 kDa membrane. Further, due to the ionic strength in sea water ‘salting out’ of steroidal hormones can occur which reduces their solubility and can lead to greater sorption to the sediment (Bowman et al., 2002; Schwarzenbach et al., 2003). This example illustrates the importance of considering the environmental conditions as this can influence solute-solute interactions.

9.5. Conclusions

This chapter reinforces the importance of this thesis by considering how solute-solute interactions can influence the fate of steroidal hormones during wastewater treatment and in the aquatic environment. Further, the removal of steroidal hormones by PA fibre, UF membranes and MIEX® were compared, with greater sorption by the PA fibres for all hormones due to the polymer coating which increases micropollutant partitioning.

Through the application of fugacity modelling and some mass balance techniques the influence of organic matter-water partitioning or solid-water adsorption can be considered. Solute-solute interactions can decrease steroidal hormone removal during wastewater treatment by reducing degradation and sorption to sludge. The presence of organic matter can reduce hormone bioavailability in the aquatic environment. Further, the strength of sorption also appears to influence hormone fate with greater bioaccumulation of weaker organic matter sorbing hormones. Consequently, organic
matter type and origin has implications for hormone fate within the environment, as the presence of organic matter rich in phenolic groups such as tannic acid is expected to reduce bioavailability of hormones in the water column.

9.6. Recommendations for Future Studies

Due to the importance of solute-solute interactions in wastewater treatment environmental modelling is essential to determine micropollutant fate within the environment. Presently, no studies have applied fugacity based models to determine the fate of natural steroidal hormones in wastewater treatment, though a model has been developed for synthetic ethinylestradiol (Mastrup et al., 2005). STPWIN can be used to provide a general overview of steroidal hormone fate, but cannot be used to accurately predict the concentration of steroidal hormones in each removal pathway (Sanderson et al., 2004). Therefore, compound specific fugacity models are required to determine steroidal hormone fate during wastewater treatment. This requires a better understanding of organic matter-water partitioning. Alternatively, a mass balance approach using log $K_{OM}$ values, similar to Carballa et al. (2007), may also be used to better understand the fate of hormones during wastewater treatment.

Further, the majority of examples presented in this chapter focuses on estradiol and estrone. While progesterone and testosterone are not as potent as estrogenic hormones, they are still of concern as they are excreted at similar levels and thus can be considered ‘pseudopersistent’. Few studies have considered the removal of progesterone and testosterone during wastewater treatment (e.g. Esperanza et al., 2007; Kolodziej et al., 2003), however, Kolpin et al. (2002) indicated that such hormones were found at average concentrations of approximately 100 ng/L in streams in the US suggesting poor removal by water treatment processes. Therefore, more studies on the fate of progesterone and testosterone within wastewater treatment and the aquatic environment are required.
10 Summary and Conclusions

Since the mid 1990’s the elevated presence of steroidal hormones in the aquatic environment has been identified as environmental pollutants which are capable of causing behavioural and developmental abnormalities in aquatic organisms. Unlike anthropogenic micropollutants the production and output of steroidal hormones cannot be minimised as hormones are excreted naturally by humans and many animals. Consequently, knowledge of the fate of steroidal hormones in both engineered and natural aquatic environments is essential. Despite the importance, few attempts have been made to better understand the interaction of steroidal hormones with organic matter. Therefore, the primary aims of this thesis were to:

1. Develop a technique to quantify solute-solute interactions at environmental concentrations
2. Demonstrate the contribution of such solute-solute interactions towards micropollutant removal mechanisms by ultrafiltration (UF)

Using solid-phase microextraction (SPME) organic matter-water partition coefficients (log $K_{OM}$) were measured in Chapter 5 for a range of steroidal hormones and organic matter types. This indicated that:

- Different organic matter types and pH did not have a significant influence on estradiol partitioning as estradiol simply did not interact with organic matter strongly.
- Stronger partitioning was observed for estrone and progesterone which both contain ketone groups in the C-17 and C-20 positions respectively. Due to the strong interaction, log $K_{OM}$ values for progesterone and estrone were significantly different as a function of organic matter type, pH, ionic strength and organic matter concentration.
- For all hormones the interaction was strongest with tannic acid which is rich in phenolic hydroxyl groups and promotes strong hydrogen bonding.
Summary and Conclusions

In Chapter 6 one-parameter and polyparameter linear free energy relationships (LFER) were applied to predict organic matter-water partitioning. When compared to experimental log $K_{OM}$ values the majority of predicted log $K_{OM}$ values were significantly different, suggesting that such models are not applicable as they lacked system constants specific to the studied organic matter types. Consequently, at present these models cannot be used properly, but it is hoped that in the future they will be of use, though much work is still required.

Using experimental log $K_{OM}$ values calculated in Chapter 5 the impact of solute-solute interaction for hormone removal by UF was predicted (Chapter 7) and this suggested that:

- For the majority of experiments, log $K_{OM}$ values indicated that solute-solute interactions were the main removal mechanism. This was confirmed by negligible flux decline and membrane sorption.
- Solute-foulant interactions also contributed to hormone removal by large molecular weight cut-off (MWCO) membranes, while membrane sorption was an important removal mechanism for progesterone.
- Hormone retention, using the example of estrone, was found to increase with increasing organic matter concentration, suggesting that organic matter was the limiting factor in this interaction.

To improve steroidal hormone removal an ion exchange-ultrafiltration (IX-UF) hybrid process was applied in Chapter 8 and this indicated that:

- Approximately 90% of estradiol was removed by the IX-UF hybrid process using 5 to 100 kDa MWCO membranes, with less removal by smaller MWCO (1 and 3 kDa) membranes (62-75%).
- This was due to deposition of magnetic ion exchange resin (MIEX®) on the membrane surface for 5 to 100 kDa MWCO membranes.
- The presence of natural organic matter (NOM) decreased hormone removal by IX-UF due to competition for MIEX® binding sites.
Finally, the implications of solute-solute interactions in the wider environment were considered in Chapter 9. In wastewater treatment solute-solute interactions can increase micropollutant discharge to the environment by preventing sorption to sludge/biomass and reducing degradation. However, once discharged into receiving waters partitioning to organic matter can reduce the bioavailability of steroidal hormones and consequently reduce uptake into aquatic organisms.

Returning to the primary aims of this thesis, a SPME technique suitable for steroidal hormones has been developed to quantify solute-solute interactions at environmental concentrations of both steroidal hormones and organic matter. This method was successfully applied to an example of advanced water treatment, namely UF, to prove the importance of solute-solute interactions. It is obvious that the implications of this research are significant not only for water and wastewater applications, but also the fate and transport of micropollutants in the environment. However, much work remains to be done to apply this method to other micropollutants and organic matter.
Summary and Conclusions
A1 Stirred Cell Ultrafiltration System

A1.1. System Design

The schematic representation of the stainless steel stirred cells used in Chapter 7 and 8 is shown in Figure A1.1. The volume of the cell is 990 mL with internal diameter of 70 mm giving a membrane surface area of 38.48 cm². The maximum pressure rating of the cell was 20 bar, however, the pressure within the cell for all experiments did not exceed 5 bar due to ultrafiltration (UF) membrane pressure restrictions. The cell was pressurised using lab air which was supplied through the top of the cell. The top of the cell also contained a thermocouple (TJ2-CPSS-M60U-250-SB), pressure transducer (PX209-300GV5) and manual pressure relief valve, as well as a high pressure relief valve in case the pressure accidentally exceeded 20 bar (Figure A1.2). The thermocouple and pressure transducer was purchased from Omega Engineering (Irlam, UK). The base of the cell contained stainless steel flow channels covered by a 1.5 mm porous stainless steel layer (SIKA-R 5 AX) purchased from GKN Sinter Metals Filters GmbH (Radevormwald, Germany) (Figure A1.3). The top and base were secured to the main part with two part clamps (Heleon Group, Middelharnis, The Netherlands) which allowed for fast access to the cell and o-rings from Wyco (Halesowen, UK) were used to seal the cell. The cell contained a magnetic stirred assembly (Millipore, Watford, UK) (Figure A1.4) and was stirred at 300 RPM using a magnetic stirrer table (Fisher Scientific, Loughborough, UK) to reduce concentration polarisation. Three stainless steel cells were used in parallel as indicated in the schematic diagram in Figure A1.5.
Figure A1.1: Stainless steel stirred cell schematic diagram
Figure A1.2: Top view of stirred cell a) schematic diagram and b) photo

Figure A1.3: Top view of stainless steel flow channel a) schematic diagram and b) photo
Figure A1.4: Stirrer assembly
a) schematic diagram side view
b) schematic diagram top view
and c) photo (Millipore Catalogue Number 15254AM 8400)

Figure A1.5: Stirred cell set-up schematic diagram where P and T represent pressure transducer and thermocouple, respectively
A1.2. pH Stability

A limitation of the stirred cell configuration was that it was not possible to adjust the pH during the experiment. As a result, the pH could be unstable over the course of the experiment. In this appendix pH stability will be assessed and the influence of organic matter concentration, dissolved oxygen content and the partial pressure of carbon dioxide will be considered to assess the causes of pH variation within the stirred cells. The influence of membrane molecular weight cut-off (MWCO) was studied as this has implications for pressure and organic matter retention.

For all experiments a carbonate buffer (1 mM NaHCO₃) was selected as it is representative of natural systems. The acid dissociation constants (pKₐ) of carbonate are 6.35 and 10.33 and consequently it exists in different forms as a function of pH. As can be seen in Equation A5.1 it changes from H₂CO₃ to HCO₃⁻ to CO₃²⁻. Buffers are generally only effective ± 1 pH unit above or below the pKₐ.

\[ H₂CO₃ + H₂O ⇄ HCO₃⁻ + H₃O⁺ ⇄ H₂O + CO₃²⁻ \]  (A1.1)

pH stability in the presence and absence of Aldrich humic acid (HA) with 10 kDa membranes is shown below in Figure A1.6. During the experiment the pH decreased for pH 9, 10 and 12, and increased for pH 7, while the other pH units remained constant. pH 7 and 10 were within 1 pH unit of the respective carbonate pKₐ values. Due to the significant retention of Aldrich HA (Figure 8.12 a in Chapter 8) it was hypothesised that the concentration of organic matter within the cell would influence pH stability due to the increased concentration of hydrogen ions. However, Figure A1.6 indicates that the presence of organic matter appears to have little influence on pH stability.

In contrast, the pH was relatively stable for the 100 kDa membrane (Figure A1.7). The stability may be related to the short experimental time (approximately 12 minutes) and low pressure (0.5 bar).
Figure A1.6: pH stability in the presence (open symbols) and absence (closed symbols) of Aldrich HA with 10 kDa membrane (1 mM NaHCO$_3$, 20 mM NaCl, 12.5 mgC/L Aldrich HA, 5 bar pressure, 60 minute experiment time)

Figure A1.7: pH stability in the presence (open symbols) and absence (closed symbols) of Aldrich HA with 100 kDa membrane (1 mM NaHCO$_3$, 20 mM NaCl, 12.5 mgC/L Aldrich HA, 0.5 bar pressure, 12 minute experiment time)
Further, the concentration of the buffer can influence its effectiveness, and a range of 20 to 50 mM is typically used (Beynon and Easterby, 1996). However, the concentration of carbonate in natural waters can range from 0.3 to 1.9 mM (Hem, 1985), therefore using NaHCO₃ at a concentration of 20 to 50 mM defeats the purpose of using a buffer which is representative of natural waters. The effectiveness of NaHCO₃ was compared at 1 and 8 mM (Figure A1.8). The pH was adjusted to 6, 7 and 8 and the experiments were conducted with a 3 kDa membrane at 5 bar pressure. The buffer concentration did influence pH stability, however, it appears that the pH remained more stable at the lower buffer concentration.

![Graph showing pH stability with NaHCO₃ concentration](image)

**Figure A1.8: Influence of NaHCO₃ concentration on pH stability with 3 kDa membrane (1 mM (open symbols) and 8 mM (closed symbols) (1 mM NaHCO₃, 20 mM NaCl, 0.5 bar pressure, 135 minute experiment time)**

As pH instability was a problem for the smaller MWCO membranes it was expected that the long experiment times and high pressure (5 bar) led to instability. Therefore, to better understand pH instability the dissolved oxygen concentration in the cell was measured and the influence of oxygen and carbon dioxide on pH with increasing pressure was modelled using Visual MINTEQ software. This will be discussed further in Sections A1.3 and A1.4.
A1.3. Dissolved Oxygen Concentration

The influence of dissolved oxygen in the stirred cells was studied as in natural waters oxygen can indirectly influence pH stability due to biological activity such as respiration (Morel and Hering, 1993). The dissolved oxygen concentration (mg/L) in the cell was measured using WTW Multiline P4 dissolved oxygen probe (Weilheim, Germany) as a function of membrane MWCO (Figure A1.9). Dissolved oxygen was measured in the feed prior to the experiment (initial concentration) and in the remaining 50 mL in the cell (concentrate) immediately after the experiment was finished (final concentration). The initial concentration did not vary as a function of MWCO as the cell was not pressured when it was measured. However, following the experiment the dissolved oxygen content was significantly higher for 1 to 10 kDa membranes. This corresponded with the increased pressure (5 bar) used for these membranes as well as the longer experiment times. Further, the presence of organic matter did not influence dissolve oxygen concentration. Due to the lack of biological activity in the stirred cells it is unlikely that dissolved oxygen influence pH instability. Instead, the instability at high pressures may be related to the introduction of carbon dioxide into the cell through the lab air. As carbon dioxide can exchange with carbonate, the increasing carbon dioxide concentration with increasing pressure can contribute to pH instability for smaller MWCO membranes. This will be demonstrated below in Section A1.4 using Visual MINTEQ modelling.
Appendix 1

Figure A1.9: Dissolve oxygen concentration (mg/L) as a function of membrane MWCO for a) testosterone and b) testosterone with Aldrich HA (1 mM NaHCO₃, 20 mM NaCl, pH 8, 100 ng/L testosterone, 12.5 mgC/L Aldrich HA)

A1.4. Visual MINTEQ Modelling

Visual MINTEQ is a chemical speciation program and version 2.5 was used in this study. As it appears that increased pressure contributes to pH instability, Visual MINTEQ was used to model pH changes of the NaHCO₃ buffer with increasing pressure. The constant parameters used for modelling are shown in Table A1.1.

Table A1.1: Parameters used for Visual MINTEQ pH modelling

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO₃²⁻</td>
<td>1 mM</td>
</tr>
<tr>
<td>Na⁺</td>
<td>1 mM</td>
</tr>
<tr>
<td>H⁺</td>
<td>1 mM</td>
</tr>
<tr>
<td>Ionic Strength</td>
<td>20 mM</td>
</tr>
<tr>
<td>Temperature</td>
<td>25°C</td>
</tr>
</tbody>
</table>
Appendix 1

The influence of pressure was studied at 0.5, 1, 3 and 5 bars. The influence of both oxygen and carbon dioxide on the pH of NaHCO₃ was considered. Therefore, the partial pressure of each gas in the studied pressure range was required. As oxygen makes up 20.95% of air the partial pressure of oxygen varies from 0.1 to 1.1 bar with a total pressure of 0.5 to 5 bar. In contrast, carbon dioxide only contributes to approximately 0.038% of air therefore the partial pressures ranges from $1.9 \times 10^{-4}$ to $1.9 \times 10^{-3}$ bar in the same total pressure range. All partial pressures are shown in Table A1.2.

Table A1.2: Partial pressure of carbon dioxide and oxygen as a function of total pressure

<table>
<thead>
<tr>
<th></th>
<th>CO₂ Partial Pressure (bar)</th>
<th>O₂ Partial Pressure (bar)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 bar</td>
<td>$1.9 \times 10^{-4}$</td>
<td>0.1</td>
</tr>
<tr>
<td>1 bar</td>
<td>$3.8 \times 10^{-4}$</td>
<td>0.2</td>
</tr>
<tr>
<td>3 bar</td>
<td>$1.1 \times 10^{-3}$</td>
<td>0.6</td>
</tr>
<tr>
<td>5 bar</td>
<td>$1.9 \times 10^{-3}$</td>
<td>1.1</td>
</tr>
</tbody>
</table>

With the partial pressures known it was possible to model pH changes with increasing pressure. Table A1.3 indicates that pH decreases with increasing pressure with carbon dioxide, while the pH remains constant in the presence of oxygen. This confirms that carbon dioxide causes pH instability, in addition to the longer experimental times for the small MWCO membranes.

Table A1.3: The pH of NaHCO₃ buffer for carbon dioxide and oxygen as a function of total pressure

<table>
<thead>
<tr>
<th></th>
<th>CO₂ pH (-)</th>
<th>O₂ pH (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 bar</td>
<td>8.5</td>
<td>8.2</td>
</tr>
<tr>
<td>1 bar</td>
<td>8.2</td>
<td>8.2</td>
</tr>
<tr>
<td>3 bar</td>
<td>7.7</td>
<td>8.2</td>
</tr>
<tr>
<td>5 bar</td>
<td>7.5</td>
<td>8.2</td>
</tr>
</tbody>
</table>

In conclusion, to prevent pH stability problems in future experiments the cells should be pressurised with an inert gas such as nitrogen. It was not possible to model the
influence of nitrogen on pH using Visual MINTEQ version 2.5, however, as it is inert it is unlikely to influence the pH.
Appendix 2

A2 Instrument Calibration

In A2 the methods of instrument calibration will be outlined, and some limitations will be discussed. The analytical instruments featured in A2 include the total organic carbon (TOC) analyser, liquid scintillation counter and UV-visible spectrophotometer.

A2.1 TOC Analyser

The organic carbon concentration in the feed, permeate and concentrate samples was measured using a TOC analyser (TOC-V CPH) in non-purgeable organic carbon (NPOC) mode (Shimadzu, Milton Keyes, UK) (Figure A2.1). NPOC refers to the TOC in a non-volatile form in the sample. As the TOC analyser was used for low organic matter applications the concentration of organic carbon in the sample could not exceed 10 mgC/L. Therefore, any samples greater than 10 mgC/L were diluted with deionised water prior to analysis.

Figure A2.1: Shimadzu total organic carbon analyser with autosampler

The TOC analyser used 680°C catalytically-aided combustion oxidation to oxidise organic matter to carbon dioxide. However, the oxidation efficiency of the TOC analyser is not usually 100%. The efficiency of oxidation can be influenced by the solution chemistry and properties of the organic matter (Spyres et al., 2000). To calculate the oxidation efficiency of each studied organic, a calibration curve (at
concentrations of 0.5, 1, 2, 5 and 10 mg/L was calculated (Figure A2.2). If the efficiency was 100% the slope would be 1. The efficiency of Aldrich humic acid (HA) was 54%, alginic acid was 73% and tannic acid was 81%. The standard used for all experiments was potassium hydrogen phthalate.

![Graph](image)

**Figure A2.2: Calibration of TOC analyser from 0.5 to 10 mgC/L organic matter (1mM NaHCO₃, 20 mM NaCl, pH 7, 0.5, 1, 2, 5 and 10 mgC/L Aldrich HA)**

**A2.2. Liquid Scintillation Counter**

A Beckman LS6500 liquid scintillation counter (Fullerton, USA) (Figure A2.3) was used to measure the concentration of radiolabelled hormone in solution. All hormones were labelled with tritium (³H) which is a beta emitter. The hormone sample was dissolved in a liquid scintillation cocktail which converted the radioactive energy into light which can be detected by the counter. The units of activity are disintegrations per minute (dpm). To improve accuracy of detection a liquid scintillation cocktail suitable for low level tritium counting in aqueous samples, Ultima Gold LLT, was selected. The average detection limits for the studied hormones is shown below in Table A2.1.
Appendix 2

Figure A2.3: Beckman LS6500 liquid scintillation counter

Table A2.1: Liquid scintillation counter detection limit for the studied hormones

<table>
<thead>
<tr>
<th></th>
<th>Average Detection Limit (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol</td>
<td>1.14</td>
</tr>
<tr>
<td>Estrone</td>
<td>1.14</td>
</tr>
<tr>
<td>Progesterone</td>
<td>1.06</td>
</tr>
<tr>
<td>Testosterone</td>
<td>1.26</td>
</tr>
</tbody>
</table>

To determine the appropriate sample volume it is important to consider sample capacity, which represents the optimal ratio of sample to cocktail volume. Above this capacity the sample can become hazy or cloudy and this prevents the counter from detecting light accurately (Hawkins, 1994). Sample capacity can be determined using Equation A2.1 where $V_S$ is the sample volume (mL) and $V_C$ is the cocktail volume (mL).

$$\text{Sample Capacity (\%)} = \frac{V_S}{V_S + V_C} \times 100 \quad (A2.1)$$

A maximum sample capacity of 13% is recommended to ensure the sample is clear to allow for accurate counting (Hawkins, 1994). Using a ratio of 1 mL sample to 7 mL cocktail 12.5% sample capacity can be achieved. To ensure that 7 mL was a suitable cocktail volume, counting efficiency of estradiol was compared with 1 mL samples in 8 and 9 mL of cocktail giving sample capacities of 11.1 and 10% respectively. The studied estradiol concentration was 10 ng/L. The results in Table
Appendix 2

A2.2 indicates that dpm is similar for 7, 8 and 9 mL of cocktail, therefore a ratio of 1 mL sample to 7 mL cocktail is suitable for analysis.

Table A2.2: Influence of cocktail volume on sample activity

<table>
<thead>
<tr>
<th>Sample Description</th>
<th>dpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mL 10 ng/L with 9 mL cocktail</td>
<td>1208.64</td>
</tr>
<tr>
<td>1 mL 10 ng/L with 8 mL cocktail</td>
<td>1203.01</td>
</tr>
<tr>
<td>1 mL 10 ng/L with 7 mL cocktail</td>
<td>1193.61</td>
</tr>
</tbody>
</table>

The presence of colour within the samples can reduce counting efficiency as it can absorb light. Therefore, an estrone calibration curve from 0.01 to 1000 ng/L was plotted as a function of organic matter concentration (Figure A2.4). The Aldrich HA concentration ranged from 0 to 125 mgC/L. Due to the log scale the influence of organic matter concentration appears to be minimal, however, in Table A2.3 the efficiency decreased from 100% with no organic matter to 86% in the presence of 125 mgC/L Aldrich HA. The counting efficiency was determined by dividing the activity at a particular organic matter concentration by the organic matter free activity. As most experiments were conducted at 12.5 mgC/L the organic matter had a negligible influence on liquid scintillation counting efficiency (99% efficiency).

![Estradiol calibration curve as a function of Aldrich HA concentration](image)

Figure A2.4: Estradiol calibration curve as a function of Aldrich HA concentration (1mM NaHCO₃, 20 mM NaCl, pH 7, 0.01 to 1000 ng/L estradiol, 0, 12.5, 25, 50 and 125 mgC/L Aldrich HA)
Table A2.3: Liquid scintillation counting efficiency as a function of organic matter concentration

<table>
<thead>
<tr>
<th>Organic Matter Concentration (mgC/L)</th>
<th>Equation</th>
<th>Correlation Coefficient (R)</th>
<th>Liquid Scintillation Counting Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mgC/L</td>
<td>$y = 457.53x$</td>
<td>1</td>
<td>100%</td>
</tr>
<tr>
<td>12.5 mgC/L</td>
<td>$y = 451.09x$</td>
<td>1</td>
<td>99%</td>
</tr>
<tr>
<td>25 mgC/L</td>
<td>$y = 439.43x$</td>
<td>1</td>
<td>96%</td>
</tr>
<tr>
<td>50 mgC/L</td>
<td>$y = 430.46x$</td>
<td>1</td>
<td>94%</td>
</tr>
<tr>
<td>125 mgC/L</td>
<td>$y = 393.83x$</td>
<td>1</td>
<td>86%</td>
</tr>
</tbody>
</table>

A2.3. UV-Visible Spectrophotometer

A Cary 100 Scan UV-visible spectrophotometer (Palo Alto, USA) (Figure A2.5) was used to measure the absorbance of a sample at a particular wavelength. The absorbance of Aldrich HA, alginic acid and tannic acid was scanned from 800 to 200 nm (Figure A2.6). The spectra for Aldrich HA and tannic acid indicate that absorbance was in the UV range (which is from 10-400 nm), while no absorbance was detected for alginic acid. This is because it lacks aromatic functional groups. For all experiments in this thesis a wavelength of 254 nm was used for consistency with the literature (Aoustin et al., 2001; Schäfer et al., 2002). Also, quartz cell with a 1 cm path length was used for all experiments.

Figure A2.5: Cary 100 Scan UV-visible spectrophotometer
Figure A2.6: Absorbance of Aldrich HA, alginic acid and tannic acid as a function of wavelength ($\lambda$)

Due to the large dilution factor required to analyse the 125 mgC/L sample in the TOC analyser, the UV-visible spectrophotometer was used to produce a calibration curve to estimate organic matter concentration based on absorbance (Figure A2.7). Aldrich HA concentrations of 0.625, 1.25, 2.5, 6.25, 12.5 and 25 mgC/L were plotted against absorbance at 254 nm. The correlation coefficient (R) was 0.999, which indicates the relationship is linear. This calibration curve was used to determine organic matter concentration in Section 7.3 in Chapter 7. This was only used in this section, with the organic matter concentration in the remainder of this thesis was measured using the TOC analyser.
UV-visible spectrophotometry can also be used to assess the aromaticity of a sample using specific UV absorbance (SUVA) (L/mg.m). This is calculated using Equation A2.2 where UVA (cm) was UV absorbance determined by the UV-Visible spectrometer divided by the path length of the quartz cell (1 cm) and DOC was dissolve organic carbon (mg/L) determined using the TOC analyser.

$$SUVA = \frac{UVA}{DOC} \times 100$$  \hspace{1cm} (A2.2)
A3 Error Calculation

To assess the error associated with organic matter-water partition coefficients (log $K_{OM}$) and stirred cell ultrafiltration a range of different statistical techniques were applied including error propagation and coefficient of variance ($C_V$). Error propagation, also called propagation of uncertainty, is a technique which allows the total error of a system to be determined by considering random error, while $C_V$ represents the relative error of a sample (Miller and Miller, 2000). This appendix will outline the error calculation techniques used for log $K_{OM}$ values and the ultrafiltration stirred cells.

A3.1. Organic Matter-Water Partition Coefficient (log $K_{OM}$)

As described in Chapter 3, log $K_{OM}$ values were determined from the slope of a linear regression using the freely dissolved hormone concentration as a function of the organic matter sorbed hormone concentration. As the log $K_{OM}$ values were calculated over a large concentration range (100 ng/L to 100 µg/L) individual log $K_{OM}$ values were required at each studied concentration. The relative error ($%E$) associated with the individual log $K_{OM}$ values are shown in Table A3.1. The relative error of Eppendorf pipettes and Hamilton syringes were taken from the company websites, while error associated with fibre variation was calculated based on difference in fibre volume ± 1 mm difference in fibre length. The total error ($%E_{Total}$) of the individual log $K_{OM}$ measurements was calculated using error propagation (Equation A3.1) and was 5.4%.

Table A3.1: Relative error ($%E$) associated with individual log $K_{OM}$ measurements

<table>
<thead>
<tr>
<th>Source of Error</th>
<th>%E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid scintillation counter</td>
<td>2.0</td>
</tr>
<tr>
<td>Eppendorf pipette (1 mL)</td>
<td>0.6</td>
</tr>
<tr>
<td>Hamilton syringe (100µL)</td>
<td>4.6</td>
</tr>
<tr>
<td>SPME fibre variation (length)</td>
<td>2.0</td>
</tr>
<tr>
<td>Electronic balance</td>
<td>0.1</td>
</tr>
<tr>
<td>Total ($%E_{Total}$)</td>
<td>5.4</td>
</tr>
</tbody>
</table>
Appendix 3

\[
%E_{Total} = \sqrt{(%E_1)^2 + (%E_2)^2 + (%E_3)^2}
\]  

(A3.1)

A3.2. Stirred Cell Ultrafiltration

The %E values associated with analytical techniques, pressure and pure water flux are shown in Tables A3.2, A3.3 and A3.4 respectively. The %E values were calculated using \( C_V \) (Equation A3.2) where \( s^2 \) was standard deviation and \( \bar{X} \) was the sample mean. Standard deviation is used to express the spread of the samples and was determined using Microsoft Excel.

\[
C_V = \frac{s^2}{\bar{X}} = %E
\]  

(A3.2)

In Table A3.2 %E represents the average variation associated with the analytical techniques. As each technique measures a single sample multiple times \( C_V \) can be determined. The error for the TOC analyser was high compared to other instruments. The variability was due to organic matter properties such as aromatic functional groups which reduce oxidation efficiency. Further, all samples had a concentration less than 10 mgC/L, and this may have also contributed to the high error observed in Table A3.2.

**Table A3.2: Relative error (%E) associated with analysis techniques**

<table>
<thead>
<tr>
<th>Analysis Technique</th>
<th>%E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid scintillation counter</td>
<td>2.0</td>
</tr>
<tr>
<td>TOC analyser</td>
<td>14.4</td>
</tr>
<tr>
<td>UV-Visible spectrophotometer</td>
<td>1.6</td>
</tr>
</tbody>
</table>

**Table A3.3: Relative error (%E) associated with pressure within the stirred cells**

<table>
<thead>
<tr>
<th>Pressure</th>
<th>%E</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 Bar</td>
<td>6.0</td>
</tr>
<tr>
<td>1 Bar</td>
<td>4.3</td>
</tr>
<tr>
<td>5 Bar</td>
<td>1.0</td>
</tr>
</tbody>
</table>
Table A3.4: Relative error (%E) associated with pure water flux

<table>
<thead>
<tr>
<th>Flux (kDa)</th>
<th>%E</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 kDa</td>
<td>5.1</td>
</tr>
<tr>
<td>3 kDa</td>
<td>6.7</td>
</tr>
<tr>
<td>5 kDa</td>
<td>11.2</td>
</tr>
<tr>
<td>10 kDa</td>
<td>7.8</td>
</tr>
<tr>
<td>30 kDa</td>
<td>5.0</td>
</tr>
<tr>
<td>100 kDa</td>
<td>10.2</td>
</tr>
</tbody>
</table>

The %E\text{\textsubscript{TOTAL}} results for retention, adsorption and specific UV absorbance (SUVA) are shown in Table A3.5 as a function of membrane molecular weight cut-off (MWCO). These were determined using Equation A3.1. The error associated with organic matter retention, adsorption and SUVA was high and this was due to the relative error of the TOC analyser which was 14.4% (Table A3.2). Further, as the MWCO increased error also increased. This was because the pressure was difficult to adjust at 0.5 bar, which led to a significant variation in pressure and consequently flux. There were only two %E\text{\textsubscript{TOTAL}} values for organic matter retention (UV-Vis) as UV-visible spectrophotometry was only used to estimate organic matter concentration for 10 and 100 kDa membranes at high organic matter concentrations.

The error associated with predicted hormone retention (R\text{\textsubscript{P%}}) in Chapter 7 was also determined using error propagation (Equation A3.1). As R\text{\textsubscript{P%}} was estimated using R\text{\textsubscript{OM%}} and log K\text{\textsubscript{OM}} values (Equation 3.19 in Chapter 3) the %E values associated with R\text{\textsubscript{OM%}} and log K\text{\textsubscript{OM}} values were considered when determining the %E\text{\textsubscript{TOTAL}} value for R\text{\textsubscript{P%}}.

Table A3.5: Error propagation (%E\text{\textsubscript{TOTAL}}) for hormone retention, organic matter retention, membrane adsorption and SUVA as a function of membrane MWCO

<table>
<thead>
<tr>
<th>MWCO (kDa)</th>
<th>Hormone Retention/Adsorption</th>
<th>Organic Matter Retention/Adsorption</th>
<th>Organic Matter Retention (UV-Vis) (R\text{\textsubscript{OM%}})</th>
<th>SUVA in permeate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 kDa</td>
<td>5.6</td>
<td>15.3</td>
<td>-</td>
<td>15.4</td>
</tr>
<tr>
<td>3 kDa</td>
<td>7.0</td>
<td>16.0</td>
<td>-</td>
<td>16.0</td>
</tr>
<tr>
<td>5 kDa</td>
<td>11.4</td>
<td>18.3</td>
<td>-</td>
<td>18.4</td>
</tr>
<tr>
<td>10 kDa</td>
<td>8.1</td>
<td>16.4</td>
<td>8.0</td>
<td>16.5</td>
</tr>
<tr>
<td>30 kDa</td>
<td>6.8</td>
<td>15.4</td>
<td>-</td>
<td>15.9</td>
</tr>
<tr>
<td>100 kDa</td>
<td>12.0</td>
<td>18.7</td>
<td>11.9</td>
<td>18.7</td>
</tr>
</tbody>
</table>
Finally, to assess reproducibility, ultrafiltration stirred cells experiments were conducted in triplicate. In Figure A3.1 the retention of estrone by 10 kDa membranes in the presence of 125 mgC/L of Aldrich humic acid (HA) was similar for all experiments, suggesting that the experiments were reproducible.

![Figure A3.1: Reproducibility of estrone retention by 10 kDa membrane (1mM NaHCO₃, 20 mM NaCl, pH 8, 100 ng/L estrone, 125 mgC/L Aldrich HA)](image)

**A3.3 Ion Exchange-Ultrafiltration Hybrid Process**

Similar to Section A3.3 %E_{TOTAL} values associated with the ion exchange-ultrafiltration (IX-UF) hybrid process was calculated using error propagation. The %E values associated with pressure and pure water flux was shown in Tables A3.6 and A3.7. These values differ from Section A3.2 as different membranes and pressures were used. However, the %E values associated with the analytical techniques were the same as Table A3.2. Similar to Section A3.2 all %E values were calculated using C_V (Equation A3.2). Further, the %E value associated with MIEX® addition using a 10 mL syringe, which was 5.3%, was included in the error propagation calculations. The %E_{TOTAL} values for hormone removal as a function of membrane (MWCO) are shown in Table A3.8 and were calculated using Equation A3.1.
Table A3.6: Relative error (%E) associated with pressure within the stirred cells

<table>
<thead>
<tr>
<th>Pressure</th>
<th>%E</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 Bar</td>
<td>11.1</td>
</tr>
<tr>
<td>1 Bar</td>
<td>5.5</td>
</tr>
<tr>
<td>3 Bar</td>
<td>2.4</td>
</tr>
<tr>
<td>5 Bar</td>
<td>1.8</td>
</tr>
</tbody>
</table>

Table A3.7: Relative error (%E) associated with pure water flux

<table>
<thead>
<tr>
<th>Flux</th>
<th>%E</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 kDa</td>
<td>5.1</td>
</tr>
<tr>
<td>3 kDa</td>
<td>6.7</td>
</tr>
<tr>
<td>5 kDa</td>
<td>2.7</td>
</tr>
<tr>
<td>10 kDa</td>
<td>7.0</td>
</tr>
<tr>
<td>30 kDa</td>
<td>1.6</td>
</tr>
<tr>
<td>100 kDa</td>
<td>12.9</td>
</tr>
</tbody>
</table>

Table A3.8: Error propagation (%E_{TOTAL}) associated with hormone retention as a function of membrane MWCO

<table>
<thead>
<tr>
<th>MWCO (kDa)</th>
<th>Hormone Retention</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 kDa</td>
<td>7.8</td>
</tr>
<tr>
<td>3 kDa</td>
<td>6.6</td>
</tr>
<tr>
<td>5 kDa</td>
<td>6.7</td>
</tr>
<tr>
<td>10 kDa</td>
<td>9.4</td>
</tr>
<tr>
<td>30 kDa</td>
<td>8.1</td>
</tr>
<tr>
<td>100 kDa</td>
<td>18.0</td>
</tr>
</tbody>
</table>
A4 The Influence of pH on Losses of Analyte Estradiol in Sample Pre-Filtration

A4 is based on a short communication accepted to the journal Environmental Engineering Science. It was included in this thesis as it provides evidence as to the importance of solute-solute interactions.

The steroidal hormone estradiol is of interest to many researchers as it is a potent hormone, and laboratory experiments have demonstrated that low concentrations of estradiol can adversely affect aquatic organisms (Thorpe et al., 2007; Thorpe et al., 2000). Using a variety of analytical techniques, including gas and liquid chromatography, estradiol can be detected at sub nanogram per litre concentration levels (Jeannot et al., 2002; Rodriguez-Mozaz et al., 2004; Williams et al., 2003). Previous studies have used 0.45 µm cellulose acetate filters to pre-treat surface and groundwater samples containing steroidal hormones, such as estradiol, in acidic and neutral solutions prior to chromatography (Drewes et al., 2005; Mansell and Drewes, 2004). Traditionally, 0.45 µm filters were used to distinguish between dissolved and particulate material (Clesceri et al., 1998; Frimmel, 1998). Within the literature cellulose acetate filters have been used to pre-treat a large variety of sample types in the analysis of different compounds prior to chromatography (Furusawa, 1999), fluorescence spectroscopy (Jung and Batley, 2004) and capillary electrophoresis (Karlsson et al., 1999) as it could remove particulate matter which may interfere with analysis. As syringe filters were used for sample pre-treatment it was important to determine if estradiol adsorbed to such cellulose acetate filters, as this would reduce compound recovery. The influence of pH was also studied, as this could affect the interaction of estradiol with the filter due to a change in estradiol charge. Estradiol has a dissociation constant (pKₐ) of 10.23 (Kwon et al., 2005), so it is negatively charged above pH 10. In addition different organic matter types, such as natural organic matter (NOM) surrogates and polysaccharides, were selected to determine whether their presence would have an impact on estradiol adsorption, and hence analyte recovery. Therefore, the purpose of this study was to determine if cellulose
acetate syringe filters were a suitable pre-treatment option for the analysis of estrogens.

**A4.1 Materials and Methods**

**A4.1.1 Chemicals and Standards**

All chemicals were of analytical grade. The background electrolyte was 1 mM NaHCO₃, 20 mM NaCl and pH was adjusted from 3 to 12 using 1 M NaOH and HCl (Sigma Aldrich, Gillingham, UK). All experiments were conducted in deionised water. Radiolabelled [2,4,6,7–³H]estradiol (3.15 TBq/mmol, 37 MBq/mL) was purchased from GE Healthcare (Little Chalfont, UK). The concentration of estradiol used in the experiments was 50, 100 and 500 ng/L. Aldrich humic acid (HA) and alginic acid (sodium salt) were purchased from Sigma Aldrich. Suwannee River reference IHSS natural organic matter (NOM) was purchased from the International Humic Substances Society (St Paul, USA), while Australian NOM was concentrated using microfiltration and reverse osmosis from Brisbane Water National Park, Australia (Schäfer, 2001). The concentration of carbon in all experiments was 12.5 mgC/L, with the exception of Aldrich HA where experiments were conducted at both 5 and 12.5 mgC/L.

**A4.1.2 Sample Preparation and Filtration**

Organic matter and estradiol were added to 100 mL flask with background electrolyte and the pH was adjusted. The solution was shaken for 48 hours using a Sartorius Certomat BS-1 incubator shaker (Göttingen, Germany) at 200 rpm and a temperature of 25 °C to ensure equilibrium between estradiol and organic matter was reached. Estradiol degradation over 72 hours was explored in Chapter 4 and found to be negligible, therefore it was unlikely estradiol degraded significantly during the experiment. 10 mL of solution was removed and filtered through a 0.45 μm cellulose acetate filter using a 10 mL syringe. The cellulose acetate syringe filters (0.45 μm LCW 916, 26 mm diameter) were purchased from Hach Lange GmbH (Düsseldorf,
Germany). To determine estradiol adsorption, any material retained on the filter was desorbed using 10 mL of methanol using a different syringe.

A4.13. Analysis

1 mL of each of the feed, permeate and methanol used to desorb the filter were transferred to a 20 mL glass scintillation vials containing 7 mL of Ultima Gold LLT (Perkin Elmer, Waltham, USA). The methanol was not evaporated prior to analysis. The samples were analysed using a Beckman LS 6500 liquid scintillation counter (Fullerton, USA). The activity of the samples in disintegrations per minute (dpm) was converted to mass of estradiol (nanograms) in feed and permeate and mass desorbed from the filter. 1 mL samples were taken initially and at 48 hours, and both were at the expected hormone concentration suggesting estradiol was completely dissolved. In addition, the mass absorbed to the filter was also calculated using mass balance. Estradiol adsorption to the plastic syringe was measured, but was shown to be negligible. Using error propagation the random error (%E_{total}) associated with the experiments was 5.31%. %E represents the variability associated with the analytical equipment, including Hamilton syringes, micropipettes and electronic balances.

A4.2 Results and Discussion

A4.21. Mass of Estradiol in Permeate

The mass of estradiol in the permeate was graphed as function of pH and organic matter type for 100 ng/L estradiol concentration (Figure A4.1a). The mass in the permeate remained constant from pH 3 to 10 with approximately 50% of the total mass of estradiol recovered in the permeate. However, at pH 11 and 12 between 80-100% of the total mass of estradiol was present in the permeate. For all estradiol concentrations (50, 100 and 500 ng/L) the same trend was observed indicating concentration had no influence on results, therefore results for 100 ng/L were shown only
Figure A4.1: a) Mass of estradiol in 10 mL of permeate, b) mass of estradiol lost to the filter determined by methanol desorption, and c) mass of estradiol lost to the filter calculated from the mass balance (1 mM NaHCO$_3$, 20 mM NaCl, 100 ng/L estradiol, 12.5 mgC/L organic matter with exception of Aldrich HA which was also studied at 5 mgC/L)

Results from filtration in the presence of organic matter were similar; the mass of estradiol in the permeate from pH 3 to 10 was around 50%. In addition, when the pH
Appendix 4

was adjusted to 11 and 12 the mass of estradiol in the permeate was between 80-100%. This was observed for all organic matter types and at all concentrations. The molecular weights of all organics studied were considerably smaller than the pore size of the cellulose acetate filter, therefore none of the organic matter could be physically retained by size exclusion. However, adsorption of organic matter to the filter may be possible.

Estradiol retention by such filters was due to adsorption to the membrane surface, and the change in retention with pH was related to charge characteristics (Nghiem et al., 2005; Schäfer et al., 2003). The charge of estradiol from pH 3 to 10 was predominantly neutral, though it became negatively charged above the dissociation constant (pKₐ, 10.23) as it was a weak acid. Cellulose acetate was negatively charged at most pH values and it became increasingly negative at alkaline pH (Childress and Elimelech, 1996). The increase in negativity could be attributed to the dissociation of functional groups on the cellulose acetate surface. When estradiol was neutral adsorption could be attributed to hydrogen bonding between the hydroxyl functional groups, as they acted as both an electron donor and acceptor (Goss and Schwarzenbach, 2003). However, when estradiol was negatively charged at pH 11 and 12 electrostatic repulsion occurred between the filter and estradiol, and significantly reduced adsorption of estradiol on the filter. Consequently 80-100% of the total mass of estradiol was in the permeate.

The percentage of estradiol present in the permeate was dependent on filtrate volume. Using a range of volumes from 2 mL to 500 mL at pH 7 with an initial concentration of 100 ng/L the percentage of estradiol in the permeate increased as the filtered sample volume increased (Figure A4.2). At 2 mL the permeate only contained 20% of the total estradiol, while at 500 mL approximately 90% of total estradiol was present in the permeate, showing a ‘breakthrough’ phenomenon. However, significant losses of estradiol from the permeate are still observed at 50 and 100 mL sample volumes, with only 70-80% of total estradiol recovered in the permeate. To put these results in context, cellulose acetate syringe filters used for
pre-treatment were often used to filter volumes of 10 mL or less (Karlsson et al., 1999).

![Graph showing percentage of estradiol in permeate as a function of sample volume](image)

**Figure A4.2:** Percentage of estradiol present in the permeate as a function of solution volume (1 mM NaHCO₃, 20 mM NaCl, pH 7, 100 ng/L estradiol)

### A4.2.2. Mass of Estradiol Loss

The mass of analyte lost to the filter was measured by desorbing analyte from the filter with methanol. Results were verified using mass balance. The mass of estradiol lost to 0.45 µm cellulose acetate filters were graphed as a function of pH and organic matter type for 100 ng/L estradiol concentration (Figure A4.1b and c) in comparison to total mass of analyte in the feed. Both the experimentally determined mass lost (based on methanol desorption) (Figure A4.1b), and mass lost based on mass balance (Figure A4.1c) showed a general trend of approximately 50% estradiol retained until pH 10, which decreased greatly at pH 11 and 12 to between 0-20% estradiol retention.

Similar to the permeate results, the trend was related to the interaction of estradiol with the filter. However, with the exception of estradiol only and alginic acid experiments, the mass of estradiol desorbed from the filter using 10 mL methanol was lower than the calculated mass loss based on the mass balance. This difference
was greatest for 5 mgC/L humic acid, as only 60–70% of adsorbed estradiol calculated from the mass balance could be desorbed by the methanol. For Australian NOM around 85% of estradiol was desorbed, while approximately 90-95% of estradiol was desorbed in the presence of 12.5 mgC/L humic acid and IHSS NOM. This suggested that in the presence of organic matter (with the exception of alginic acid) methanol was not sufficient to desorb the analyte.

Previous studies have demonstrated estradiol could interact strongly with organic matter including NOM surrogates and tannic acid (Yamamoto and Liljestrand, 2003; Yamamoto et al., 2003). In addition, NOM surrogates, particularly humic acid, could adsorb to cellulose acetate membranes through hydrophobic interactions (Childress and Deshmukh, 1998). As a result the interaction of estradiol with organic matter had an influence on the desorption of adsorbed estradiol from the filter using methanol. In Chapter 6 experimental organic matter-water partition coefficient (log $K_{OM}$) for estradiol and humic acid at pH 7 was 4.21, which was greater than the estradiol octanol-water partition coefficient (log $K_{OW}$), 4.01 (Hansch et al., 1995; Nghiem et al., 2004), indicating the preference of estradiol to sorb to humic acid compared to the solvent. Therefore, estradiol could remain bound to the filter in the presence of humic acid. In contrast, experimental log $K_{OM}$ value in Chapter 6 for alginic acid at pH 7 was 3.96, and as this was lower than log $K_{OW}$ value it was likely any estradiol interacting with alginic acid would be desorbed by methanol, resulting in no difference between mass balance and experimental (methanol) desorption of estradiol from the filter in the presence of alginic acid.

A4.3. Conclusions

The results suggest that as much as 50% of the analyte estradiol in a sample could be lost to cellulose acetate 0.45 µm filters at pH 3 to 10. This was related to the adsorption of estradiol to the membrane in this pH range. Further, the presence of organic matter, particularly humic acid, reduced desorption of estradiol from the filter. Adsorption could be controlled by filtering the micropollutant when it was dissociated and hence charged. In the case of estradiol the pH could be adjusted to 11
prior to pre-filtration with cellulose acetate syringe filters. Alternatively, different filter material such as glass fibre filters (Holbrook et al., 2004; Williams et al., 2003) or hydrophilic HVLP (Rodriguez-Mozaz et al., 2004) could be selected. However, further studies are required to assess the loss of steroidal hormones to different types of filter material as well as the impact of solute-solute interactions. Results illustrated using the example of estradiol were applicable to other micropollutants, although the specific interaction with the cellulose acetate filter in the presence of organic and inorganic matrices need to be studied on an individual basis as such interactions are micropollutant specific. Previous studies have shown that other hormones, such as estrone, progesterone and testosterone behave similarly to estradiol (Nghiem et al., 2004). The pKₐ of estrone is 10.34 (Kwon et al., 2006), therefore adsorption to the filter could be minimised above pH 10. However, progesterone and testosterone do not dissociate, and therefore pH adjustment could not be used to prevent the problem.
**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AER</td>
<td>Anion exchange resin</td>
</tr>
<tr>
<td>AFM</td>
<td>Atomic force microscopy</td>
</tr>
<tr>
<td>CMC</td>
<td>Critical micelle concentration</td>
</tr>
<tr>
<td>COM</td>
<td>Colloidal organic matter</td>
</tr>
<tr>
<td>CPMAS</td>
<td>Cross-polarisation magic-angle spinning</td>
</tr>
<tr>
<td>DOC</td>
<td>Dissolved organic carbon</td>
</tr>
<tr>
<td>DOM</td>
<td>Dissolved organic matter</td>
</tr>
<tr>
<td>dpm</td>
<td>Disintegrations per minute</td>
</tr>
<tr>
<td>DVB</td>
<td>Divinylbenzene</td>
</tr>
<tr>
<td>E1</td>
<td>Estrone</td>
</tr>
<tr>
<td>E2</td>
<td>$17\beta$ Estradiol</td>
</tr>
<tr>
<td>EDX</td>
<td>Energy dispersive X-ray</td>
</tr>
<tr>
<td>EPS</td>
<td>Extracellular polymeric substances</td>
</tr>
<tr>
<td>ESEM</td>
<td>Environmental scanning electron microscope</td>
</tr>
<tr>
<td>FA</td>
<td>Fulvic acid</td>
</tr>
<tr>
<td>FIB-SEM</td>
<td>Focused ion beam scanning electron microscope</td>
</tr>
<tr>
<td>FQ</td>
<td>Fluorescence quenching</td>
</tr>
<tr>
<td>HA</td>
<td>Humic acid</td>
</tr>
<tr>
<td>IHSS</td>
<td>International Humic Substances Society</td>
</tr>
<tr>
<td>IX</td>
<td>Ion exchange</td>
</tr>
<tr>
<td>LFER</td>
<td>Linear free energy relationship</td>
</tr>
<tr>
<td>LSER</td>
<td>Linear solvation energy relationship</td>
</tr>
<tr>
<td>MF</td>
<td>Microfiltration</td>
</tr>
<tr>
<td>MIEX®</td>
<td>Magnetic ion exchange</td>
</tr>
<tr>
<td>MWCO</td>
<td>Molecular weight cut-off</td>
</tr>
<tr>
<td>nd-SPME</td>
<td>Negligible depletion solid-phase microextraction</td>
</tr>
<tr>
<td>NF</td>
<td>Nanofiltration</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>NOM</td>
<td>Natural organic matter</td>
</tr>
<tr>
<td>NPOC</td>
<td>Non-purgeable organic carbon</td>
</tr>
<tr>
<td>PA</td>
<td>Polyacrylate</td>
</tr>
<tr>
<td>PAH</td>
<td>Polycyclic aromatic hydrocarbons</td>
</tr>
<tr>
<td>PCB</td>
<td>Polychlorinated biphenyl</td>
</tr>
<tr>
<td>PDMS</td>
<td>Polydimethylsiloxane</td>
</tr>
<tr>
<td>POM</td>
<td>Particulate organic matter</td>
</tr>
<tr>
<td>QSAR</td>
<td>Quantitative structure-activity relationships</td>
</tr>
<tr>
<td>RMS</td>
<td>Root mean square</td>
</tr>
<tr>
<td>RO</td>
<td>Reverse osmosis</td>
</tr>
<tr>
<td>RPM</td>
<td>Revolutions per minute</td>
</tr>
<tr>
<td>S.D</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SDS</td>
<td>Sodium dodecyl sulphate</td>
</tr>
<tr>
<td>SE</td>
<td>Solubility enhancement</td>
</tr>
<tr>
<td>SPME</td>
<td>Solid-phase microextraction</td>
</tr>
<tr>
<td>SUVA</td>
<td>Specific UV absorbance</td>
</tr>
<tr>
<td>TOC</td>
<td>Total organic carbon</td>
</tr>
</tbody>
</table>
Abbreviations and Symbols

TPR  Templated polymer resin
UF   Ultrafiltration
UVA  Ultraviolet absorbance

Symbols

Chapter 2

$C_S$  Concentration of micropollutants adsorbed to particulate organic matter at equilibrium (Freundlich Isotherm)
$C_W$  Concentration of freely dissolved micropollutants in solution at equilibrium
$K_D$  Solid-water distribution coefficient (L/kg).
$K_F$  Freundlich constant
$K_L$  Langmuir constant
$K_{OC}$  Organic carbon normalised-water partition coefficient (L/kg)
$K_{OM}$  Organic matter-water partition coefficient (L/kg)
$K_{OW}$  Octanol-water partition coefficient (dimensionless)
$n$  Empirical constant used to reflect non linearity (Freundlich Isotherm)
$pK_a$  Acid dissociation constant
$Q_{MAX}$  Maximum amount of micropollutant adsorbed to the organic matter (Langmuir Isotherm)

Greek Symbols

$\phi_{OM}$  Micropollutant fugacity in organic matter
$\phi_{W}$  Micropollutant fugacity in water

Chapter 3

$A$  Membrane area (m$^2$ for flux experiments and cm$^2$ for adsorption experiments)
$C_{Bi}$  Concentration within the cell ignoring membrane adsorption (ng/L or mgC/L)
$C_C$  Concentrate concentration (ng/L or mgC/L)
$C_F$  Concentration of steroidal hormone on fibre (ng/L)
$C_{FD}$  Feed concentration (ng/L or mgC/L)
$C_{OM}$  Concentration of steroidal hormone sorbed to organic matter (ng/kg).
$C_{Pi}$  Permeate concentration based on the 4$^{th}$ sample (ng/L or mgC/L)
$C_V$  Coefficient of variance
$C_W$  Concentration of freely dissolved steroidal hormone in aqueous solution (ng/L)
$D$  Diffusivity (m$^2$/h)
$D_S$  Solute diffusion constant (m$^2$/s)
$\%E$  Relative error
$\%E_{total}$  Total random error
$f_{W}$  Fraction of freely dissolved hormone in solution at equilibrium (%)
$J$  Flux (L/m$^2$.hr)
$J_0$  Pure water flux (L/m$^2$.hr)
### Abbreviations and Symbols

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_1$</td>
<td>Uptake rate of steroidal hormone to fibre (min)</td>
</tr>
<tr>
<td>$K_1^1$</td>
<td>Uptake rate of steroidal hormone to fibre (ng.min)</td>
</tr>
<tr>
<td>$K_2$</td>
<td>Release rate of steroidal hormone from fibre (min)</td>
</tr>
<tr>
<td>$K_B$</td>
<td>Boltzmann’s constant (J.K)</td>
</tr>
<tr>
<td>$K_{FW}$</td>
<td>Fibre-water partition coefficient (dimensionless)</td>
</tr>
<tr>
<td>$K_{OM}$</td>
<td>Organic matter-water partition coefficient (L/kg)</td>
</tr>
<tr>
<td>$K_{OW}$</td>
<td>Octanol-water partition coefficient (dimensionless)</td>
</tr>
<tr>
<td>$M$</td>
<td>Molecular weight (Da)</td>
</tr>
<tr>
<td>$m_{ADS}$</td>
<td>Mass of hormone (ng/m2) or organic matter (mg/m2) adsorbed per unit membrane area</td>
</tr>
<tr>
<td>$m_{DOM}$</td>
<td>Mass of dissolved organic matter in solution (kg)</td>
</tr>
<tr>
<td>$m_f$</td>
<td>Mass of steroidal hormone on fibre (ng)</td>
</tr>
<tr>
<td>$m_{OM}$</td>
<td>Mass of steroidal hormone sorbed to organic matter (ng)</td>
</tr>
<tr>
<td>$m_{TOT}$</td>
<td>Initial mass of steroidal hormone in aqueous solution (ng)</td>
</tr>
<tr>
<td>$m_W$</td>
<td>Mass of steroidal hormone freely dissolved in aqueous solution at equilibrium (ng)</td>
</tr>
<tr>
<td>$n$</td>
<td>Sample size</td>
</tr>
<tr>
<td>$n_i$</td>
<td>Slope of linear regression</td>
</tr>
<tr>
<td>$pK_a$</td>
<td>Acid dissociation constant</td>
</tr>
<tr>
<td>$r$</td>
<td>Pore radius (m)</td>
</tr>
<tr>
<td>$R_{H}$</td>
<td>Hormone retention by the membrane (%)</td>
</tr>
<tr>
<td>$R_{OM}$</td>
<td>Organic matter retention by the membrane (%)</td>
</tr>
<tr>
<td>$R_{PS}$</td>
<td>Predicted hormone retention due to solute-solute interactions (%)</td>
</tr>
<tr>
<td>$s^2$</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>$T$</td>
<td>Temperature (K)</td>
</tr>
<tr>
<td>$t$</td>
<td>Time (hours)</td>
</tr>
<tr>
<td>$</td>
<td>t</td>
</tr>
<tr>
<td>$V$</td>
<td>Permeate volume (L) (flux measurement)</td>
</tr>
<tr>
<td>$V_B$</td>
<td>Volume in remaining in the cell, and is calculated by subtracting the cumulative permeate volume from the feed volume (L)</td>
</tr>
<tr>
<td>$V_C$</td>
<td>Concentrate volume (L)</td>
</tr>
<tr>
<td>$V_{FD}$</td>
<td>Initial feed volume (L)</td>
</tr>
<tr>
<td>$V_F$</td>
<td>Volume of fibre coating (L)</td>
</tr>
<tr>
<td>$V_{Pi}$</td>
<td>Permeate volume (L)</td>
</tr>
<tr>
<td>$V_W$</td>
<td>Volume of aqueous solution (L)</td>
</tr>
</tbody>
</table>

**Greek Symbols**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\bar{x}$</td>
<td>Sample mean</td>
</tr>
<tr>
<td>$\chi$</td>
<td>Organic matter-water partition coefficient (L/kg) in two-sample t-test</td>
</tr>
<tr>
<td>$\eta$</td>
<td>Dynamic viscosity (Pa.s)</td>
</tr>
</tbody>
</table>

### Chapter 4

- $K_{FW}$  | Fibre-water partition coefficient (dimensionless) |
- $K_{OM}$ | Organic matter-water partition coefficient (L/kg) |
- $K_{OW}$ | Octanol-water partition coefficient |
- $pK_a$  | Acid dissociation constant |
### Abbreviations and Symbols

#### Chapter 5

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>( f_{\text{Neutral}} )</td>
<td>Fraction of neutral species (%)</td>
</tr>
<tr>
<td>( K_{\text{OM}} )</td>
<td>Organic matter-water partition coefficient (L/kg)</td>
</tr>
<tr>
<td>( K_{\text{OW}} )</td>
<td>Octanol-water partition coefficient</td>
</tr>
<tr>
<td>( pK_a )</td>
<td>Acid dissociation constant</td>
</tr>
<tr>
<td>( R )</td>
<td>Correlation coefficient</td>
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</table>

#### Chapter 6

<table>
<thead>
<tr>
<th>Symbol</th>
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<tbody>
<tr>
<td>( A )</td>
<td>Overall hydrogen bond acidity (hydrogen donor)</td>
</tr>
<tr>
<td>( a )</td>
<td>Difference in hydrogen bond acidity of organic matter and water</td>
</tr>
<tr>
<td>( B )</td>
<td>Overall hydrogen bond basicity (hydrogen acceptor)</td>
</tr>
<tr>
<td>( b )</td>
<td>Difference in hydrogen bond basicity of organic matter and water</td>
</tr>
<tr>
<td>( c )</td>
<td>Solute specific free energy constant</td>
</tr>
<tr>
<td>( D_{\text{OW}} )</td>
<td>pH dependent octanol-water partition coefficient</td>
</tr>
<tr>
<td>( E )</td>
<td>Excess molar refraction (cm(^3)/mol)</td>
</tr>
<tr>
<td>( e )</td>
<td>Difference in capacity for organic matter and water to interact through polarization</td>
</tr>
<tr>
<td>( f_{\text{Neutral}} )</td>
<td>Fraction of neutral species (%)</td>
</tr>
<tr>
<td>( G )</td>
<td>Gibbs free energy</td>
</tr>
<tr>
<td>( \Delta G_{\text{OM}} )</td>
<td>Transfer of Gibbs free energy from one phase to another, such as organic matter to water</td>
</tr>
<tr>
<td>( G_{\text{cavity-OM}} )</td>
<td>Free energy required to form and close a cavity in organic matter</td>
</tr>
<tr>
<td>( G_{\text{cavity-water}} )</td>
<td>Free energy required to form and close a cavity in water</td>
</tr>
<tr>
<td>( G_{\text{cohesion}} )</td>
<td>Cohesive free energy</td>
</tr>
<tr>
<td>( G_{\text{interaction-OM}} )</td>
<td>Free energy of micropollutant interaction with organic matter</td>
</tr>
<tr>
<td>( G_{\text{interaction-water}} )</td>
<td>Free energy of micropollutant interaction with water</td>
</tr>
<tr>
<td>( \Delta H )</td>
<td>Enthalpy</td>
</tr>
<tr>
<td>( \Delta S )</td>
<td>Entropy</td>
</tr>
<tr>
<td>( S )</td>
<td>Dipolarity/polarizability</td>
</tr>
<tr>
<td>( s )</td>
<td>Difference in capacity for organic matter and water to interact through dipole-dipole interactions</td>
</tr>
<tr>
<td>( s^2 )</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>( T )</td>
<td>Temperature</td>
</tr>
<tr>
<td>(</td>
<td>t</td>
</tr>
<tr>
<td>( V )</td>
<td>McGowan’s characteristic volume (cm(^3)mol/100)</td>
</tr>
<tr>
<td>( v )</td>
<td>Ease of cavity formation</td>
</tr>
</tbody>
</table>

#### Greek Symbols

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \chi )</td>
<td>Experimental organic matter-water partition coefficient (L/kg)</td>
</tr>
<tr>
<td>( \mu )</td>
<td>LFER modelled organic matter-water partition coefficient (L/kg)</td>
</tr>
</tbody>
</table>
Abbreviations and Symbols

Chapter 7

J  Flux (L/m².hr)
J₀  Pure water flux (L/m².hr)
K_{OC}  Organic carbon normalised-water partition coefficient (L/kg)
K_{OM}  Organic matter-water partition coefficient (L/kg)
K_{OW}  Octanol-water partition coefficient (dimensionless)
m_{DOM}  Mass of dissolved organic matter in solution (kg)
m_{OM}  Mass of steroidal hormone sorbed to organic matter (ng)
pKₐ  Acid dissociation constant
Rₚ₈  Hormone retention by the membrane (%)  
R_{DOM%}  Organic matter retention by the membrane (%)  
R_{P%}  Predicted hormone retention due to solute-solute interactions (%)  

Chapter 8

A  Membrane area (m²)
dₚ  Average MIEX® particle diameter (m)
J  Flux (L/m².hr)
J₀  Pure water flux (L/m².hr)
ΔP  Pressure (bar)
pKₐ  Acid dissociation constant
Mₚ  Mass of MIEX® deposited (kg)
Rₚ  Hydraulic resistance due to fouling (m)
Rₘ  Hydraulic resistance of the clean membrane (m)
Rₜ  Total hydraulic resistance of the membrane (m)

Greek Symbols

α  Specific resistance of the deposit on the membrane (m/kg).
ε  Porosity (-)
η  Dynamic viscosity of water (Pa.s)
ρₚ  MIEX® density (kg/m³)

Chapter 9

Cₘ  Amount sorbed specific to surface area (ng/cm²).
Cₘ₃  Concentration of freely dissolved micropollutant in solution (ng/L)
Kₘ  Solid-water distribution coefficient (L/kg).
Kₘ₃  Membrane-water partition coefficients (L/cm²)
K_{OC}  Organic carbon normalised-water partition coefficient (L/kg)
K_{OM}  Organic matter-water partition coefficient (L/kg)
K_{OW}  Octanol-water partition coefficient (dimensionless)

Appendix 2

Vₐ  Liquid scintillation cocktail volume (mL)
Vₜ  Sample volume (mL)

Greek Symbols
Abbreviations and Symbols

\( \lambda \)  
Wavelength

**Appendix 3**

\( C_V \)  
Coefficient of variance

\%E  
Relative error

\%E_{total}  
Total random error

\( R_p\% \)  
Predicted hormone retention due to solute-solute interactions (%)

\( s^2 \)  
Standard deviation

**Greek Symbols**

\( X \)  
Sample mean

**Appendix 4**

\%E  
Relative error

\%E_{total}  
Total random error

\( K_{OM} \)  
Organic matter-water partition coefficient (L/kg)

\( K_{OW} \)  
Octanol-water partition coefficient (dimensionless)

\( pK_a \)  
Acid dissociation constant
References


References


References


References


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