Sorption of steroidal hormones by electrodialysis membranes

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Abstract

The mechanisms of sorption of four steroidal hormones – estradiol, estrone, progesterone and testosterone – to electrodialysis (ED) membranes were investigated as a function of solution pH and presence of humic acid (HA). Hormone-membrane partition coefficients (log $K_{AEM/CEM}$) determined through sorption isotherm experiments suggested that hormone sorption was due to hydrogen bonding and cation–π interactions between hormone and membrane functional groups. Progesterone sorption at pH 7 (922 µg/cm³) during ED was greater than estrone sorption (591 µg/cm³) due to its greater cation-exchange membrane (CEM) bonding affinity. Estrone sorption at pH 11 (487 µg/cm³) was reduced due to estrone dissociation and electrostatic repulsion with negatively charged CEMs. Permeation of estrone (30-100 ng/cm².h) through the anion-exchange membranes (AEMs) was observed. At pH 11, charge repulsion between estrone and HA coupled with AEM electrostatic attraction resulted in increased sorption. Partial membrane desorption was noted in isotherm (20-30%) and ED desorption (3.8%) experiments and was dependent on the initial mass sorbed, solution pH and resultant electrostatic interactions.

Keywords: Electrodialysis; Hormones; Adsorption; Ion exchange membranes; Organic matter.

1. Introduction

The presence of steroidal hormones at low concentrations (0.1-10 ng/L) in effluents from conventional wastewater treatment plants (WWTPs), receiving waterways and drinking water have received widespread attention [1-3]. The impact of hormones are prominent as they have higher endocrine disrupting potency than other endocrine disrupting chemicals (EDCs) [4]. Numerous studies have linked exposure to trace levels of EDCs to declining male sperm count and increases in occurrence of testicular, prostate, ovarian and breast cancer [5, 6]. EDCs also have potential to interfere with the endocrine system of fish, amphibians, birds, reptiles and mammals [7, 8].

ED is a competing process to pressure driven membrane processes such as reverse osmosis (RO) for brackish water and water reuse applications. However, any contaminant that RO is designed to retain occurs in elevated concentrations in the concentrate making its discharge to the environment questionable. These concentrates contain salt, nutrients and inorganic and organic

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contaminants such heavy metals and steroidal hormones [9]. Currently there has been little research on the treatment of these concentrates [10], but the treatment of this waste stream will improve the health of receiving waters and reduce the risk of increased build up of contaminants if these wastes are recycled through wastewater treatment plants. While the treatment of steroidal hormones by membrane processes such as microfiltration (MF), nanofiltration (NF) and RO have been widely reported [11, 12], studies on the fate of hormones in ED are limited. Pronk *et al*. [13] observed considerable sorption of 17α-ethinylestradiol (75%) to membranes during batch ED experiments for the treatment of urine. However, the mechanisms governing hormone sorption by ion-exchange membranes are not understood.

The complexation of polar organic compounds with organic matter (OM) has previously been reported [14]. HA, a component of OM, can cause serious fouling in ED due to its negative charge and subsequent sorption by AEMs [15]. Previous studies have shown that hormone sorption to membranes in other membrane processes are dependent on solution pH and properties of the membrane, hormone and OM [16, 17]. The influence of OM on hormone sorption in ED is unknown.

The purpose of this study was to elucidate the fate of steroidal hormones in ED and to determine the influence of solution pH, OM and hormone type on these interactions. An understanding of the partitioning of hormones between water and ion-exchange membranes (log $K_{AEM/CEM}$) is important for the prediction of their fate in ED. Therefore, differences in sorption of the hormones estradiol, estrone, progesterone and testosterone to ion-exchange membranes were investigated in sorption isotherm experiments. The behaviour of progesterone and estrone during batch and continuous ED experiments were evaluated to identify differences in sorption between undissociated (progesterone at pH 7 and 11, estrone at pH 7) and dissociated compounds (estrone pH 11). ED experiments were conducted with and without HA.

2. Materials and Methods

2.1. Chemicals

All chemicals used were of analytical grade. The background solution was comprised of 5 g/L NaCl and 84 mg/L NaHCO₃ (Fisher Scientific, UK). NaOH and HCl used for pH adjustments (1 mol/L) and membrane desorption experiments (0.002 mol/L) and Na₂SO₄ (0.5 mol/L) used in the electrode rinse were purchased from Fisher Scientific (UK). Radiolabeled [2,4,5,7-³H] 17β-estradiol, [2,4,5,7-³H] estrone, [2,4,5,7-³H] progesterone and [2,4,5,7-³H] testosterone (> 98.5% purity; 37 MBq/mL) were purchased from GE Healthcare (UK). Non-labelled hormones (≥ 98% purity) were purchased from Sigma Aldrich (UK). Stock solutions of radiolabeled (100 µg/L) and non-labelled (990 mg/L and 1000 mg/L) hormones were prepared in methanol (CH₃OH) (Fisher Scientific, UK). Physicochemical properties of the hormones are outlined in Table 1.

The OM used was HA sodium salt (Sigma Aldrich, UK). While the concentration of OM in treated wastewater and natural waters is highly variable (0.5-100 mg C/L) [18], 12.5 mg C/L was used for experiments containing HA. The negatively charged HA (neutral-basic pH) includes carboxylic, phenolic, alcohol/aldehyde acids and methoxyl functional groups [19].

[Table 1]

2.2. Sorption isotherm and desorption experiments

Radiolabeled (100 ng/L) and non-labelled hormones were added to 100 mL background solutions (adjusted to pH 7) to make the following concentrations: 0.1, 0.5, 1, 10, 100 and 2500 µg/L. A 2 cm² segment of AEM or CEM (3 replicates/membrane) was added to each solution bottle and shaken in an incubator shaker (Certomat BS-1, Sartorius) at 200 RPM and 25°C for 100 hours. Samples (0.5 mL) were taken from each bottle before membrane addition and periodically during each experiment for hormone analysis. Solutions without membrane addition were shaken to determine possible hormone sorption to the glass bottles. Hormone desorption from the membranes

used in the 1 µg/L isotherm experiments was determined by AEM or CEM addition to 100 mL solutions of 0.002 mol/L NaOH and HCl and ultrapure water (UW) shaken for 288 hours.

2.3. Electrodialysis system, membranes and protocol

ED experiments were carried out using a BEL-500 ED stack (Berghof, Germany) with six Neosepta AEMs and seven CEMs (supplied by Eurodia, Germany; manufactured by ASTOM Corporation, Japan) with an available membrane area of 58 cm^2 each. The membranes contain alkylammonium (AEM) and sulfonic acid (CEM) ion-exchange groups, attached to a polystyrene-divinylbenzene matrix (PS-DVB) on a polyvinyl chloride (PVC) gel supported by PVC cloth [20]. The thicknesses of the AEMs and CEMs were 0.14 and 0.17 mm, respectively. The volumes of the AEMs and CEMs were 4.9 and 6.9 cm^3, respectively [21]. The stack was connected to a DC power supply (Model GPR-1810HD, GW Instek, Taiwan) with an applied voltage of 10 V fixed for all ED experiments. The feed, diluate, concentrate and electrode rinse flow rate was 1.5 L/min (I/P Variable speed pump system, Masterflex, USA).

Continuous (diluate and concentrate recirculated to one feed container) and batch (separate diluate and concentrate containers) experiments were undertaken. Feed solutions (2500 µg/L progesterone or estrone, 4L total) for continuous ED experiments were prepared in the background solution. The hormone concentration used in the ED experiments was greater than the concentration found in natural waters due to the high sorption capacity of the membranes. To determine the influence of solution pH on hormone sorption during continuous ED experiments, the feed pH was maintained constant by the addition of 1 mol/L HCl and/or NaOH. Before the continuous experiments with HA were performed, the feed was stirred for 24 hours to allow for hormone-HA equilibrium. Sorption within the diluate and concentrate was evaluated in batch experiments undertaken after the completed continuous experiments (continuous solution separated into diluate and concentrate). Due to estrone dissociation at pH 11, extended batch ED experiments (estrone concentration 2500 µg/L) were carried out to evaluate possible estrone breakthrough. Desorption of estrone in ED was evaluated, whereby the diluate and concentrate was filled with background electrolyte solution (adjusted to pH 7) and the system was rerun in batch desalination mode. Samples were collected at the beginning of each ED experiment and periodically for hormone (0.5 mL) and UV-Vis absorbance (3 mL) analysis.

2.4. Analytical methods

Hormone samples (0.5 mL) were mixed with 3.5 mL Ultima Gold® LLT (Perkin Elmer, UK) and analysed using a scintillation counter (LS 6500, Beckman Coulter, USA). Hormone concentration was ascertained from a linear regression performed on calibration standards (0.01, 0.1, 1, 10, 100 and 1000 ng/L). The pH, electrical conductivity and temperature of samples periodically taken from the feed, diluate and concentrate during ED experiments were measured (Multiline P4 pH electrode, WTW, Germany). UV-Visible Spectrometry (Varian Cary 100 Scan, UK) was used to determine the absorbance of HA (wavelength of 254 nm) in samples.

3. Results and Discussion

3.1. Hormone sorption in batch sorption isotherm tests

Hormone concentration decreased significantly in the isotherm experiments indicating membrane sorption with two sorption processes: (1) Initial surface sorption and (2) diffusion limited sorption within the membrane (Figure S1). The amount of hormone sorbed (log $C_{AEM/CEM}$) increased as the solution phase concentration (log C_w^t) increased (R > 0.99) (Figure 1). Isotherm deviation from linearity at 2500 µg/L indicates that membrane sites were beginning to be saturated. The hormone-membrane partition coefficients (log $K_{AEM/CEM}$) are given in Table 2.

Photodegradation and biotransformation of hormones from aqueous samples have been reported [22]. Control sorption experiments using covered solutions and biocide addition were carried out to measure hormone sorption to and/or volatilisation from the sample bottles. There was no significant difference between the control (e.g. Estradiol, Covered: AEM 155.2 ± 3.8 ng/cm^3,

CEM 85.5 ± 2.7 ng/cm^3; Biocide: AEM 153.4 ± 3.7 ng/cm^3, CEM 80.0 ± 3.7 ng/cm^3) and experiments without degradation prevention (AEM 159.0 ± 2.8 ng/cm^3; CEM 84.9 ± 1.2 ng/cm^3). Sorption to glassware was minimal with the bulk lost within 48 hours (% of initial hormone mass, Estradiol: 3.2 ± 1.1%, Estrone: 2.7 ± 0.9%, Progesterone: 3.4 ± 2.3%, Testosterone: 3.9 ± 1.2%). Log $K_{AEM/CEM}$ values were adjusted accordingly to account for this loss.

[Figure 1Figure]

[Table 2]

3.2. Hormone sorption mechanisms

Pronk *et al*. [13] postulated that hormone sorption to ion-exchange membranes was related to hydrophobicity. Poor correlation between the log K_{ow} (Table 1) and log $K_{AEM/CEM}$ values (Figure S3, SI) suggests other mechanisms contribute to sorption at neutral pH. Since the hormones are undissociated at pH 7 (pH of isotherm experiments) electrostatic interactions are not possible. Previous studies have suggested hydrogen bonding as the mechanism for the adsorption of hormones to membranes [12, 23]. The possible hydrogen bonding formations between the hormones that exhibited strongest AEM (estrone) and CEM (progesterone) sorption are illustrated in Figure 2. Hormones can be hydrogen-donors (contain phenolic OH groups) or hydrogen-acceptors (contain C=O groups). The AEM functional group (N(CH$_3$)$_3$) can bond with molecules containing hydrogen-donor and acceptor groups [24] and presents more opportunities for bonding than the CEM, thus accounting for the higher log K_{AEM} values. Since the AEM functional group is dissociated and may be strongly hydrated, another interaction mechanism is proposed which is cation–π interactions [25]. The interaction of RNH$_3^+$ with double bonds is thought as a form of X-H··π hydrogen bonds. These interactions can further explain the higher hormone sorption to the AEMs. Estrone and estradiol sorption to the AEM would be facilitated through bonding between the AEM CH$_3$ hydrogen-donor group and the C-17 C=O and C-17 OH groups, respectively, coupled with minor contribution from the predominantly hydrogen-donor C-3 OH group [26]. The higher log K_{AEM} for estrone (0.53 ± 0.13 L/cm^3) compared to estradiol (0.39 ± 0.10 L/cm^3) suggests the bonding strength of C-17 OH in estradiol is lower compared to C-17 C=O in estrone. Testosterone exhibited the lowest sorption by AEM due to the poor hydrogen accepting ability of its C-17 OH group [26].

[Figure 2]

Studies on the determination of steroids using molecularly imprinted polymers (MIPs) found that the C-17 OH group is more important for interactions compared to the C-3 OH group due to steric constraints between the MIPs and C-3 OH groups [27]. Hormone sorption to ion-exchange membranes would be influenced by hormone structure and the space available for interaction. The lower log K_{AEM} of progesterone can be attributed to the steric constraints around the C-20 C=O group available for approaching the AEM compared to the C-3 C=O group [28]. Although estrone and progesterone both contain C-17,20 C=O groups, studies have demonstrated that the C-3 C=O moiety in progesterone is a triple hydrogen acceptor (i.e. can accept hydrogens directed from 3 positions) and provides for more space for approaching the CEM hydrogen-bond donors [28, 29]; thus explaining the greater log K_{CEM} for progesterone (0.22 ± 0.13 L/cm^3) than estrone (log K_{CEM} 0.04 ± 0.01 L/cm^3), testosterone (log K_{CEM} -0.16 ± 0.05 L/cm^3) and estradiol (log K_{CEM} -0.24 ± 0.05 L/cm^3). These results suggest that the C-17 OH group in estradiol and testosterone is not as strong as the C-20 and C-17 C=O group in progesterone and estrone, respectively. This is substantiated by Gancia *et al*. [30] who quantitatively estimated the hydrogen bonding strengths of hydrogen donor (log K_α, C=O) and acceptor (log K_β, OH) functional groups in a range of chemical structures. It was found that the hydrogen bonding strength of the C-17,20 C=O group (log K_β 1.52-1.61) was greater

than the C-17 OH group (log K_α 0.91), thus explaining the higher log K_{AEM} for estrone (log K_β 1.61) compared to estradiol (log K_β 1.36).

3.3. Hormone sorption in Electrodialysis

3.3.1. Effect of solution pH

ED experiments were carried out to elucidate the mechanisms of hormone sorption in ED. The mass of progesterone and estrone sorbed per unit volume of membrane within the ED stack (μg/cm^3) during continuous ED experiments is shown in Figure 3. Progesterone sorbed more than estrone at pH 7 as a result of the greater sorption of progesterone to the CEMs and the larger volume of CEMs within the ED stack compared to the AEMs. The mass of progesterone sorbed at pH 7 (922 ± 28 μg/cm^3) was similar to the mass sorbed at pH 11 (874 ± 26 μg/cm^3) due to progesterone being undissociated under both pH conditions. While sorption kinetics (Figure S2) demonstrated rapid sorption within 4 hours, constant hormone mass sorbed was not reached indicating membrane diffusion. After the feed solution was separated into diluate and concentrate, progesterone sorption to the membranes continued within the diluate and concentrate (Figure 4). Pronk *et al.* [13] assumed that neutral compounds sorb to both AEMs and CEMs, which is confirmed by these results.

[Figure 3]

The mass of estrone sorbed at pH 11 (487 ± 24 μg/cm^3) was less than the mass sorbed at pH 7 (591 ± 30 μg/cm^3) due to estrone dissociation (pK_a 10.4; Table 1). At pH 7, estrone sorption would occur on both the AEMs and CEMs facing the diluate and concentrate. This continues at pH 11 for the neutral fraction, while dissociated estrone no longer sorbs to the negatively charged CEMs due to electrostatic repulsion. At pH 11 estrone sorption within the diluate (116.4 ± 5.9 μg/cm^3) was greater than within the concentrate (19.8 ± 1.0 μg/cm^3, Figure 4), indicating preferential transport towards the positively charged AEMs facing the diluate. Therefore, AEM penetration by dissociated estrone is possible at pH 11.

[Figure 4]

While estrone flux was low (30-100 ng/cm^{2}h), breakthrough into the concentrate was noted after 10 hours of extended batch ED experiments (Figure 5), confirming estrone diffusion through the AEM pores, of which ED membranes have an approximate radius of 3 nm [31], to the concentrate. The low flux also indicates that after estrone molecules penetrate the AEM they find more binding sites within the membrane to interact with. These results correlate with the slow diffusion kinetics demonstrated in Figure S2 and are in accordance with literature [13], where permeation of dissociated organic contaminants increased with membrane sorption. Therefore, it is postulated that estrone permeation is dependent on sorption to the membrane surface, diffusion through the AEM, desorption and diffusion from opposing membrane boundary layer.

[Figure 5]

3.3.2. Effect of organic matter

Solute-solute interactions, such as hormone and OM complexation, have implications on hormone removal during wastewater treatment [17]. The mass of progesterone sorbed decreased in the presence of HA (pH 7: 758 ± 23 μg/cm^3, pH 11: 739 ± 22 μg/cm^3; Figure 3). The same trend was noted with estrone at pH 7 (535 ± 16 μg/cm^3), while at pH 11 estrone sorption was slightly higher with HA (508 ± 15 μg/cm^3). Neale *et al.* [32] reported high partitioning of hormones to HA (log K_{OM}: Progesterone: pH 7 4.59 ± 0.25 L/kg, pH 10 4.48 ± 0.24 L/kg; Estrone: pH 7 4.82 ± 0.26 L/kg). This was attributed to interaction between the C-17 and C-20 C=O hydrogen-acceptor groups of estrone and progesterone, respectively, and the OH hydrogen-donor groups of HA. As ionic strength has implications for OM charge and conformation, as well as charge and solubility of trace organics, partitioning of progesterone and estrone to HA within the ED feed solutions would be reduced at a higher ionic strength due to charge negative charge shielding [33]. However, studies on the influence of ionic strength on the partitioning of trace organics to OM present conflicting results with some reporting no significant difference with increasing ionic strength [34] and others reporting a slight decrease in partitioning [32, 35].

The mass of hormone predicted to partition to HA as a percentage of the initial hormone feed mass was significant (Progesterone: pH 7 48.6%, pH 11 37.7%; Estrone: pH 7 82.6%). Experimental hormone sorption by the membrane L_{FD} (Progesterone: pH 7 84.6%, pH 11 82.0%; Estrone: pH 7 63.2) was greater than the predicted sorption PL_{FD} (Progesterone: pH 7 7.1%, pH 11 4.1%; Estrone: pH 7 8.4%), indicating the negligible contribution of solute-solute interactions to the membrane sorption. However, HA deposits on ion-exchange membranes, can cause increases in electrical resistance of the membranes and would reduce the area available for progesterone and estrone sorption to the membrane surface. Thus, the decrease in progesterone sorption in the presence of HA at pH 7 and pH 11 and estrone at pH 7 (Figure 3) is contributed to the deposition of uncomplexed HA on the AEMs (HA sorption in hormone ED experiment: Progesterone: pH 7 19.7%, pH 11 15.2%; Estrone: pH 7 17.8%, pH 11 16.0%). At pH 11, charge repulsion between dissociated estrone and HA coupled with electrostatic attraction between estrone and the AEMs resulted in the increase in membrane sorption in the presence of HA.

3.4. Desorption of hormones

Changes in solution chemistry influence the sorption process and can potentially release hormones back into solution, particularly during backwashing and cleaning of membranes [11]. Analyses were carried out to determine whether hormones could be desorbed from the membranes used in the sorption isotherm experiments. Partial desorption (20-30% initial mass sorbed) occurred in the presence of HCl, NaOH and UW. Desorption from the CEMs, on average, was similar (HCl: 19.2 ± 5.1%; NaOH: 18.8 ± 8.3%; UW: 18.7 ± 0.8%) while it varied for the AEMs (HCl: 13.3 ± 4.5%; NaOH: 18.3 ± 12.6%; UW: 11.8 ± 3.8%). These results imply that waste attained from membrane cleaning processes may contain potentially high concentrations of trace organics. Membrane desorption was not only dependent on the initial mass sorbed but also on solvent pH and electrostatic interactions between the hormones and membranes. More estradiol (25.8 ± 0.3%) and estrone (24.7 ± 0.2%) was desorbed from the CEM with NaOH (pH ~10.8) compared to progesterone and testosterone, due to estradiol and estrone dissociation and subsequent electrostatic repulsion with the negatively charged CEM. Desorption of estrone from membranes used during continuous and batch ED experiments was investigated to determine if hormone desorption was facilitated by applied voltage and desalination. After 2 hours, 18.7 μg/cm^3 of estrone was desorbed (3.8% of initial mass sorbed), indicating that desorption of estrone during desalination (at pH 7) is limited. However, the possibility that trace organics can desorb into the diluate exists.

4. Conclusions

The quantification of partition coefficients indicated strong sorption of steroidal hormones to the ion-exchange membranes and was postulated to be due to hydrogen bonding interactions and cation–π interactions between the hormone and membrane functional groups. Membrane sorption was dependent on hormone type, the position and strength of bonding of the hormone functional groups as well as the membrane bonding capacity. Electrostatic repulsion between dissociated estrone (which behaves similar to a charged organic acid) at alkaline pH and negatively charged CEMs reduces membrane sorption during ED. Adsorption/partitioning and diffusion mechanisms played a role in trace organic sorption with breakthrough of estrone noted after membrane saturation occurred. The permeation of trace organics is a possible environmental and health risk where removal is essential. The decrease in progesterone sorption in the presence of HA (pH 7 and 11) and estrone at pH 7 was attributed to uncomplexed HA sorption reducing the area available for hormone sorption to the membrane surface.

Supplementary Information Available

For further information on the calculation of hormone sorption to the membranes, the water-membrane partition coefficients, hormone-HA complexation and sorption/desorption kinetics during isotherm and ED experiments, refer to Supplementary Information.

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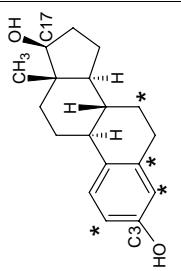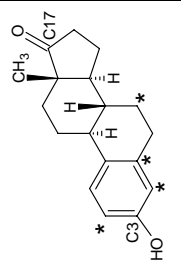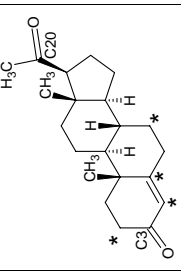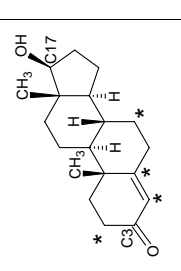
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List of Tables

Table 1. Physicochemical characteristics of the hormones studied.

Table 2. Membrane-water partition coefficients (Log $K_{AEM/CEM}$, L/cm^3) for the steroidal hormones.

Table 1

Property	[2,4,5,7-³H] 17β-estradiol	[2,4,5,7-³H] Estrone	[2,4,5,7-³H] Progesterone	[2,4,5,7-³H] Testosterone
Formula	$C_{18}H_{24}O_2$	$C_{18}H_{22}O_2$	$C_{21}H_{30}O_2$	$C_{19}H_{28}O_2$
Molecular Weight (g/mol)	272.4	270.4	314.5	288.4
Solubility in water (mg/L 25°C) [36]	13	30	8.81	30
pK_a [37]	10.23	10.34	n/a	n/a
Log K_{ow} [38]	4.01	3.13	3.87	3.32
Hydrogen acceptors [39]	2	1	0	1
Hydrogen donors [39]	2	2	2	2
Structure [a]				

[a] Asterix on hormone structure indicates location of tritium (³H) radiolabel.

Table 2

	Log K_{AEM} (L/cm³)[a]	Log K_{CEM} (L/cm³)[a]
Estradiol	0.39 (± 0.10)	-0.24 (± 0.05)
Estrone	0.53 (± 0.13)	0.04 (± 0.01)
Progesterone	0.37 (± 0.14)	0.22 (± 0.13)
Testosterone	-0.18 (± 0.03)	-0.16 (± 0.05)

[a] ± indicates 95% C.I.

List of Figures

Figure 1

Figure 2

Anion exchange membrane (AEM)

A Estrone

Alkylammonium functional group

Cation exchange membrane (CEM)

B Progesterone

Sulfonic acid functional group

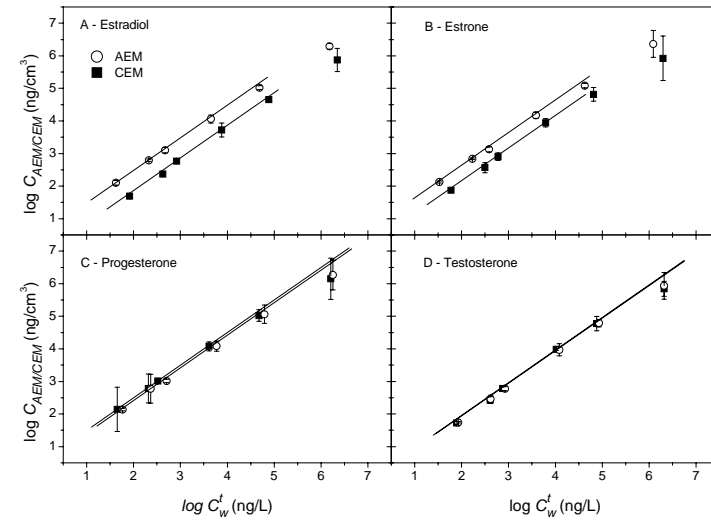Hydrogen acceptor   Hydrogen donor   Bipolar
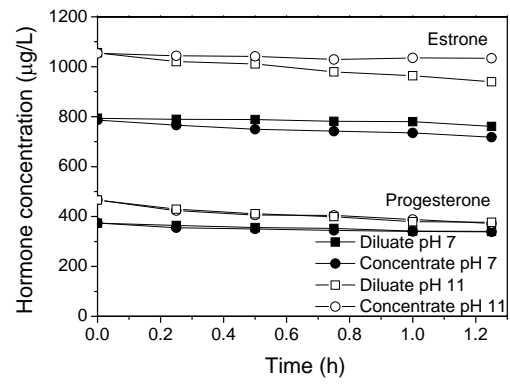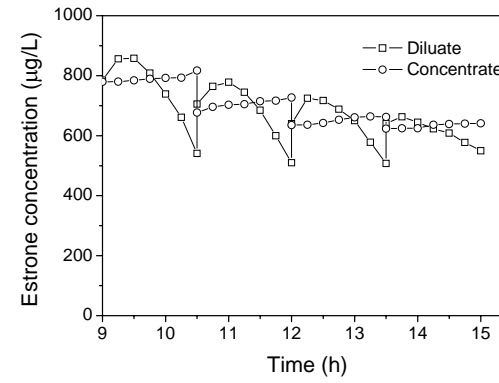
Figure 3

Figure 4

Figure 5

Supplementary Information

Sorption of steroidal hormones by electrodialysis membranes

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Overview of Supporting Information

In this Supporting Information we present:

1. Sorption kinetics during 1 µg/L steroidal hormone sorption isotherm experiments;

2. Information regarding the determination of the water-membrane partition coefficients (log $K_{AEM/CEM}$);

3. Calculation of the mass of hormone sorbed to HA and the quantification of solute-solute interactions between hormones and OM during electrodialysis;

4. Progesterone and estrone sorption kinetics during continuous electrodialysis experiments in the absence of humic acid;

5. Correlation between hormone hydrophobicity (log K_{ow}) and water-membrane partition coefficients (log $K_{AEM/CEM}$).

All information found in this Supplementary Information is also referred to in the manuscript.

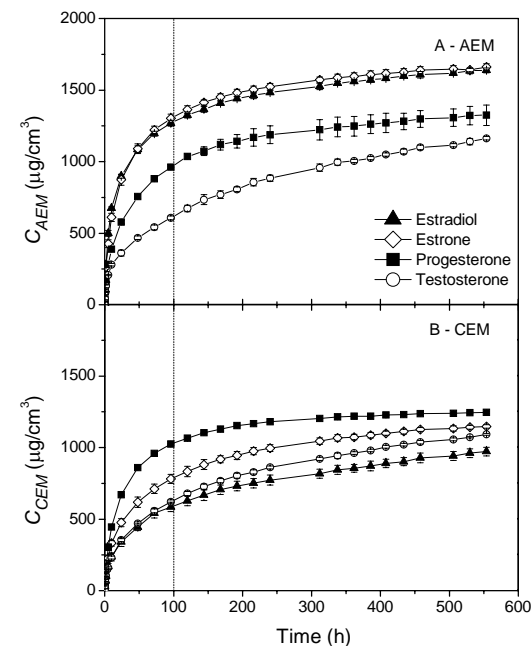**Steroidal hormone sorption kinetics during 1 µg/L sorption isotherm experiment**

Figure S1. Hormone sorption kinetics for the (A) AEM and (B) CEM (1mM NaHCO$_3$, 85.5 mM NaCl, pH 7, 1 µg/L hormone; vertical dotted line indicates time chosen for log $K_{AEM/CEM}$ determination: 100 h).

Partition coefficient ($K_{AEM/CEM}$) determination

The partition coefficient, $K_{AEM/CEM}$ (L/cm^3) for each hormone between the respective membrane (AEM or CEM) and the bulk solution was evaluated using eqn (S1).

$$K_{AEM/CEM} = \frac{C_{AEM/CEM}}{C_w^t} = \frac{m_{AEM/CEM}}{V_{AEM/CEM}} \cdot \frac{V_w}{m_w^t} \qquad \textbf{(S1)}$$

where $C_{AEM/CEM}$ is the hormone concentration sorbed per unit volume of membrane at time t = 100 h (µg/cm^3 or ng/cm^3), C_w^t is the hormone concentration (µg/L or ng/L), $m_{AEM/CEM}$ is the mass of

hormone sorbed to each membrane (μg or ng), $V_{AEM/CEM}$ is the AEM or CEM membrane volume (cm^3), V_w is the solution volume (L) and m_w^t is the mass of hormone freely dissolved in aqueous solution (subscript w) (μg or ng).

Due to error associated with the $K_{AEM/CEM}$ measurements $K_{AEM/CEM}$ was determined over the entire concentration range. Log $K_{AEM/CEM}$ was derived from the slope (n_i) of the linear regression of $C_{AEM/CEM}$ as a function of log C_w^t when the sorption isotherms (plotted on log scale) according to eqn (S2) were linear.

$$\log C_{AEM/CEM} = \log K_{AEM/CEM} + n_i \log C_w^t \qquad \textbf{(S2)}$$

Standard deviation (± S.D.) and confidence intervals (± C.I.) associated with the log $C_{AEM/CEM}$ and Log $K_{AEM/CEM}$ values, respectively, were calculated.

Quantification of solute-solute interactions during Electrodialysis

The implication of solute-solute interactions between progesterone and estrone and HA on membrane sorption during ED was estimated. The mass of hormone sorbed to HA at equilibrium (m_{ADS-HA}, μg) was calculated using eqn (S3).

$$m_{ADS-HA} = m_{FD}^e \cdot \frac{K_{OM}}{V_{FD}} \cdot m_{OM} \qquad \textbf{(S3)}$$

where m_{FD}^e is the mass of hormone freely dissolved in the ED feed solution (μg) of volume (V_{FD}, L), K_{OM} is the hormone-OM partition coefficient (L/kg) determined by Neale *et al*. [1] and m_{OM} is the mass of OM (kg). The log K_{OM} for estrone above pH 10 could not be determined, due to limitations in extracting dissociated compounds [32]. Therefore, the mass of estrone partitioned to HA during ED at pH 11 could not be estimated.

Using m_{ADS-HA} it was possible to predict hormone sorption during ED due to hormone-HA interactions (PL_{FD}, %) using eqn (S4).

$$PL_{FD} = \frac{m_{ADS-HA} \cdot L_{FD}}{m_{FD}^0} \times 100\% \qquad \textbf{(S4)}$$

where L_{FD} is the loss of hormone within the feed as a percentage of the initial concentration (%) and m_{FD}^0 is the initial mass of hormone in the feed (μg).

Steroidal hormone sorption kinetics during continuous Electrodialysis in the absence of humic acid
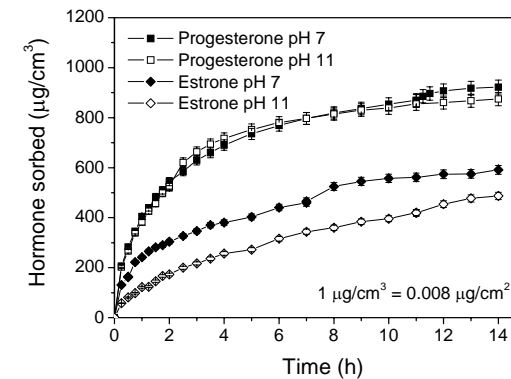

Figure S2. Mass of progesterone and estrone sorbed during ED per unit volume of membrane (μg/cm^3) (1mM NaHCO$_3$, 85.5 mM NaCl, 10 V; initial concentration of progesterone: 1057 ± 10.1 μg/cm^3 (pH 7), 1051 ± 23.4 μg/cm^3 (pH 11); initial concentration of estrone: 880 ± 12.7 μg/cm^3 (pH 7), 889 ± 5.4 μg/cm^3 (pH 11)).

Correlation between hormone hydrophobicity (log K_{ow}) and water-membrane partition
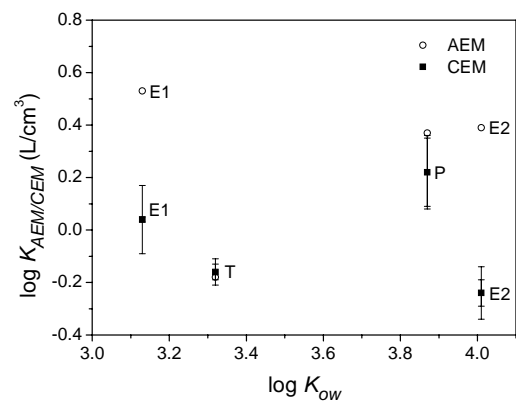
coefficients (log $K_{AEM/CEM}$)

Figure S3. Correlation between log K_{ow} and log $K_{AEM/CEM}$ for the steroidal hormones (Estrone E1,

Estrodiol E2, Progesterone P, Testosterone T).