Mechanistic evaluation of the sorption properties of endocrine disrupting chemicals in sewage sludge biomass

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Mechanistic evaluation of the sorption properties of endocrine disrupting chemicals in sewage sludge biomass

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ABSTRACT: This study investigated the sorption behaviour of two endocrine disrupting chemicals; 17β-estradiol (E2) and 17β-ethinylestradiol and their thermodynamic properties in an activated sludge biomass. The partition coefficient values measured for E2 and EE2 at varying temperatures range from 245 - 604 L/kg (log K_d 2.39 -2.78) and 267 - 631 L/kg (Log K_d 2.43 – 2.80), respectively. The K_d values were inversely related to temperature. The average percentages of E2 and EE2 adsorbed to the solid phase at 4.3 % dry solid were 87.2 % and 92.5 %, respectively. Sorption of E2 and EE2 to the activated sludge biomass was found to be spontaneous and entropy retarded with ΔG values in the range of -13 to -16 KJ/mol and ΔS value of -105.2J/mol/K and 96.7 J/mol/k for E2 and EE2, respectively. The enthalpy changes for E2 and EE2 were - 45.7KJ/mol and - 43.4KJ/mol respectively, demonstrating that the sorption process is exothermic. The values of the enthalpy changes also show that the mechanism of sorption is physisorption with some element of chemisorption.

Keywords: *Enthalpy; Entropy; Partitioning coefficient; Sludge; Sorption*

INTRODUCTION

The endocrine disrupting effects of certain chemicals found in sewage effluent and sludge continue to be a major issue of scientific interest, political and public concern. The steroid estrogens particularly estrone (E1), 17β-estradiol (E2) and 17β-ethinylestradiol (EE2) have been detected in sewage effluents, sludge and receiving rivers across the globe (Huang and Sedlak, 2001; Nakada *et al.*, 2006; Gomez *et al.*, 2007; Kanda and Churchley, 2008; Combalbert and Hernandez –Raquet, 2010; Plosz *et al.*, 2010; Rosal *et al.*, 2010). E2 and EE2 are also very significant in terms of their estrogenic activity as they are among the most potent endocrine disruptors (Jobling *et al.*, 1998). Various research efforts have shown that removal of these steroid hormones in activated sludge is predominantly through biological degradation, as well as sorption onto solids (Andersen *et al.*, 2003; Langford and Lester, 2003; Ying *et al.*, 2003; Kreuzinger *et al.*, 2004; Ifelebuegu *et al.*, 2006; Bonin and Simpson, 2007; Hashimoto and Murakami, 2009; Chen and Hu, 2009; Kumar *et al.*, 2009; Schaar *et al.*, 2010; Stasinakis *et al.*, 2010). The sorption behaviour of

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these compounds in wastewater treatment can be predicted to a large extent by the partitioning coefficient values (Lai *et al*., 2000; Schwazenbach *et al*., 2003; Ternes *et al*., 2004; Andersen *et al*., 2005; Keenan *et al*., 2008). An understanding of the partitioning of the steroid hormones between the solid and the aqueous phases of an activated sludge biomass, as well as the mechanics of the sorption process is essential to understand the fate of these compounds in the unit processes of a wastewater treatment plant and their route into the environments when the biosolids are used for agricultural purposes.

The sorption isotherms describe the relationship between the solid and the aqueous phases from which the partitioning coefficients are determined (Abdel-Ghani and Elchaghaby, 2007; Yoochatchaval *et al.,* 2008; Abdel-Ghani *et al.,* 2009; Chen *et al.,* 2010). Previous studies have examined partitioning coefficients of a number of organics (Soltanali and Hagani, 2008; Gong *et al.,* 2009). Ternes *et al.* (2004) conducted a series of batch tests using sewage sludge to determine partitioning coefficients (K_d) for a number of pharmaceuticals and personal care products. They found that log K_d values

of the compounds ranged from 0 to 2.7 with EE2 having a log K_d value of 2.5. Clara *et al.,* (2004) found that the log (K_d) for steroid estrogens were in the ranges of 2.64–2.97 and 2.71–3.00 for E2 and EE2, respectively. Andersen *et al.* (2005) determined distribution coefficients, K_d with activated sludge biomass for the steroid estrogens estrone, E2 and EE2 in batch experiments and they found log K_d values of 2.6, 2.7, 2.8, respectively. Ren *et al.,* 2007 studied the adsorption of EE2 on inactivated sludge and they found that the process was spontaneous and enthalpy driven. Similar observations were also made by Feng *et al.,*(2010).

This study focused on determining the partitioning of E2 and EE2 in a blended primary and secondary sludge from a conventional activated sludge plant and evaluating the thermodynamic properties of the sorption process. These are important parameters in modeling of the sorption of steroid hormones to activated sludge biomass, as well as numerically evaluating sorption as a mechanism of removal of steroid hormones. E2 and EE2 were chosen for research experiments as they represent both natural and synthetic estrogens, respectively. The study was carried out in 2009 at the Coventry University Environmental Science Laboratory in the UK.

MATERIALS AND METHODS
Materials and reagents
E2 and EE2 were used as the model solute for this research. They were purchased from Sigma Aldrich (UK). All reagents used were all of HPLC grade provided from Fischer Scientific (UK). The aqueous solution of E2 and EE2 were prepared by dissolving the required amounts in methanol.

Sewage sludge
The sludge samples used for this research were obtained from a UK sewage treatment plant treating wastewater for a population equivalent of 381,000. The plant consists of a conventional activated sludge plant with ferric dosing for phosphorus removal. The primary and secondary sludge were sampled and blended for the partitioning experiments. Previous research studies have shown that the K_d values for the partitioning of steroid hormones are similar for both primary and secondary sludge from an activated sludge plant (Ternes *et al.,* 2004). The measured percentage dry solid of the blended sludge was 4.3 % with a COD/TSS of 99%, TOC of 28.14 % and a pH of 6.8. The homogenised sludge was used without any pH adjustment and was treated to abiotic conditions by autoclaving at 120 °C for 25 min to inhibit any biological action during the batch experiments.

Sorption experiments
Sorption equilibrium
In the initial step, the sorption equilibrium was determined. 50 mL of the homogenised sludge spiked with approximately 1mg/Lwas stirred in a glass flask at 120 rpm for 2 h to determine the time to reach equilibrium. Samples were withdrawn at intervals for analysis.

Batch experiments
Batch equilibrium experiments were used to determine sorption coefficients of the E2 and EE2 on the mixed sludge. 50 mL of sludge aliquots were added into 250 mL glass flasks with mix standard solutions of E2 and EE2 at concentrations ranging from 500 to 2500 μg/L. To achieve equilibration, the mixtures were stirred using a magnetic stirrer for 2 h and centrifuged at 4000 rpm for 15 min to separate the aqueous phase from the sludge. The sludge was then filtered and the supernatant solutions were removed and analyzed for the equilibrium concentrations of each endocrine disrupting chemicals (EDCs). The solid phase EDCs concentrations were determined on mass balance basis. The experiments were conducted at varying temperatures of 15 °C 20 °C, 25 °C and 30 °C. The batch experiments were conducted in duplicates with two blank samples spiked with the solvent. The amount of each EDC adsorbed was calculated as the difference between the initial concentration and the equilibrium concentration of EDC in solution. The sludge/aqueous partition coefficient (K_d L/kg) for each compound were calculated using the relationship:

$$K_d = \frac{C_s}{C_{aq}} \tag{1}$$

Where C_s is the concentration of EDC adsorbed by sludge in mg/kg and while *Caq* is the concentration of the compound in aqueous phase in mg/L

The percentage of EDC adsorbed to sludge
The percentage of EDC adsorbed to sludge and that left in the liquid phase were also determined using Eq. 2.

$$\% EDC_s \ in \ sludge = \frac{C_s}{C_{total}} * \frac{100}{1} \tag{2}$$

Where the concentration in the solid phase is C_s, while C_{total} is the sum of the concentration in the aqueous and the solid phase.

Determination of thermodynamic properties

The experimental data obtained were used to determine the thermodynamic feasibility of the sorption process. The concentrations used are higher than the actual levels in sewage sludge however; it has been shown that thermodynamic properties of sorption process are concentration independent (Annamalai and Puri, 2002). For the determination of "H and "S a plot of $\ln K_d$ against $1/T$ was done and the values determined from the slope and intercept of the plot according to the Eq. 3

$$\ln Kd = -\left(\frac{\Delta H}{R}\right)\left(\frac{1}{T}\right) + \frac{\Delta S}{R} \qquad (3)$$

Where, R is the gas constant (8.314 J/mol/K) and T is the temperature in Kelvin.

ΔG was obtained at the various temperatures from Eq. 4.

$$\Delta G = \Delta H - T\Delta S \qquad (4)$$

Analytical procedures
SPE clean Up of extracts

The extracts (supernatant) from the centrifuged aqueous solution were passed through a clean-up process by SPE using LC-18 cartridges set up on a SPE vacuum manifold after pre-conditioning. The cartridges were conditioned with 5 mL of acetone, 5 mL of methanol and 5 mL of D.I water at a flow rate of 5 mL/min. The sample was then loaded at about 5 mL/min and subsequently washed with 10 mL of D.I water. Thereafter, the vacuum system was used to dry the cartridges for 45 min. The analytes was then eluded from the SPE cartridges with 10 mL acetone. The extracts obtained were concentrated to an approximate volume of 1mL under a gentle stream of nitrogen and then reconstituted with Methanol: H_2O 55:45 (v/v) to a final volume of 2 mL for HPLC analysis.

Analysis of EDCs

A Perkin-Elmer LS-50 Luminescence spectrometer was used to determine the wavelength range of the estrogens and Fluorescence detector wavelengths of 280 nm (excitation) 310 nm (emission) were selected based upon fluorescence peaks observed during excitation–emission matrix (EEM) analysis of the compounds in the HPLC eluent. Similar wavelengths were recorded by Van Emmerik *et al.*, (2003) and Yoon *et al.*, (2003). The HP 1050 HPLC –System consisted of a 79852A Quaternary Pump equipped with a 79855A 21-vial position autosampler, a G1303A vacuum helium degasser,

computer and monitor, chemstation, 7125 injector valve and a 1046A fluorescence detector, using methanol and water of a 55:45 ratio as mobile phase. The analysis was conducted for 14 min, with peaks for E2 and EE2 eluting at 9.5 and 10.5 min, respectively. The methods detection limit was evaluated as described in method USEPA (1984). MDL were 800 ng/L and 845 ng/L for E2 and EE2, respectively.

RESULTS AND DISCUSSION
Sorption equilibrium

The time for the aqueous phase to reach equilibrium was established as previously described. The apparent equilibrium time was under one hour as shown in Fig. 1. The aqueous concentration remained unchanged in the last hour of the test.

Sorption isotherms of E2 and EE2

The partitioning of the compounds between the aqueous and solid phase was assumed to follow a linear isotherm with finite transfer rate as demonstrated by Schafer *et al.* (2002). The result represented in Figs. 2 and 3 shows that sorption of E2 and EE2 onto the activated sludge was well described by linear regression similar to the Freundlich isotherm model.

Partition coefficients

The slope of the isotherms represents the partition coefficients. K_d values measured for E2 and EE2 at varying temperatures were within the range of 245 - 604 L/kg (log K_d 2.39 -2.78) and 267 - 631 L/kg (Log K_d 2.43 – 2.80) respectively. Detailed values and their corresponding correlation coefficients are presented in Table 1. Previous studies have also reported adsorption coefficients within this range. Ternes *et*
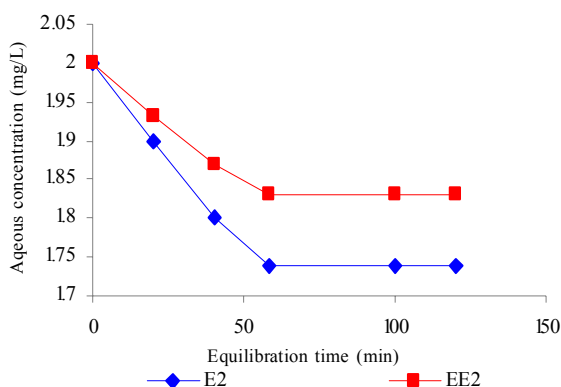

Fig. 1: Concentration of E2 and EE2 in the aqueous phase over time for the determination of time to reach equilibrium
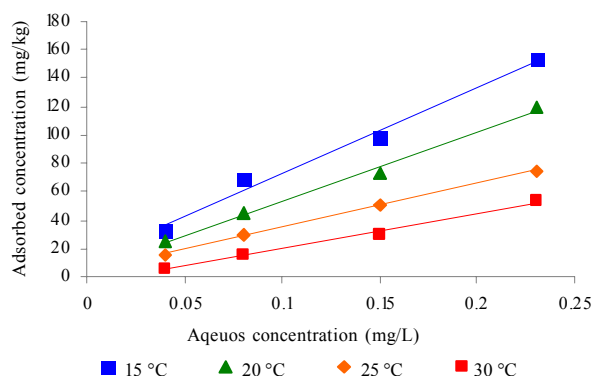
Fig. 2: E2 sorption isotherm for the blended sludge at varying temperatures for the determination of partitioning coefficients
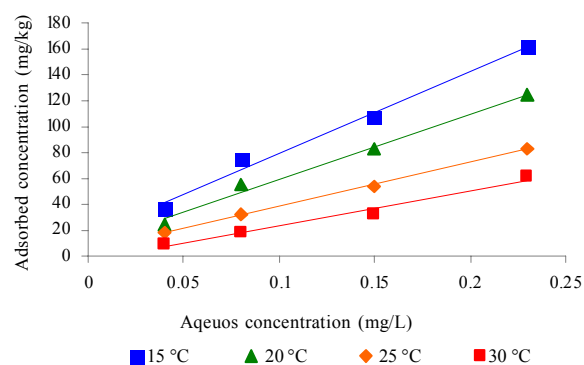

Fig. 3: EE2 sorption isotherm for the blended sludge at varying temperatures for the determination of partitioning coefficients

Table 1: Partitioning coefficients of E2 and EE2 at varying temperatures

	Temperature (°C)	K_d (L/Kg)	r^2
E2	15	604	0.987
	20	487	0.996
	25	312	0.998
	30	245	0.995
EE2	15	631	0.988
	20	508	0.990
	25	337	0.998
	30	267	0.981

Table 2: Gibbs free energy of sorption for E2 and EE2

Temperature (°C)	E2 - ΔG (KJ/mol)	EE2 - ΔG (KJ/mol)
15 °C	-15.42	-15.53
20 °C	-14.89	-15.04
25 °C	-14.37	-14.46
30 °C	-13.84	-14.08
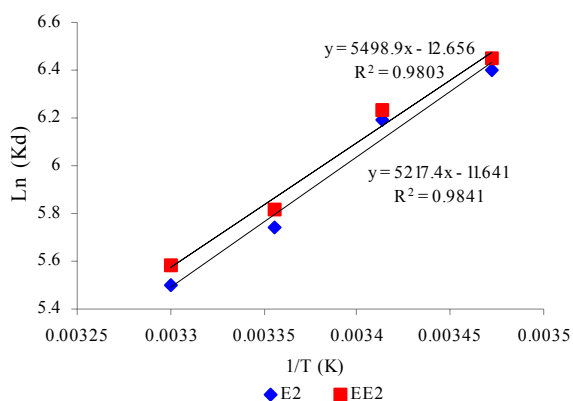
Fig. 4: Plot of $\ln K_d$ versus inverse of temperature for enthalpy and entropy change determination

al. (2004) reported K_d values for EE2 as 278-379 L/kg. Similar values were also obtained by Andersen *et al.* (2005). This study also demonstrates that the partitioning coefficients are inversely related to temperature.

The percentage of EDC adsorbed to sludge

The percentage of EDC adsorbed to sludge and that left in the liquid phase were also determined using Eq. 2. The average percentages of E2 and EE2 adsorbed to the solid phase at 4.3 % dry solid were 87.2 % and 92.5 %, respectively. A similar study by Keenan *et al.* (2008) reported a > 90 % sorption rate of estrogens to the solid phase. Also, Ivashechkin *et al.* (2004) investigated Radioactive-labelled BPA and showed that over 75 % of BPA in sludge was bound to solids (log K_d=2.09-2.30; log K_{oc}=2.72-3.11). The high sorption rate of E2 and EE2 to the solid phase can be attributed to the high hydrophobicity of their functional group and the higher % dry solid of the homogenized sludge blend. EE2

showed a higher affinity to the solid phase, this could explain while EE2 is more recalcitrant compared to E2 and other natural estrogens during the wastewater treatment process.

Thermodynamic properties

The natural logarithm of the K_d values obtained from the linear regressions in Figs. 2 and 3 were plotted against the inverse of temperature. The plot Fig. 4 gave a linear relationship with correlation coefficient of 0.980 for E1 and 0.984 for EE2. Enthalpy and entropy changes were obtained from Eq. 3 from which the Gibbs free energy of the sorption of the compounds were computed. The Gibbs free energy at the different temperatures is presented in Table 2. The "G values are in the range -13 to -16 KJ/mol. Similar values were obtained by Feng *et*

al. (2010) and this demonstrates that the sorption of E2 and EE2 to the activated sludge biomass is spontaneous. The enthalpy changes for E2 and EE2 were -45.7KJ/mol and -43.4KJ/mol, respectively, demonstrating that the sorption process is exothermic. The values of the enthalpy changes also suggest that sorption is likely both physisorption and chemisorption. According to Weber and DiGiano (1996), the heat of adsorption for physisorption is in the range of 5- 20 KJ/mol while that of chemisorptions is in the region of 100-400 KJ/mol. The enthalpy values are between the two ranges indicating that the mechanism is predominately physisorption mechanism with some chemical adsorption as the enthalpy values are closer to the physisorption range. The entropy changes were -105.2 J/mol/K for E2 and -96.7 J mol/K for EE2 implying that the sorption of E2 and EE2 to conventional activated sludge biomass is entropy retarded. This barrier is however, superseded by the enthalpy changes.

CONCLUSION

The following conclusions can be drawn from this study:

- The average percentages of E2 and EE2 adsorbed to the solid phase at 4.3 % dry solid were 87.2 % and 92.5 %, respectively with EE2 showing a slightly higher affinity to the solid phase.
- The "G values are in the range -13 to -16 KJ/mol. This demonstrates that the sorption of E2 and EE2 to the activated sludge biomass is spontaneous.
- K_d values measured for E2 and EE2 at varying temperatures were within the range of 245 - 604 L/kg (log K_d 2.39 -2.78) and 267 - 631 L/kg (Log K_d 2.43 – 2.80), respectively.
- This study has also demonstrated that the partitioning coefficients were inversely related to temperatures.
- The enthalpy changes for E2 and EE2 were - 45.7 KJ/mol and -43.4 KJ/mol respectively, demonstrating that the sorption process is exothermic. The values of the enthalpy changes also suggest that the mechanism of sorption is predominantly physisorption and some elements of chemisorption.
- The entropy changes were -105.2 J/mol/K for E2 and -96.7J/mol/K for EE2 implying that the sorption of E2 and EE2 to conventional activated sludge biomass is entropy retarded which is superseded by the enthalpy changes.

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