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1 **Removal of estrone (E1), 17β-estradiol (E2), and 17α-ethinylestradiol (EE2) from**
2 **wastewater by liquid-liquid extraction**

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7
8 **Abstract**

9 With the recent wide spread concerns of the environmental and public health effects of
10 endocrine disrupting chemicals (EDCs), it is becoming important to develop new techniques
11 to remove these substances from wastewater. EDCs find their way to the environment mainly
12 via effluents from WWTPs. They are often cited as moderately hydrophobic, hence they have
13 tendency to distribute to organic solvents and can then be removed using liquid-liquid
14 extraction (LLE) techniques. However, despite being a mature chemical engineering unit
15 operation, LLE has not been studied for the removal of EDCs in water. This study
16 investigated the removal of three EDCs of concerns including estrone (E1), 17β-estradiol
17 (E2), and 17α-ethinylestradiol (EE2) using decamethylcyclopentasiloxane (D5) as an
18 extraction solvent in three water matrix types (Milli-Q, tap water, and a secondary treated
19 wastewater). The study showed that all three EDCs were distributed to D5 but at varying
20 distribution coefficients: $K_{E1} = 2.66$, $K_{E2} = 0.61$ and $K_{EE2} = 1.67 \pm 5\%$ at pH 6 and 20°C.
21 Due to the high pKa values of the three EDCs, pH had no significant effect on K_{EDCs} up to
22 about pH 9.5 but higher pHs reduced the distribution ratios up to almost zero at pH 12. Van't
23 Hoff Equation described the effect of temperature on K_{EDCs} and showed that the process was
24 endothermic. The overall estrogenic potency of the three EDCs in mixtures was quantified
25 with an E2 equivalent potency, which was found to distribute well into the solvent at a K_{E2EQ}

26 = 1.43. The study suggests that LLE is an effective method to remove estrogenic potency of
27 wastewater.

28 **Keywords:** Endocrine disrupting chemical; liquid-liquid extraction; distribution coefficient;
29 steroid estrogen; decamethylcyclopentasiloxane.

30

31 **1. Introduction**

32 Exposure to endocrine disrupting chemicals (EDCs) via water is becoming a serious problem
33 to humans and the wildlife. EDCs interfere with the body's endocrine system by influencing
34 the synthesis, release, transport, metabolism and excretion of hormones in the body [1]. They
35 affect the thyroid and adrenal gland functions and can act as estrogens, antiestrogens and
36 antiandrogens [1]. Exposure to EDCs has been associated with several diseases involving the
37 reproductive [2-5], immune [6, 7] and neurological [8, 9] systems and has also been
38 associated with developmental dysfunctions [10, 11]. EDCs have been found in almost all
39 water matrices including treated and untreated wastewaters, surface waters, groundwaters,
40 and even drinking waters [12-15]. Wastewater treatment plants (WWTPs) have been reported
41 as the major source of EDCs [16-20]. Research studies have also reported that wildlife in
42 areas close to treated wastewater discharge points and sewage treatment plants have been
43 particularly affected by exposure to EDCs via hormonal changes, identified by feminisation
44 of local fish, and near extinction of some aquatic animal species [21-23].

45

46 Due to the considerable interest in the subject from the research community and advancement
47 in analytical techniques, the list of chemicals suspected of acting as endocrine disruptors has
48 grown significantly in the past decade. In the field of water policy, recently the European
49 Union Priority Substances Directive 2013/39/EU has amended the list of priority substances
50 by identifying new substances and enforces that actions have to be taken to reduce or

51 eliminate emissions of the priority substances. The directive has also introduced a “*watch*
52 *list*” as a new mechanism for identifying priority substances in the future and currently has
53 included in the first “*watch list*” one pharmaceutical (diclofenac) and two EDCs (17-beta-
54 estradiol (E2) and 17 α -ethinylestradiol (EE2)). Although these three substances were not
55 designated as priority substances at this point of time, their regulation is not ruled out in the
56 future [24]. EE2, E2 in addition to estrone (E1) are very potent estrogenic compounds as
57 shown by *in vitro* [25, 26] and *in vivo* [27, 28] studies. The removal of these three EDCs E1,
58 E2 and EE2 from water is studied in this research. Their molecular structures are shown in
59 Figure 1.

60

61 Conventional WWTPs have been typically designed to remove the organic carbon load and
62 nutrients (N and P) but no attention was given to the specific removal of EDCs. However,
63 given the significant research carried out and knowledge gained so far on the fate of EDCs in
64 the treatment process and their effects on humans and the environment, additional treatment
65 modules to the existing WWTPs have been proposed and investigated in the recent decade.
66 These include physical, biological and chemical advanced oxidation methods [29, 30]. Some
67 researchers have studied the adsorption of EDCs by activated carbon (AC) and found that AC
68 is effective in removing EDCs in the lab as well as pilot and full-scale plants [29]. However,
69 operational conditions should be strictly controlled and large amount of AC is required in
70 full-scale plants, making this method expensive [31]. Advanced oxidation processes (AOPs),
71 such as ozonation and non-thermal plasma, have also been studied and proven to achieve
72 good removal of EDCs in wastewater [30, 32-34]. However, the effects of oxidation products
73 are still not fully understood, which may delay the wide utilisation of such methods. EDCs
74 may also be removed by biodegradation processes [35] but numerous investigations showed
75 significant variability between the treatment processes [36]. Membrane techniques,

76 specifically reverse osmosis (RO) and nanofiltration (NF), have attracted great attention for
77 EDCs removal in wastewater treatment [37-39], while microfiltration (MF) and ultrafiltration
78 (UF) had limited performance due to their large pore sizes [40]. Chemical fouling and
79 biofouling remain the major drawbacks of membrane application in wastewater though.

80

81 EDCs are generally hydrophobic organic molecules, hence they have tendency to distribute in
82 organic phases. In fact, E1, E2 and EE2 (the three EDCs of interest in this research) are
83 weakly soluble in water and because of their hydrophobicity they possess high octanol/water
84 distribution coefficients (Table 1). This suggests that their removal in a liquid-liquid
85 extraction (LLE) process is meaningful and potentially efficient. So far there was no
86 investigation on the removal of EDCs from water matrices using LLE as an alternative
87 treatment technology.

88

89 **Figure 1**

90

91 **Table 1**

92 [29, 41, 42]

93

94 The choice of a suitable solvent is a crucial step in the development of an LLE method. The
95 solvent should be non-toxic and environmentally benign as well as it has low volatility and
96 solubility so the associated losses are minimal. In this study, the organic solvent
97 decamethylcyclopentasiloxane (D5) was chosen to carry out the removal of the EDCs from
98 the aqueous phase because of its non-toxic nature [43], low water solubility and low volatility
99 (Table 2). In addition, D5 is expected to present extraction capabilities for EDCs and has

100 already been shown to be a suitable wastewater treatment medium when combined with
101 ozone [44].

102

103 The key operating parameters (pH, temperature, initial aqueous concentration of EDCs and
104 volume ratio (D5/water)) affecting the distribution of E1, E2 and EE2 were studied. Different
105 aqueous EDC-containing matrices including Milli-Q water, tap water and a secondary treated
106 wastewater were used in this study. The results obtained in this study provide a basis for
107 further investigation into the recovery of EDCs from wastewater matrices or their degradation
108 using reactive techniques such as LLE combined ozonation, proven to be effective for the
109 removal of chloro-organics and textile dyes in wastewater [44, 45].

110

111 **Table 2**

112 [43, 44]

113

114 **2. Materials and Methods**

115 **2.1. Reagents**

116 Esterone (E1), 17 β -estradiol (E2), and 17 α -ethinylestradiol (EE2) with purity higher than
117 99% were purchased in powder form from Sigma-Aldrich (Dorset, UK). Stock solutions of
118 1,000 mg/L EDCs in methanol were prepared and stored in a freezer at -21 °C. A mixture of
119 EDCs standard stock solution was prepared in methanol at a concentration of 10 mg/L and
120 stored in sealed amber glass vial also at -21°C. Working solutions were prepared daily by an
121 appropriate dilution of the stock solutions. HPLC grade acetonitrile and methanol were
122 purchased from Fisher Scientific (Loughborough, UK). Ultra pure water was obtained from a
123 Milli-Q water purification system (Millipore Q system, 18 M Ω .cm, Bedford, MA, USA).
124 Decamethylcyclopentasiloxane (D5) solvent was purchased from Dow Corning, UK.

125 2.2. HPLC analysis

126 2.2.1. LC conditions

127 The HPLC analysis was performed using an Agilent 1200 Series HPLC system, equipped
128 with an on-line-degasser, a quaternary pump, an autosampler and a thermostated column
129 compartment. Both Fluorescence detector (model G1321A, Agilent, USA) and Diode Array
130 detector (model G2180BA, Agilent, USA) were used for the detection of EDCs. Reverse
131 phase chromatographic separation of the EDCs was achieved by a Hypersil GOLD C18
132 column (150×4.6mm×5µm) (Thermo Scientific, Hertfordshire, UK) that was thermostatically
133 held at 30 °C. Agilent ChemStation software was used for the control of the HPLC system
134 and data acquisition. Fluorescence excitation and emission wavelengths were set at λ_{ex}
135 200 nm and λ_{em} 315 nm for the detection of both E2 and EE2 while the diode array detector
136 was fixed at λ_{DAD} 200 nm for E1 detection. These conditions were determined in a
137 preliminary work carried out in this study. The total run time was 6 min and the injection
138 volume was 20 µL using a mobile phase flow rate of 1 mL/min. Steroids in the aqueous phase
139 were quantified using external calibration methods and their identification was based on the
140 EDCs' respective reference standard retention times ($\text{RT}_{\text{E2}} = 4.05$ min, $\text{RT}_{\text{EE2}} = 4.82$ min,
141 $\text{RT}_{\text{E1}} = 5.05$ min), when eluted with 50:50 (v/v) acetonitrile: Milli-Q water.

142

143 2.2.2. Standards of EDCs

144 Calibration curves of E1, E2 and EE2 were generated by serial dilution of the three EDC
145 stock solutions using Milli-Q water to cover a concentration range of 1 to 10,000 µg L⁻¹. The
146 standard curves were calculated by linear regression of the plots concentration versus peak
147 area. The resulting calibration curves were linear with r^2 values of at least 0.998. The

148 calibration curve equations and limits of detection for each EDC are shown in Table 3.
149 Checks of the calibration curves have also been carried out routinely.

150

151 **Table 3**

152

153 **2.3. Liquid-liquid extraction**

154 The liquid-liquid extraction experiments were carried out using a total working volume of 40
155 mL. Special attention was given to maintaining the operating temperature constant
156 throughout the experiments since the distribution of EDCs is thermodynamically sensitive to
157 temperature. A Grant LTD6G refrigerated water bath was used to control the temperature.

158

159 *2.3.1. LLE procedure*

160 Working solutions of EDCs were instantly prepared from the stock solutions at ambient
161 temperature by dilution with Milli-Q water. The initial concentration was set at 1 mg/L if not
162 otherwise differently stated. Once the aqueous mixture and D5 solvent have reached the
163 target temperature separately, extraction of the EDCs started by mixing the desired volumes
164 of both phases in a flask tightly sealed from the atmosphere and obscured from light. The
165 flask is then placed in a temperature controlled water bath and continuous mixing of the two
166 phases was made using magnetic stirrer at a predefined stirring rate. Once the extraction step
167 was completed, settling by natural gravity was carried out at the same temperature as
168 extraction to avoid any alteration to the EDC's distribution between the two phases. A
169 settling time of 5 minute was found sufficient to allow the two phases to clearly separate with
170 a well-defined interface.

171

172 Samples of 1 mL from the aqueous phase were collected using a micropipette from the
173 bottom of the flask and each of these was added directly to a 2 mL capped glass HPLC vial
174 that was placed in the autosampler's tray for analysis. All measurements were performed at
175 least in triplicate.

176

177 2.3.2. Distribution coefficient

178 The distribution coefficient of each EDC was determined according to Equation 1.

$$179 \quad K_{EDC} = \frac{C_{EDC_org}}{C_{EDC_aq}} = \frac{1}{R_v} \left(\frac{C_{EDC_aq0}}{C_{EDC_aq}} - 1 \right) \quad \text{Equation 1}$$

180 where: K_{EDC} is the distribution coefficient of the corresponding EDC, R_v is the volume ratio
181 between D5 and the aqueous phase, C_{EDC_aq0} and C_{EDC_aq} are the concentrations of the
182 corresponding EDC in the aqueous phase at time 0 and after equilibrium respectively
183 determined by HPLC. C_{EDC_org} is the concentration of the corresponding EDC in the organic
184 phase at equilibrium determined from a mass balance.

185

186 2.3.3. Water matrices

187 To study the effect of the water matrix make up, tap water and secondary treated wastewater
188 samples were used in addition to Milli-Q water. They were spiked with EDCs from the stock
189 solutions to the target concentration in the same manner as for Milli-Q water. Calibration
190 curves ($R^2 > 0.998$) were determined for the EDCs in each matrix and were used for the
191 quantification of EDCs in the corresponding aqueous phase after LLE. The effects of pH,
192 D5/water volume ratio, initial concentration of EDCs and temperature were also investigated
193 for tap water and wastewater using the same procedure as for Milli-Q water.

194

195 A volume of 5 L treated urban wastewater from the catchment area north-western part of
196 Swansea (UK) was sampled from the final effluent of the Welsh Water/Dŵr Cymru treatment
197 plant at Gowerton (Wales, UK). The effluent was treated with a conventional activated
198 sludge process followed by UV treatment at its outfall. The sample used in this study was
199 collected downstream of the UV unit. The wastewater samples were filtered under vacuum
200 through a 0.22 μm filter and stored in amber glass bottles at 6°C before being LLE treated.
201 The characteristics of the wastewater were pH=7.89, COD=25 mg/L and BOD=15 mg/L. Tap
202 water samples were taken directly from the water tap in the laboratory (Swansea University,
203 Wales, UK) and their characteristics were chloride = 6.3 mg/L, sulphate = 13.6 mg/L, and
204 pH=7.48.

205

206 **3. Results and discussion**

207 **3.1. Preliminary experiments**

208 A series of LLE preliminary experiments were initially performed to determine suitable
209 operating conditions for the extraction experiments. Aqueous solutions containing 1 mg/L
210 EDC were mixed with D5 (1:1 v/v) at 20°C under different stirring speeds (200, 400 and 600
211 rpm) and various extraction times (5, 15, 30 and 60 min). Different settling times of 5, 15 and
212 30 minutes were also tested for each experiment before an aqueous sample was collected for
213 analysis. The preliminary experiments showed that: (i) the distribution coefficients of the
214 three EDCs reached constant values in less than 30 min extraction time for all mixing speeds
215 used; (ii) the settling time (i.e. separation of the two phases) was very rapid with only 5 min
216 were sufficient to have two clear layers and the distribution coefficients did not change if
217 longer settling times were used. Following these results, the operating conditions for all
218 further LLE experiments were set to 30 min of extraction time, 200 rpm of stirring speed and
219 5 min of settling.

220

221 **3.2. Distribution coefficients**

222 The distribution coefficients, K_{EDCs} , for the three EDCs were determined using different
223 initial concentrations up to 5 mg/L in Milli-Q water. The other parameters were maintained
224 constant at D5/water volume ratio of 1:1, initial pH of 6.0 and temperature of 20°C. For each
225 EDC, the equilibrium concentrations in the aqueous and the organic phases were determined
226 from HPLC analysis and mass balance respectively. The results presented in Figure 2, show
227 that for each EDC, the relationship between the equilibrium concentration in D5 and water
228 was linear. The slopes of the linear lines give the values of the distribution coefficients K_{EDCs}
229 which were found equal to 2.66, 0.61, and $1.67 \pm 5\%$ for E1, E2 and EE2 respectively. The
230 distribution coefficients of E1 and EE2 were higher than one indicating that E1 and EE2 were
231 more distributed in the organic phase than the aqueous phase. This was not the case for E2
232 since its distribution coefficient was less than one. The distribution coefficient of E1 was the
233 highest, possibly due to a strong interaction of the lone pair of electrons on the carbonyl
234 oxygen of the estrone molecule with the silicon atoms. On the other hand, the presence of the
235 alkyne group increases the electronegativity of EE2 which enhances the interaction with D5
236 and leads to relatively higher distribution coefficient than E2.

237

238

Figure 2

239

240

241

242 **3.3. Effect of initial pH**

243 The distribution of an ionisable compound between the organic and aqueous phases depends
244 on its degree of ionisation, which in turn depends on the aqueous phase pH and solute

245 dissociation constant (pK_a). Considering the distribution ratio, D , defined as the ratio of
246 concentration of EDC in all chemical forms in the organic phase to the concentration of EDC
247 in all chemical forms in the aqueous phase, its change with pH can be determined by
248 Equation 2. Note that the distribution coefficient, K_{EDC} , (Equation 1) relates to one form of
249 the EDC only (i.e. neutral form in our case).

250

$$251 \quad D_{EDC} = \frac{K_{EDC}}{1 + 10^{pH - pK_a}} \quad \text{Equation 2}$$

252 where K_{EDC} is the distribution coefficient as defined by Equation 1 and pK_a is the acid/base
253 dissociation constant.

254

255 The effect of pH on the distribution of E1, E2 and EE2 between water and D5 was studied
256 using an initial concentration of 1mg/L in Milli-Q water for each EDC and a solvent to water
257 volumetric ratio of one. The pH range was from 2 to 12; high pHs were used because of the
258 high pK_a values of the EDCs (~ 10.7 , Table 1). The experimental results show that the trends
259 of the distribution ratios of the three EDCs as function of pH were similar (Figure 3(a)). This,
260 as pH increased from 2 to approximately 9.5, the distribution ratios for all EDCs remained
261 almost constant, but a further increase of pH to 10, 11 and 12 resulted in significant drop of
262 D_{EDCs} to almost zero at pH 12. Figure 3(b) shows that at $pH < 9.5$, the EDCs have neutral
263 molecular forms (i.e. non-dissociated forms), which are very weak acids, whilst at $pH > 9.5$,
264 the dissociated forms (i.e. the conjugate bases) started to dominate and the amounts of both
265 forms become equal at $pH = pK_a$. The EDCs are converted to their ionised forms at high pH
266 by losing protons to the hydroxide ions. At higher pHs than pK_a s, the EDCs become more
267 soluble in water than in the organic solvent because of their polar character, which explains
268 the lower distribution ratios obtained at pHs 11 and 12. On the other hand, at pHs less than
269 near pK_a s (approx. 9.5), most of the EDC molecules are non-dissociated hence their

270 distribution ratios were not affected by pH and the distribution ratios, D_{EDCs} , are equal to the
271 distribution coefficients, K_{EDCs} . The experimental results were fitted using Equation 2 and as
272 clearly shown in Figure 3(a), good agreement was obtained between the experimental data
273 and the model indicating the validity of the equation. It can be predicted using Equation 2
274 that for a pH = 10.5, K_{EDCs} will decrease from their values at pH 6 by 35, 38 and 39% for E1,
275 E2 and EE2 respectively. The degree of molecular dissociation of the EDCs defines their
276 hydrophilicity or lipophilicity. Based on the principle “like dissolves like”, the non-
277 dissociated (i.e. neutral) molecular form (pH < ~9.5) of the three EDCs have higher affinities
278 to non-polar organic phase, so they are more distributed to the organic solvent (D5). On the
279 opposite, when the EDCs become ionised (pH > pK_a), their affinity to the polar solvent
280 increases and hence they preferentially distribute to the aqueous phase. Given that the pH at
281 which the distribution of the EDCs into D5 becomes low is relatively higher than that
282 expected in a real wastewater, pH adjustment will not be required for this technique to be
283 effective.

284

285 **Figure 3**

286

287 **3.4. Effect of Temperature**

288 The effect of temperature on the distribution coefficients at pH 6 of the three EDCs was
289 studied using temperatures in the range 5-30°C. Since the concentration of EDCs is low, the
290 system was assumed dilute hence Van't Hoff's equation (Equation 3) was used to describe
291 the effect of temperature on K_{EDC} .

292
$$K_{EDC} = A_{EDC} \exp\left(-\frac{\Delta H_{EDC}}{RT}\right) \quad \text{Equation 3}$$

293 where: K_{EDC} is the solvent-water distribution coefficient, T is temperature (K), ΔH_{EDC} is the
294 standard enthalpy change of the process (J/mol), R is the universal gas constant (8.314 J.mol⁻¹
295 \cdot K⁻¹) and A_{EDC} is a constant related to the entropy of the process.

296

297 The linearisation of Van't Hoff's equation is given by Equation 4 and a plot of $\ln(K_{EDC})$
298 versus $1/T$ gives a linear line with a slope of $(-\Delta H_{EDC}/R)$ and intercept $\ln(A_{EDC})$. Such plots
299 are presented on Figure 4 for each EDC, which are indeed linear with R^2 values of at least
300 0.998 indicating the validity of the model.

$$301 \quad \ln(K_{EDC}) = \left(-\frac{\Delta H_{EDC}}{R} \right) \frac{1}{T} + \ln(A_{EDC}) \quad \text{Equation 4}$$

302

303

304

Figure 4

305

306 The standard enthalpy of the extraction process (ΔH) for each EDC was deduced from the
307 slopes of the lines and these in addition to the values of A_{EDC} are summarised in Table 4. The
308 enthalpy values of the three EDCs are positive indicate that the extraction process is
309 endothermic and the distribution coefficients increase with the operating temperature under
310 ambient pressure. The overall rate of increase of the distribution coefficients as function of
311 temperature in the range 5 to 30°C was calculated at 1.89%/°C for K_{EI} , 3.43%/°C for K_{E2} and
312 2.83%/°C for K_{EE2} . Other studies on liquid-liquid extraction have also highlighted a similar
313 effect of temperature on the distribution coefficients between organic and aqueous phases.
314 For example Saïen and Daliri [46] who have used a cumene–isobutyric acid–water and Saïen
315 et al. [47] who have used (4-methylpentan-2-one)-acetic acid-water have found that the
316 distribution coefficients increased as temperature increased by 3.02%/°C and 1.84%/°C
317 respectively, which are comparable to the results obtained in this study.

318

319

Table 4

320

321

322 3.5. Effect of the solvent/water volume ratio

323 The solvent-to-feed ratio is important in LLE since it defines the economics of the process

324 and aids researchers and process operators to use efficiently the solvent for a given maximum

325 extraction percentage. In this study, the effect of the solvent/water volume ratio was

326 investigated by varying simultaneously the volumes of D5 and the aqueous solution while

327 keeping the total volume (D5+water) constant at 40 mL and extraction time of 30 minutes.

328 The range of D5/water volume ratio was selected from 1:4 to 4:1 and the initial aqueous EDC

329 concentration was 1 mg/L. The removal efficiency of EDCs' extraction from Milli-Q water

330 solution is shown in Figure 5. The figure shows that as the D5/water volume ratio increased,

331 the removal efficiency also increased. The extraction percentages of E1, E2 and EE2 have

332 respectively increased from 38%, 18% and 29% at a D5/water volume ratio of 1:4 to 94%,

333 71% and 88% at a D5/water volume ratio of 4:1. By changing the D5/water volume ratio in

334 the range 1:4 to 4:1, the distribution coefficients remained constant at values comparable to

335 those obtained in Section 3.2 within $\pm 5\%$. As the volume of the organic phase increases in

336 relation to the aqueous phase, the extraction efficiency also increases. While high extraction

337 efficiency is desirable, handling large quantities of solvent in one stage is costly and can be

338 impractical. The solvent volume should then be appropriately selected for an optimal EDC

339 extraction and for this, multi-stage extraction is more preferred than a single-stage extraction.

340 In crosscurrent extraction, the aqueous EDC solution from one extraction stage is fed to the

341 next stage while the loaded solvent is removed by settling from the stage and fresh solvent is

342 added to the next stage. In this way, even if the distribution coefficient of the EDCs in each

343 stage is low, as is the case of E2, the overall system can have higher extraction efficiency
344 using a lower solvent total volume. With a linear extraction equilibrium line, as obtained in
345 this study, the removal efficiency of the EDC may be calculated using Equation 5 if a
346 multistage crosscurrent extraction is used.

$$347 \quad E = 1 - \left(\frac{1}{1 + K_{EDC}r} \right)^N \quad \text{Equation 5}$$

348 where: E is the removal efficiency, K_{EDC} is distribution coefficient, r is volumetric ratio of
349 organic solvent to water and N is number of crosscurrent stages.

350

351 Figure 5(a) shows that the theoretical model, given by Equation 5, fits well the experimental
352 results indicating its validity. Figure 5(a) inset shows the changes of the removal efficiency as
353 the number of theoretical stages increases. According to Figure 5(a) inset, almost five stages
354 are required to achieve 90% removal of E2 whilst only about 2.5 stages are required to
355 remove 90% of E1 and EE2. Because of its low distribution coefficient, E2 may be used as a
356 “key” component to determine the required number of stages for this multicomponent system
357 and its removal at a given efficiency (e.g. 90%) implies that the other two EDCs are also
358 removed of at least the same or higher removal percentages. For example, for a 90% E2
359 removal and using a ratio $r = 0.5$, nine stages will be required to achieve this level of E2
360 removal and under these conditions, the removals of E1 and EE2 are >99.5%. Figure 5(b)
361 shows the combined effect of N and r on the removal efficiency of E2. As r or N increases,
362 the removal efficiency also increases. Figure 5(b) also shows that low number of stages is
363 required when r is high. For example, when $r = 1$ only five stages are required to achieve
364 90% E2 removal as compared to nine stages when $r = 0.5$.

365

366

Figure 5

367

368

369 3.6. Removal of E2 equivalent estrogenic potency

370 Given that the EDCs will ultimately occur as mixtures in sewage effluents and natural waters

371 and since their estrogenic potencies are different, the total estrogenic potency of the EDCs in

372 mixtures should be used instead of individual components to design a liquid-liquid extraction

373 system. In this study, the “toxic equivalent” (TE) approach was used to take into account of

374 the total estrogenic potency. The three EDCs used in this study present different potencies

375 with EE2 being the most potent followed by E2 then E1. To facilitate calculation, the

376 estrogenic potency of the mixture can be estimated as estradiol equivalent (E2EQ) based on

377 individual EDC concentration and its relative potency. The relative estrogenic potencies of

378 EE2 and E1 were determined by Thorpe et al. [48] and their values are 1.8 and 0.68 for EE2

379 and E1 respectively. These relative estrogenic potencies were determined by comparing the

380 median effect concentration EC50 value of the EDC to that of E2. Based on the TE approach,

381 the estrogenic potency of the mixture as estradiol equivalent can be calculated by Equation 6.

382 The removal of the E2EQ was then studied and its distribution between the solvent and water

383 is presented on Figure 6. The value of the distribution coefficient of the estrogenic potency,

384 K_{E2EQ} , was found equal to 1.43 (Figure 6(a)) indicating that D5 was effective to remove the

385 overall estrogenic potency imparted by E1, E2 and EE2. Figure 6(b) shows that as the solvent

386 to water volumetric ratio r increased, removal of the estrogenic potency has also increased.

387 Equation 5 was applied to the experimental results and as shown in Figure 6(b), the equation

388 fitted well the experimental results. For a 90% removal of the estrogenic potency and using a

389 solvent to water ratio of 0.5, only four stages will be required as opposed to nine stages if this

390 level of removal was specified for individual E2. This suggests that lumping the estrogenic

391 effect in one parameter such as that of the estradiol equivalent is a more realistic approach not

392 only in terms of the measurement of the estrogenic potency of the water but also it provides
393 for better and economical process design (i.e. less stages are required for E2EQ). It can hence
394 be suggested that a multistage liquid-liquid extraction system using
395 decamethylcyclopentasiloxane as a water-immiscible solvent, which can be recovered and
396 continuously reused, offer an efficient process for the removal of estrogenic potency of water.

397

$$398 \quad C_{E2EQ} = 0.68 \times C_{E1} + C_{E2} + 1.8 \times C_{EE2} \quad \text{Equation 6}$$

399 where: C_{E2EQ} is the equivalent concentration of E2 that would give the same potency as the
400 mixture, C_{E1} , C_{E2} and C_{EE2} are the concentrations of E1, E2 and EE2 respectively.

401

402

403

Figure 6

404

405 3.7. Effect of the water matrix

406 The effect of the water matrix on the extraction of EDCs was studied using in addition to
407 Milli-Q water, tap water and a secondary treated wastewater. Figure 7 shows that the trends
408 and values of D_{EDCs} as function of pH obtained for tap and waste waters were similar to that
409 obtained for Milli-Q water with D_{EDCs} remained constant at $\text{pH} < \sim 9.5$ (i.e. $D_{EDCs} = K_{EDCs}$)
410 followed by a drop to almost zero at pH 12. For a given pH, the values of D_{EDCs} were within
411 about $\pm 10\%$ from the average values obtained for the three waters (Figure 7). This indicates
412 that the water matrix had low effect on the extraction of the EDCs, which can be neglected.

413

414 The effect of the initial EDC concentration was also studied using different concentrations of
415 EDCs in tap water and wastewater (0.5 - 4 mg/L) at pH 6. The resulting distribution
416 coefficients were also found little-affected by the water matrix and their values almost

417 matched those obtained in Milli-Q water within ± 9 % from the average values (data is not
418 shown). Moreover, when tap water and wastewater were used, the solvent to water
419 volumetric ratio was found to have no effect on K_{EDCs} similarly to Milli-Q water. The effect
420 of temperature on the distribution coefficients using tap water and wastewater was also
421 studied and similar results to Milli-Q water were also found. These results support even
422 further the suggestion that decamethylcyclopentasiloxane is a suitable solvent for the liquid-
423 liquid extraction of E1, E2 and EE2 from wastewater.

424

425 **Figure 7**

426

427 **3.8. Recovery and reuse of the solvent**

428 Decamethylcyclopentasiloxane was used not only because of it being benign and stable but it
429 is also recoverable and has good separation properties. In this study, after each LLE
430 experiment, the used D5 was firstly separated from the aqueous phase by gravity and
431 collected for further reuse. To make sure that the recovered D5 was EDC-free, the solvent
432 was first cleaned up by exposing it to ozone (20 g/m^3 ozone in oxygen for 10 min in 100 mL
433 of the solvent with stirring at 350 rpm). Traces of ozone in the solvent after clean-up were
434 flushed out by air injection into the solvent accompanied by mixing at 800 rpm; the removal
435 of ozone from the recovered D5 was checked by spectrophotometric measurement at 260 nm
436 and a mixing time of 1h was largely sufficient to flush all ozone traces out of the solvent.
437 Ozone was used to clean up D5 because ozone is very effective in degrading the EDCs [49]
438 and at the same time D5 was found resistant to ozone [50]. A series of successive three LLE
439 experiments were carried out using the recovered and cleaned D5 at a solvent to water
440 volumetric ratio of one, 1 mg/L EDC, and 20°C . The results showed that the distribution
441 coefficients of the three EDCs did not change significantly from one experiment to the other.

442 The values of K_{EDC} were comparable to those obtained for fresh solvent within an average
443 error of 6% for all EDCs. This indicates that the solvent can be reused without significant
444 loss to its performance. The results also show that the ozone clean-up procedure did not affect
445 the solvent performance.

446

447 **4. Conclusion**

448 In the present study, a novel LLE method was studied for the extraction of three endocrine
449 disrupting chemicals of significant importance E1, E2 and EE2 using Milli-Q water, tap
450 water and a secondary treated wastewater. Decamethylcyclopentasiloxane as a water-
451 immiscible solvent was found effective to extract the EDCs and can be recovered by gravity
452 separation and reused in further extractions of the EDCs. The distribution of the EDCs was
453 not affected by pH up to a pH around 9.5 as well as by the water matrix. The distribution
454 coefficients at pH 6 for the three EDCs were $K_{E1} = 2.66$, $K_{E2} = 0.61$ and $K_{EE2} = 1.67 \pm 5\%$.
455 Since the compounds are expected to be present in mixtures, the E2 equivalent estrogenic
456 potency (E2EQ) was used to characterise the overall estrogenic effect imparted by the
457 mixture. E2EQ was found to distribute well to the solvent with a K_{E2EQ} equal to 1.43. It was
458 suggested that the removal of 90% of E2EQ using a solvent to water ratio of 0.5 can be done
459 in a four cross flow extraction stages. Temperature effect showed that the extraction process
460 was endothermic and higher temperatures favour the extraction of the EDCs. The results
461 obtained in this study prove that liquid-liquid extraction is a suitable technique to recover
462 organic substances from wastewater and the technology has potential not only to protect the
463 aquatic environment, by removal of hazardous substances, but also to recover valuable
464 resources in wastewater. However, future studies are required to further develop and optimise
465 the LLE process so it can be adapted in large scale wastewater treatment plants either alone
466 or combined with other techniques such as ozonation or membranes.

467

468 **5. References**

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Tables

Table 1: Physico-chemical properties of E1, E2 and EE2 [29, 41, 42]

EDC	CAS	Molecular mass (g/mol)	Water solubility (mg/L at 20°C)	Vapour pressure (mmHg)	pKa	Log κ_{ow}
Estrone (E1)	53-16-7	270.4	13	$2.3 \cdot 10^{-10}$	10.77	3.43
17 β -estradiol (E2)	50-28-2	272.4	13	$2.3 \cdot 10^{-10}$	10.71	3.94
17 α -ethinylestradiol (EE2)	57-63-6	296.4	4.8	$4.5 \cdot 10^{-11}$	10.46- 10.7	4.15

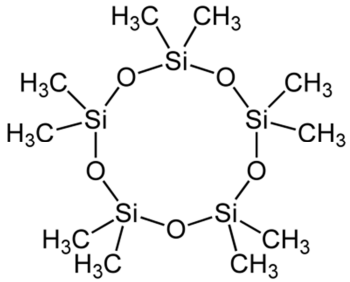
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Table 2: General physical properties of decamethylcyclopentasiloxane [43, 44]

Molecular Structure	Property	Value
	Molecular Formula	$C_{10}H_{30}O_5Si_5$
	CAS No.	541-02-6
	Density (kg/m^3)	0.955 at 20°C
	Viscosity (cp)	3.9 at 25°C
	Molar mass (g/mol)	370.77
	Flash point (°C)	70
	Water solubility ($\mu g/L$)	17 at 25°C
	Vapour pressure (Pa)	11 at 20°C
	Interfacial tension with water (mN/m)	18.9 at 20°C

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Table 3: E1, E2 and EE2 LC calibration curves

	E1	E2	EE2
Calibration curve equation	$C_{E1} = 13.33 \times A_{E1} - 36.93$	$C_{E2} = 8.55 \times A_{E2} - 42.42$	$C_{EE2} = 9.17 \times A_{EE2} - 6.70$
Correlation factor	0.9995	0.9996	0.998
Limit of detection ($\mu\text{g/L}$) ^a	5.01	5.14	2.97
Limit of quantification ($\mu\text{g/L}$) ^b	16.70	17.16	9.91

C_{E1} , C_{E2} and C_{EE2} : Concentration of E1, E2 and EE2 ($\mu\text{g/L}$)

A_{E1} , A_{E2} and A_{EE2} : Peak area of E1, E2 and EE2 (mAU.min)

^a $\text{LOD} = \text{SD}^b \times 3$

^b $\text{LOQ} = \text{SD}^c \times 10$

^c SD: Standard deviation

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Table 4: ΔH_{EDC} and A_{EDC} for E1, E2, and EE2 extraction with D5

EDCs	ΔH_{EDC} (kJ/mol)	A_{EDC}
E1	9.5	163
E2	25.2	24563
EE2	23.4	32761

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Figures

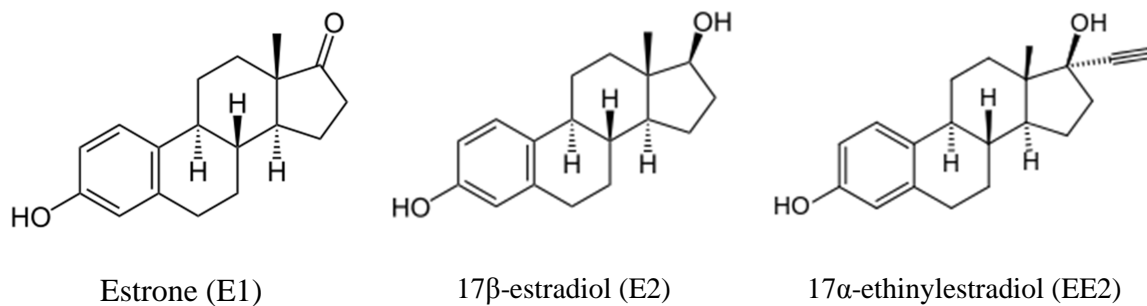
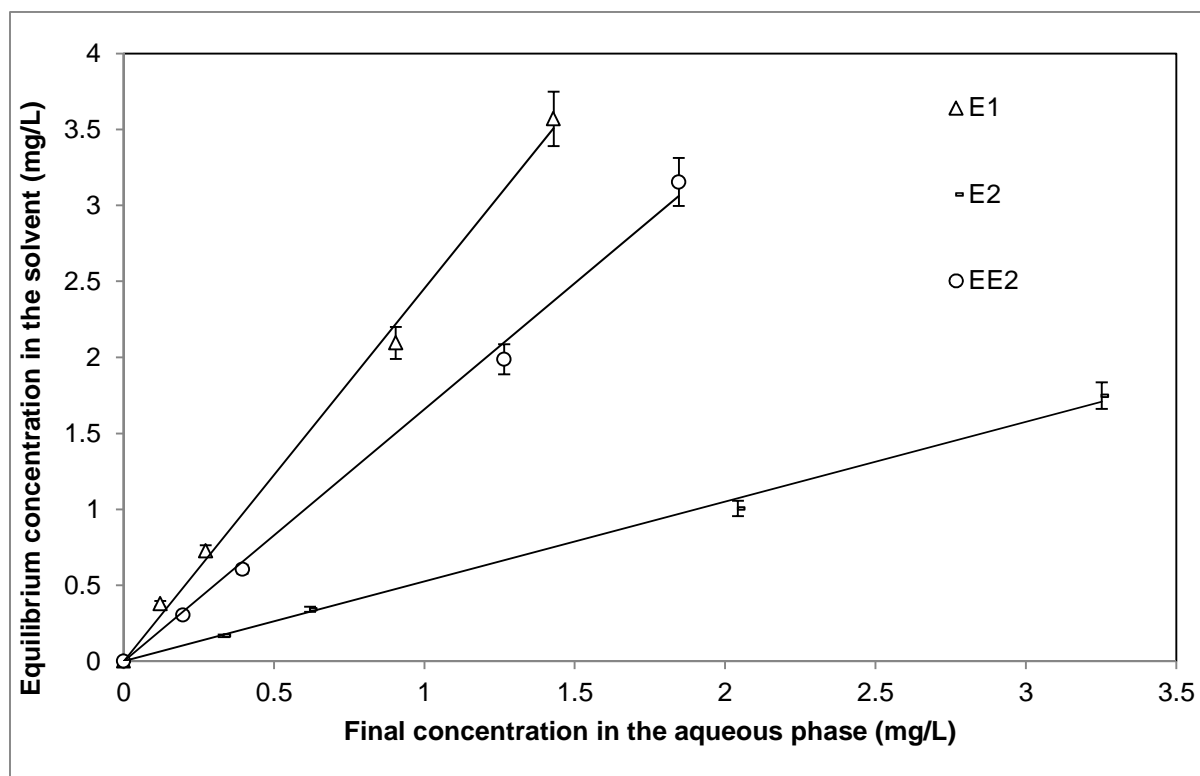


Figure 1: Molecular structure of natural (Estrone (E1), Estradiol (E2)) and synthetic (17α-ethinylestradiol) estrogenic compounds.

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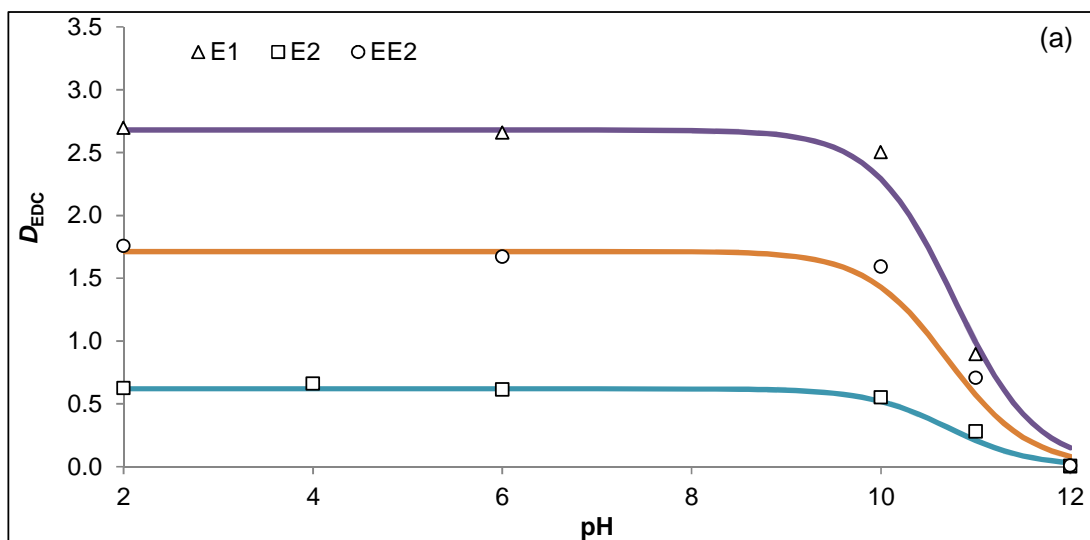
Figure 2: Final concentrations in the organic phase after liquid-liquid extraction of E1, E2 and EE2 as function of final concentrations in the aqueous phase; (D5/water 1:1 v/v; initial concentrations 0 to 5 mg/L; pH 6; 20°C).

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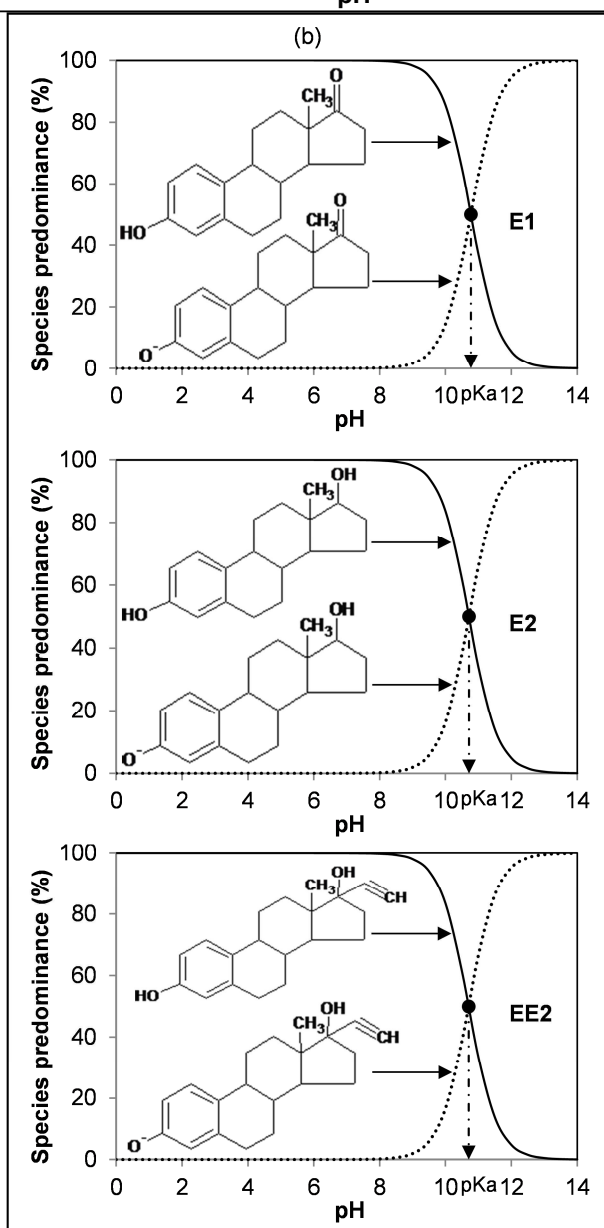
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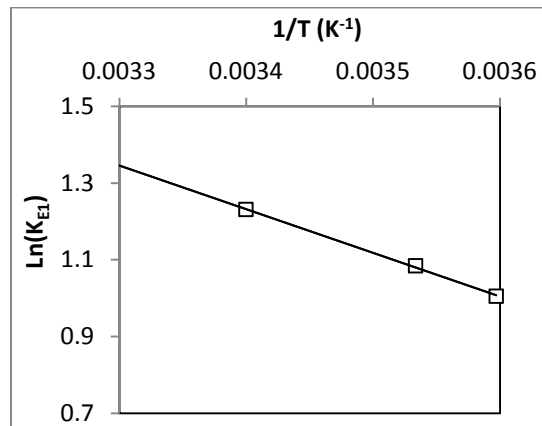


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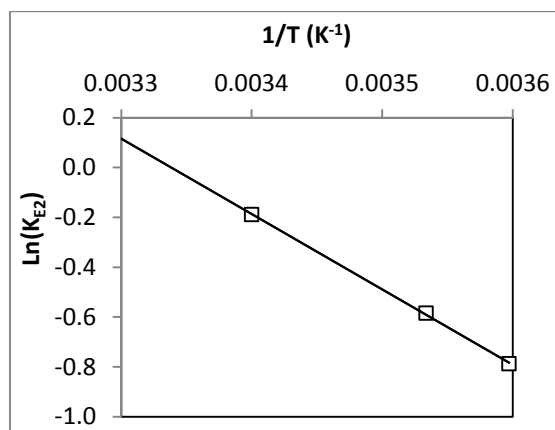
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Figure 3: (a) Effect of pH on the distribution coefficients of E1, E2 and EE2 between D5 and Milli-Q water at 20°C (marker: experimental data, continuous line: model); (b) Theoretical degree of ionisation of E1, E2 and EE2 molecules in water as function of pH.

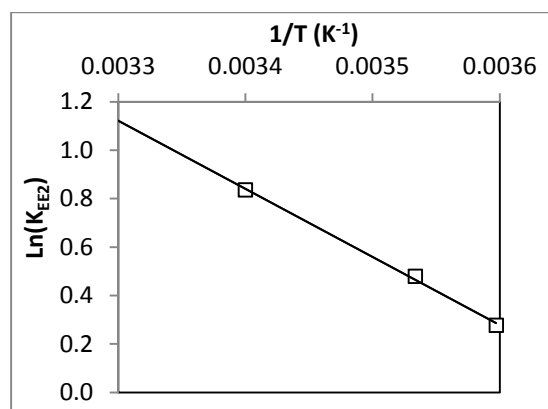
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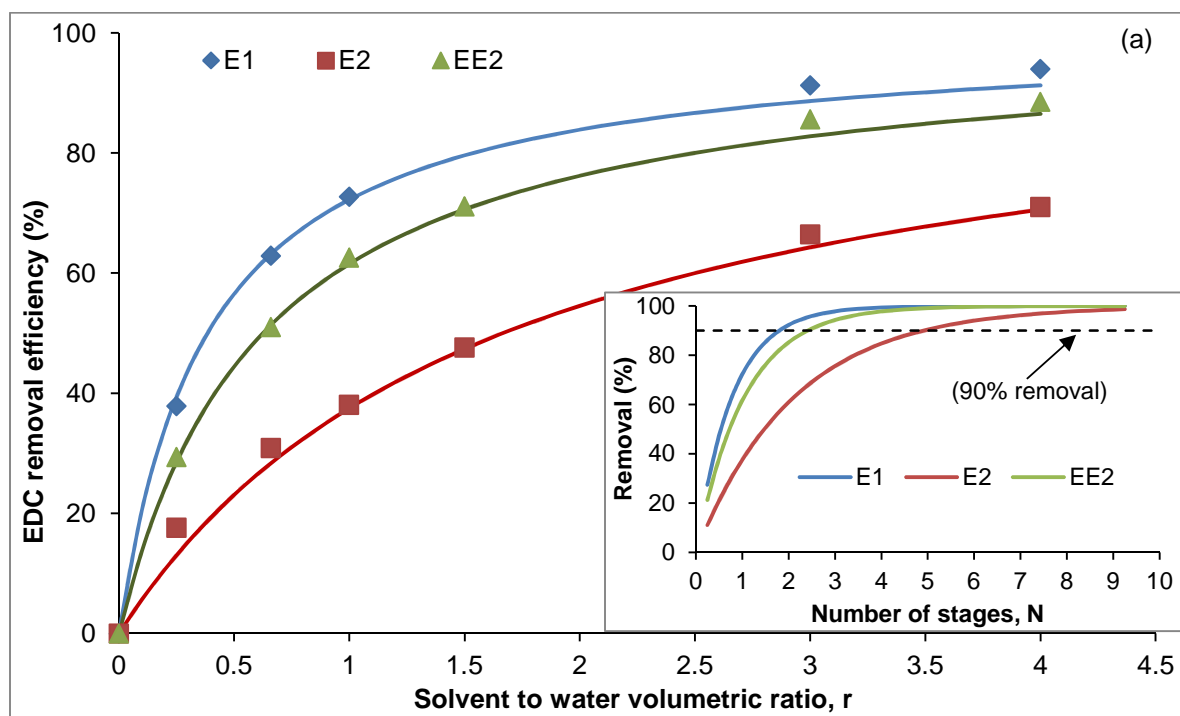


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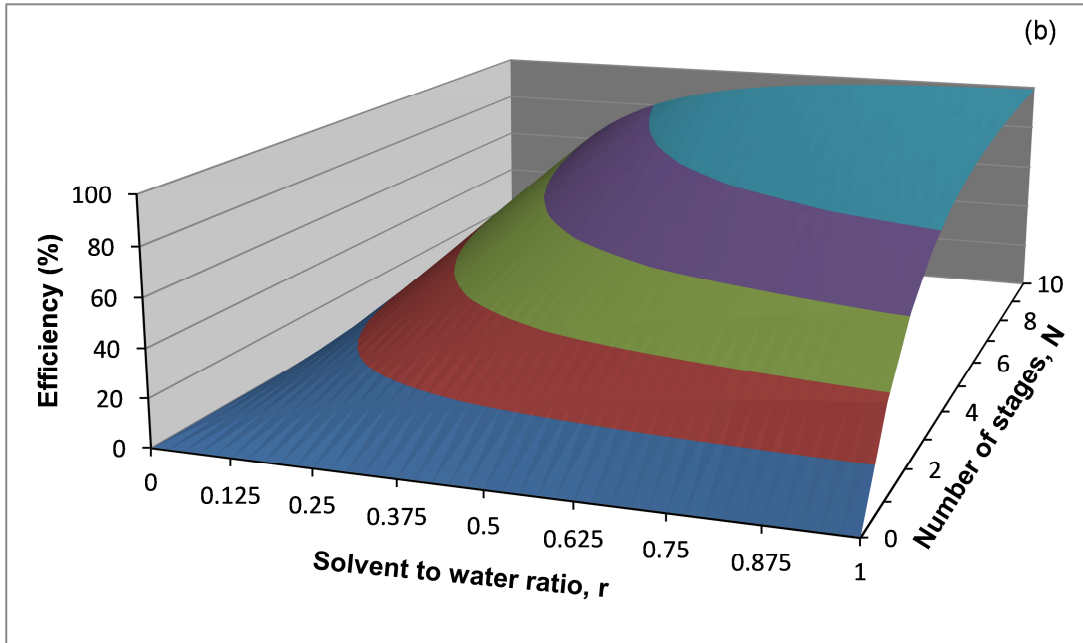
Figure 4: Van't Hoff plots of the distribution coefficients of E1, E2, and EE2 (initial concentration 1mg/L; pH 6.0; volume ratio 1:1; temperature range 5-30°C).

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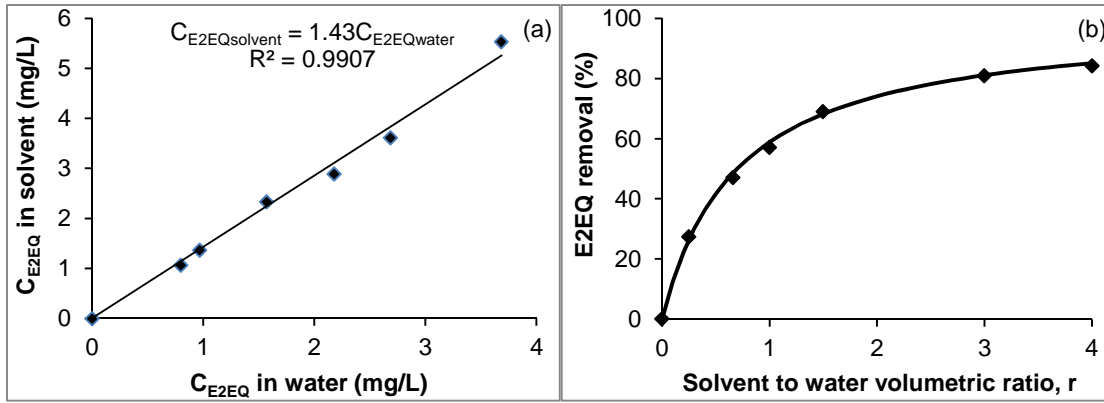


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783 **Figure 5:** (a) Effect of solvent to water ratio on EDC removal efficiency in one stage; inset:
784 effect of theoretical number of stage on removal efficiency (solvent to water ratio, $r=1$);
785 continuous lines are from theoretical calculation and marker symbols from experimental data,
786 (b) 3D representation of the effects of r and N on E2 removal efficiency.

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Figure 6: (a) Distribution of the estrogenic potency measured as E2 equivalent; (b) effect of

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the solvent-to-water ratio on E2EQ removal in a one stage extraction

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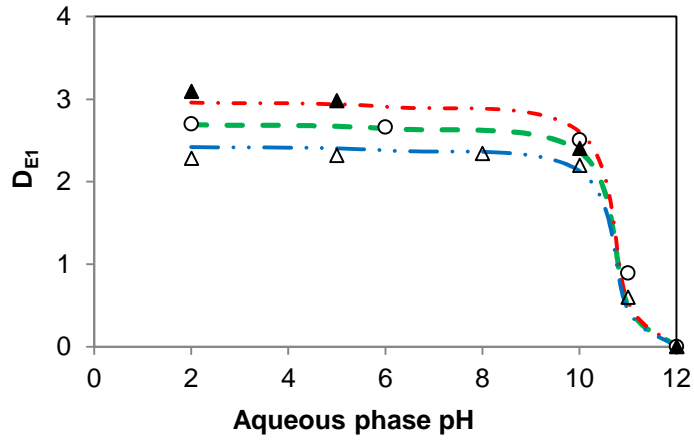
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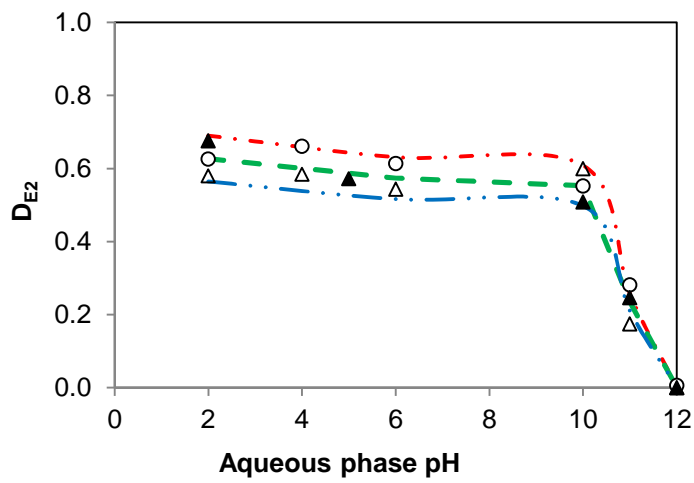
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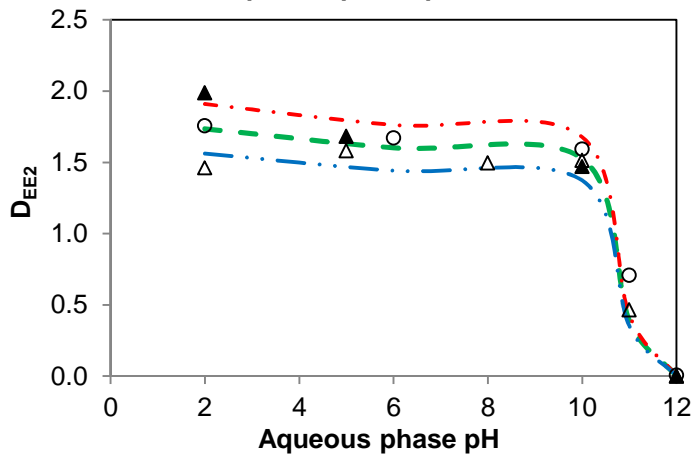
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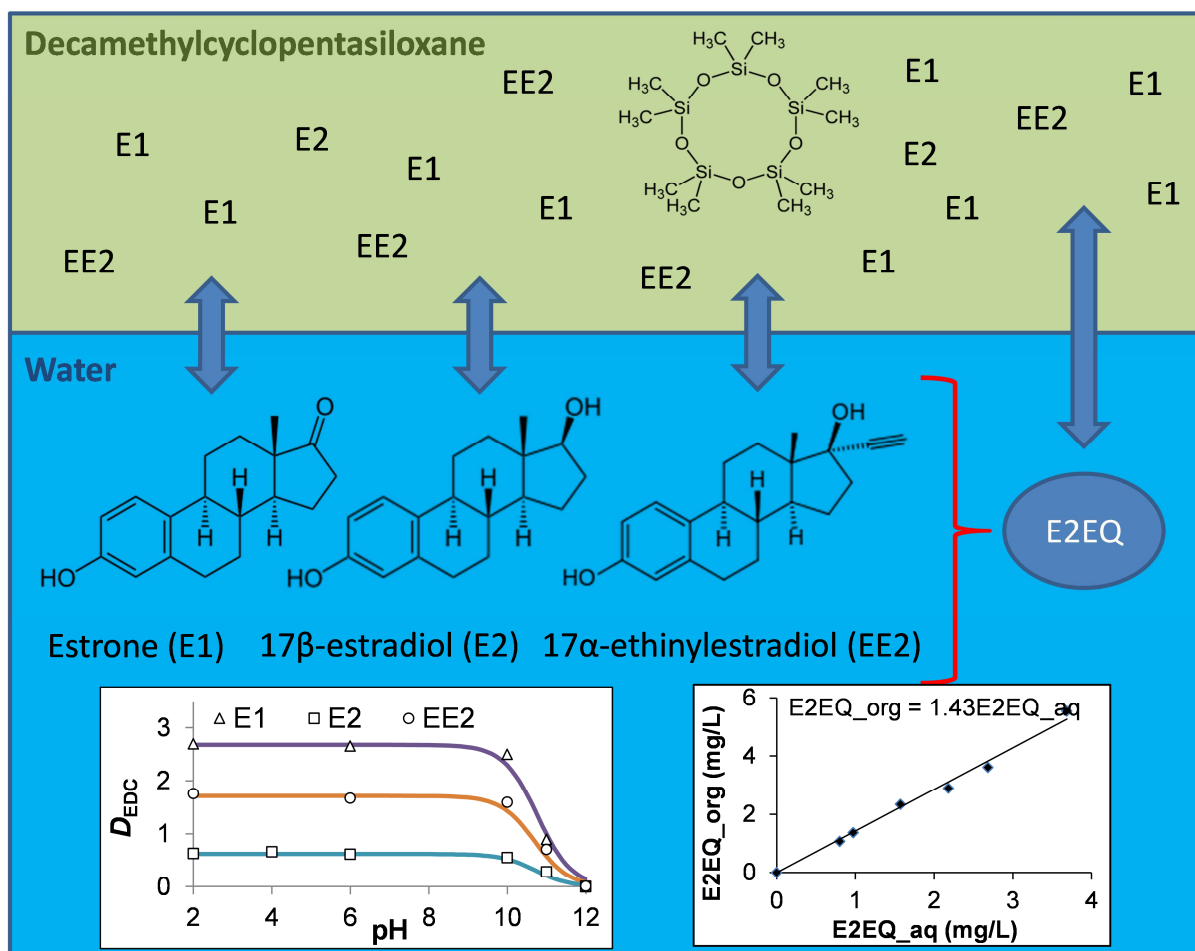
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Figure 7: Effect of the water matrix on the distribution ratios at different pHs.

Symbols: ○ MQ-water △ Tap water ▲ Wastewater - - - Average - · - · Average+10% - · · - Average-10%

843 **Graphical Abstract**

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Highlights

- Huge concerns about occurrence of endocrine disrupting chemicals (EDCs) in water
- EDCs have tendency to distribute to organic solvents
- Decamethylcyclopentasiloxane was effective to extract E1, E2 and EE2
- The distribution coefficients were not affected by pH up to pH 9.5
- Liquid-liquid extraction was effective to remove estrogenic potency from water.