

Pedro Dinis Torres Silva

NºMec:94183

Remoção de Etinilestradiol utilizando líquidos iónicos suportados como adsorventes

Relatório de Projeto

Licenciatura em Bioquímica

2020/2021



| Pedro Dinis Torres Silva | Remoção de Etinilestradiol<br>utilizando líquidos iónicos suportados<br>como adsorventes |
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| IV IVICC.74105           | Relatório de Projeto   |
|                          | Trabalho realizado sob a orientação científica da  |
|                          | Doutora Márcia Carvalho Neves, Investigadora   |
|                          | Auxiliar do Departamento de Química da   |
|                          | Universidade de Aveiro, e coorientação da  |
|                          | Doutora Ana Catarina Sousa, Professora Auxiliar  |
|                          | do Departamento de Biologia da Universidade de   |
|                          | Évora.   |

This work was financially supported by the project IonCytDevice (POCI-01-0145-FEDER-031106, PTCD/BTA-BTA/31106/2017) funded by FEDER, through COMPETE2020 - Programa Operacional Competitividade e Internacionalização (POCI), and by national funds (OE), through FCT/MCTE).

#### **Palavras-chave**

Etinilestradiol, Desregulador Endócrino, Contaminação Ambiental, Líquidos Iónicos Suportados

#### Resumo

O Etinilestradiol é um estrogénio sintético usado por todo o mundo em pilulas contracetivas. Este composto tem sido associado a múltiplos impactos negativos na vida selvagem, nomeadamente, feminização de populações de peixe. As concentrações de Etinilestradiol em meios aquáticos tem vindo a aumentar nas últimas décadas como resultado do aumento da sua utilização aliado a tratamento deficiente em estações de tratamento de águas residuais.

No presente trabalho, Líquidos Iónicos Suportados (LISs) foram estudados como potenciais adsorventes de Etinilestradiol. Modelos Cinéticos e Isotérmicas foram usados para comparar o LIS com melhor rendimento ([Si][N<sub>3118</sub>]Cl), com o Carvão Ativado. A capacidade máxima de adsorção do [Si][N3118]Cl foi determinada, sendo inferior à do Carvão Ativado, 44,81 mg/g e 52,41 mg/g respetivamente, no entanto, o LIS demonstrou melhor potencial para uso comercial devido à sua maior rapidez em adsorção de EE2, atinge equilíbrio em 5 min em contraste com o

# KeywordsEthinyl Estradiol, Endocrine Disruptor,<br/>Environmental Contamination, Supported Ionic<br/>Liquids

Abstract

Ethinyl Estradiol is a synthetic estrogen used worldwide in contraception pills. This compound has been shown to negatively impact wildlife in numerous ways most notably inducing fish feminization. Ethinyl Estradiol concentration has been increasing in water matrices in the last decades as result of higher usage and deficient elimination in Wastewater Treatment Plants.

> In this work, Supported Ionic Liquids (SILs) were studied as potential Ethinyl Estradiol adsorbents. Kinetic models and Isotherms were used to compare the best performing SIL, [Si][N<sub>3118</sub>]Cl, with Activated Carbon. [Si][N<sub>3118</sub>]Cl maximum adsorption capacity was determined to be lower than that of Activated Carbon, 44.81 mg/g and 52.41 mg/g respectively, but the SIL showed better potentiality for commercial use due to faster EE2 adsorption, reaching equilibrium in 5 min in contrast with Activated Carbon which took 360 min.

#### List of abbreviations

- %AE Adsorption Efficiency
- C0 Initial concentration
- CEC Contaminant of Emerging Concern
- $Ce-Equilibrium\ concentration$
- Ct Concentration at t time
- EE2 Ethinyl Estradiol
- EDC Endocrine Disrupting Chemical
- HRT Hydraulic Retention Time
- IL Ionic Liquid
- k1 Rate constant of first order
- $k2-Rate\ constant\ of\ second\ order$
- K<sub>F</sub> Freundlich equilibrium constant
- K<sub>S</sub> SIPS equilibrium constant
- LOEQ Lowest-observed-effect concentration
- LOQ Limit of Quantification
- MEC Measured Environmental Concentration
- PhACs Pharmaceutical Active Compounds
- PNEC Predicted No Effect Concentration
- PZC Point of Zero Charge
- qe Concentration of adsorbate in solid phase in equilibrium
- $q_t$  Concentration of adsorbate in solid phase at t time
- qmáx Maximum adsorption capacity
- RQ-Risk Quocient
- SIL Supported Ionic Liquid
- SPE Solid-Phase Extraction
- SRT Sludge Retention Time
- WWTP Wastewater Treatment Plant

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## 1 Introduction

The Contamination of waterbodies with contaminants of emerging concern (CECs) is a growing issue worldwide[1]. These substances vary greatly in structure and function, with unique physiochemical properties that makes them biologically active at low concentrations, posing challenges to their detection and also to their removal [1]. These substances of emerging concern include personal care products and pharmaceuticals, as for example steroid hormones[2] like estrogens. These CECs are being found in increasing concentrations in the environment, potentially disrupting ecosystems and affecting human health[3].

This project focuses on the development and characterization of new materials capable of selectively removing a potent estrogen (Ethinyl Estradiol) from aqueous solutions.

#### 1.1 Ethinyl estradiol, source and characterization.

Ethinyl Estradiol (EE2), IUPAC name (8R,9S,13S,14S,17R)-17-ethynyl-13-methyl-7,8,9,11,12,14,15,16-octahydro-6H cyclopenta[a]phenanthrene-3,17-diol, is a fine white to creamy white powder or crystals. This synthetic steroid weights 296.4 g/mol, is insoluble in water, soluble in ethanol and its 2D structure is shown on figure 1[4].



Figure 1 Ethinyl Estradiol 2D Structure

EE2 Octanol-Water Partition Coefficient is 4.12, this metric quantifies relative hydrophobicity and is very important when designing EE2 removal methods. Being higher than 4, this steroid has high potential of sorption to sludge[5] as well as other hydrophobic matrices, which makes their removal more challenging.

Table 1- Ethinyl Estradiol Physicochemical Properties

| Physicochemical properties |                   |  |  |  |  |
|----------------------------|-------------------|--|--|--|--|
| Name                       | Ethinyl Estradiol |  |  |  |  |
| CAS                        | 57-63-6           |  |  |  |  |
| Molecular Formula          | $C_{20}H_{22}O_2$ |  |  |  |  |
| Melting Point (°C)         | 182–183           |  |  |  |  |
| Molecular Weight (g/mol)   | 296.4             |  |  |  |  |
| LogKow                     | 4.12              |  |  |  |  |
| рКа                        | 10.4              |  |  |  |  |
| Vapor Pressure (Kpa)       | $6.00E^{-09}$     |  |  |  |  |
| Solubility (mg/L)          | 4.83              |  |  |  |  |
| Volatility (mmHg at 25°C)  | $1.95E^{-09}$     |  |  |  |  |

First developed in 1938, EE2 is currently synthesized by causing a estrone to react with sodium acetylide in liquid ammonia with subsequent hydrolysis (Nef reaction), the reaction of a estrone and ethinyl magnesium bromide is also an option (Grignard reaction)[4].

This compound is used in a variety of medications including hormone replacement therapies, palliative treatments for breast and prostate cancer, acne vulgaris mitigation amongst others. However, it is most used and known as the main estrogenic ingredient in oral contraceptive pills[1].

As with most oral medications, it takes a lot of EE2 administred orally to get the effective amount into the bloodstream, in fact, only 20-48% of the average daily dose of women in oral contraceptives is metabolized [6], an average of 35  $\mu$ g of EE2 and metabolites are excreted daily[7] along with natural steroid hormones, ending up in Waste Water Treatment Plants (WWTPs) where, current removal technologies for CECs have showed to be inefficient[1,8].

Ethinyl estradiol removal efficiencies were found to vary greatly and the need for improved removal methods is evident [1,5]. Subsequently, EE2 is discharged in the environment by way of WWTP effluents or use of sludge as fertelizer, this being considered the main source of environmental contamination[1].

EE2 is a potent endocrine disrupting chemical (EDC) with higher estrogenic activity than his natural counterparts while being more resistant to degradation than estradiol (E2)[1].

The risk quocient (RQ) posed by EE2 has been estimated[9] using the measured environmental concentrations (MECs) and the predicted no effect concentrations (PNECs) by using the formula:

$$RQ = \frac{MEC}{PNEC}$$

Du et al. calculated the risk quotient (RQ) which was used to rank the risk posed by steroid estrogens contamination and also the optimized risk quotient (RQf) at the continental scale. This RQf was based on the mean RQ value and the frequency of measured concentration of steroid estrogens exceeding the corresponding PNEC. This approach allows to evaluate the potential risks which are close to the natural scenario. According to the authors "RQf values highlight the most frequently detected SEs and could be classified into 5 risk level for categorizing the potential risks of SEs: high risk ( $\geq$ 1), medium risk [0.1, 1), low risk [0.01, 0.1), negligible (0, 0.01), and safe (0)". The obtained results disclosed that EE2 exibited high risks for all continents analysed.

#### 1.2 Geographical distribution and Environmental levels

Several studies reported the occurrence of EE2 in the environment. Du et al. [9] compiled the occurrence data on steroid estrogens including ethinyl estradiol, by reviewing 145 studies with data from 51 countries from January 2015 to March 2020. Most of the studies were conducted in Asian countries (61 studies) followed by Europe (38 studies), North America (22 studies), South America (11 studies), Africa (9 studies), Oceania (3 studies) and Antarctica (2 studies).



Figure 2-Percentage of articles from each continent from January 2015 to March 2020, adapted from [9]

Figure 2 highlights the research deficit on Africa and South America, even though these regions have high population and numerous water quality issues. In fact, Africa and South America mean concentrations of estrogens in natural water bodies were the highest recorded, with the average surpassing 100 ng/L in both cases.

Generally, EE2 is detected on WWTPs influents and effluents but also on groundwaters, surface waters and even in tap water. According to the data compiled by Du et al., EE2 detection frequency in drinking and tap water was 15-20%, whereas on natural water bodies and WWTP effluents it was about 40-45% and 55-60%, respectively.

Since EE2 environment contamination is a by-product of oral contraceptives usage, this substance can be detected even in remote locations providing the area is habited by women on birth control pills, in fact, EE2 was even detected in Antarctica in wastewater from research stations.

A 2021 study indicates that EE2 concentration in WWTP effluents is on average 12.3 ng/L with the maximum recorded measured value being 549 ng/L[5], although usually dropping to low ng/L levels when observing surface waters, concentrations of EE2 can reach a maximum of 34 ng/L in this type of matrix[10]. These values are much higher than the lowest-observed-effect concentration (LOEC), 0.2 ng/L[5].

Table 2 congregates data on the levels of estrogens detected in WWTPs influents and effluents. Generally, the levels are much higher in influents than in effluents across-theboard. Nevertheless, even after treatment at WWTP, the effluents still present detectable levels of these substances, this reality highlights the urgency in the development of alternative methods of preventing EE2 pollution.

| Countries/Regions                  | Compounds   | Concentration range (ng/L)   | Reference                    |
|------------------------------------|---|--|------------------------------|
| Europe                             | 17β-E2, EE2   | influent (<0.2-3000),<br>effluent (0.1-85)   | Tiedeken et al. (2017)       |
| Latin America                      | E1, 17β-E2, E3, EE2,<br>17β-E2-3S, E3-16G,<br>17β-E2-17G, 17β-E2-<br>17Ac | influent (0.9-23000),<br>effluent (0.74-2650)  | Peña-Guzmán et al.<br>(2019) |
| Africa                             | Ε1, 17β-Ε2, Ε3, ΕΕ2   | influent (8-9833),<br>effluent (7-4608)  | Madikizela et al. (2020)     |
| Asia, Europe, and<br>North America | Ε1, 17β-Ε2, Ε3, ΕΕ2   | influent ( <loq-802),<br>effluent (<loq-275)< td=""><td>Tran et al. (2018)</td></loq-275)<></loq-802),<br> | Tran et al. (2018)           |

Table 2- Differences in estrogen levels in WWTPs influents and effluents, data extracted from [9]

LOQ: Limit of Quantification

#### 1.3 WWTPs usual treatment methods, potential drawbacks

WWTPs are designed to receive wastewater i.e., water contaminated by human activities and to discharge water with minimum environmental pollution potential. To achieve this objective these plants use a variety of biological, chemical or mechanic processes in a series of consecutive steps[11]. Each step utilizes different methods and is optimized differently accordingly to factors like region specific needs and cost[5]. Figure 3 shows a typical WWTP layout.



Figure 3-Types of wastewater treatment system throughout the wastewater treatment plant, figure adapted from [7]

The **primary treatment** main purpose is to prepare the influent for the secondary and tertiary phases, by removing big solids and heavy sediments that might damage the equipment in later stages. This is achieved by using screens and a grit chamber. Additionally, oils in excess are also removed in this phase.

In the **secondary treatment**, a number of different microorganisms are used in order to degrade organic matter and remove nutrients[7].

The **tertiary treatment** is the final step of the process, in this phase the nitrogen present is removed and the water is disinfected by UV radiation or chlorination[7], these procedures ensure that the discharged water is sterile and safe to discharge.

When analyzing worldwide data, the average removal rate for EE2 in WWTPs is 68.3%, as expected, this rate varies between WWTP facilities [5].

Biodegradation is generally cited as the main removal mechanism, present mainly in the first and second treatment phases. Biodegradation rates depends on factors like redox condition, temperature, organic loading, and initial organic concentration. Sludge retention time (SRT), hydraulic retention time (HRT) and temperature are possibly the easiest factors to be optimized. There is evidence of positive correlation between the hydraulic retention time and removal efficiency, the same occurs with temperature. For sludge retention time there is no clear correlation[5].

Table 3 gathers data on the average effectiveness of the most popular treatment processes, it is shown that the type of treatment is significant in EE2 removal.

| Treatment process                 | Removal average % |
|-----------------------------------|-------------------|
| Activated sludge process          | 71.5              |
| Anaerobic-anoxic-oxic/anoxic-oxic | 75.2              |
| Oxidation ditch                   | 83.6              |
| Sequence batch reactor            | 69.1              |
| Membrane bioreactor               | 71.5              |
| Biofilm-process                   | 55.3              |
| CW/SP/Lagoon/RBF                  | 59.4              |
| Primary Treatment                 | 47.5              |
| Others                            | 81.3              |

Table 3- EE2 removal averages for different WWTP treatment processes, data extracted from [5]

Biofilm-process: trickling filter (TF); biofilter (BF); rotating biological treatment (RBT); fixed bed (FB); moving bed biofilm reactor (MBBR). CW/SP/Lagoon/RBF: constructed wetland/stabilization pond/lagoon/reed-bed filter. Others: Daewoo nutrient removal process + chlorination (DNR + Cl); Bio-Best-Bacillus process + chlorination (B3+Cl); up-flow anaerobic sludge bed

Others: Daewoo nutrient removal process + chlorination (DNR + Cl); Bio-Best-Bacillus process + chlorination (B3+Cl); up-flow anaerobic sludge bed (UASB); activated sludge + oxidation ditch (AS + OD); activated sludge + trickling filter (AS + TF); trickling filter + oxidation ditch (TF + OD); rotating biological treatment + activated sludge (RBT + AS).

In activated sludge process (ASP) a bacterial biomass suspension is used to remove pollutants[12], there is evidence that ASP EE2 removal efficiency might be improved in low organic substrate concentrations[13]. Selecting microbiota populations can also be a good practice, ammonia-oxidizing bacterium (AOB) shown a strong correlation with EE2 removal[5] while nitrifiers produced high biotransformation rates, additionally EE2 is better biodegraded under aerobic conditions[13].

The Anaerobic-anoxic-oxic/anoxic-oxic process, the oxidation ditch and the sequence batch reactor are variations of the ASP, with oxidation ditch exhibiting the best results of this group. The Primary treatment EE2 removal values rates are the lowest followed by the biofilm process which uses attached microbial growth and more passive processes like constructed wetland and reed bed filter which make use of microbial life, vegetation and sun radiation to treat water[14].

Despite some treatments producing relatively better results, these have drawbacks that must be accounted for, oxidation processes depend on close monitoring of variables like water pH and contact time while, similarly to ozonation, they are responsible for producing a number potentially dangerous oxidation products that require further treatment steps to remove[13,15].

Membrane Bioreactor (MBR) is one of the most effective and popular treatments, this treatment has the advantage of being customizable; a specific membrane can be used to adsorb and subsequently degrade estrogens. As with other treatments already discussed, MBRs efficiency can be influenced operational conditions which can impair its results, additionally the usual increases that this method provides in biodegradation rates are not as significant with more recalcitrant compounds[13].

Both UV disinfection an chlorination are able to further increase removal of EE2, with the latter showings better results[7] but also more drawbacks like the formation of chlorination by-products, toxic gases, etc.

Another challenge with current treatment methods is that these lose efficiency as pharmaceutically active compounds (PhACs) concentrations decrease [1] which in EE2 case poses a serious problem considering that the LOEC is in the ng/L range meaning that even if the effluent concentration is a fraction of the influent levels, it is still too high to be discharged safely.

#### 1.4 Impact of ethinyl estradiol on aquatic life

EE2 typically enters the environment through WWTPs and STPs discharges, runoff and leaching from landfills. Once in the environment, EE2 bioaccumulates due to its low volatility, relative high resistance to biodegradation and hydrophobicity[10], contributing to its presence in natural habitats[16]. With the first observations of fish feminization dating to the late 1970s[17], it is now clear that exposure to EDCs has negative impacts on most living organisms including plant species, were there is evidence of bioaccumulation in roots and shoots as well as altered growth in a concentration dependent manner[3].

Fish populations exposed to EDCs have indeed much higher rates of infertility and hermaphroditism, there is also evidence of behavior changes as well as decreasing biomass [3], likewise, these effects hinder second generation survival[18] and are positively correlated with EDCs concentrations and exposure time[3].

Immune function impairment is also a common effect affecting male fish in EE2 concentrations as low as 33 ng/L [16,20], similarly behaviors like courtship and aggression are also influenced EE2 levels[17,21][19].

A study evaluating the response of crucian carps to relevant EE2 concentrations, found that even if no obvious apparent changes are observed, EE2 lead to relevant changes in several metabolic pathways including amino acid metabolism, lipid metabolism as well as the activation of antioxidant defense mechanisms[20].

The effects of EE2 pollution will most likely continue to be present and worsen as long as improved mitigation measures are not taken.

#### 1.5 Advanced treatment processes

Advances in various technologies have sprang new methods of CECs removal, Soares Filho et al [21] selected operation parameters that enable 97% removal efficiency of EE2 through electrocoagulation, another study found a photocatalyst that led to 98% degradation of EE2 when exposed to four hours of UV radiation, however the method lost efficiency in subsequent cycles[22].

Adsorption techniques have already proven useful in a variety of areas including medicine, industrial production and analytical chemistry, similarly, water treatment processes can greatly benefit from the low cost and high performance of these techniques.

Silica is one of the most researched and developed materials in state of the art material engineering, due to its high specific surface area, large pore–size, chemical inertness as well as good thermal stability and low manufacture cost, moreover, Silica matrixes are highly customizable since a large number of functional groups can be added in order to improve selectivity and versatility[23].

Designer silicates have shown high adsorption, selectivity and reusability, while being adequate for fast removal of organic, inorganic, and microbial contaminants from aqueous solutions[24].

Activated carbon and electro-adsorption are able to remove EE2 to non-detected levels[25]. Carbon nanotubes and graphene oxide were also used as EE2 adsorbents, although it was found that with these materials, sediments inhibit adsorption crippling removal efficiencies[26].

#### 1.6 Alternative Technologies: SILs - characterization, advantages.

In response to the urgency of improved ways to prevent and reverse environmental pollution with CECs, and in line with the recent advances in adsorption technologies, with materials like Mesoporous silica as well as the advantageous physiochemical properties of Ionic-Liquids, the use of Supported ionic-liquid-materials (SILs) has emerged as a possibly effective and environmentally friendly way of addressing the challenges described[27].

ILs are liquid molten salts at temperatures below 100 °C, composed by inorganic or organic anions and organic cations[27]. Sometimes called "designer solvents", ILs physiochemical properties like melting point, viscosity, density, solubility, and hydrophobicity can be tailored to a large number of use cases by changing the ion combinations[28]. Figure 4 mentions some of the most common ions present in ILs.



Figure 4-Common cations and anions in ionic liquids, figure from [29]

Additionally, ILs excellent chemical, thermal, and electrochemical stability, nonflammability, negligible volatility and solvation ability[27], further contribute to ILs usefulness as solvents and as part of extraction and purification methods[27].

Techniques based on IL properties have already shown great results, IL-based aqueous biphasic systems (ABS) were already proven to successfully remove no steroidal anti-inflammatories (NSAIDs), antibiotics as well as pain and antidepressant medications from a variety of matrixes[30]–[33].

After previous activation, Silica can be functionalized with ILs[34], when immobilized, ILs lose the liquid state but retain their chemical functionality, Silica's large surface area enables adsorption of a wide variety of pollutants while providing a solid matrix which can be easily manipulated[35], furthermore reusability of SILs seems to be higher when compared to other ILs based extraction methods[27].

The inherent advantages of SILs make these materials attractive alternatives in Solid Phase Extraction Techniques [35], these are adequate remove a large number of harmful chemicals, [Si][N<sub>3114</sub>]Cl was able to adsorb 0.08 mmol of acetylsalicylic acid per gram of material[36], additionally, SILs have already been successfully used to determinate trace levels of steroid hormones in water and urine samples[37].

# 2 Experimental section

#### 2.1 Materials

Table 4 lists the materials used in the experimental section of this work. There are two main sections, the screening test to evaluate the performance of the SILs and subsequent synthesis and characterization of the SIL that showed a higher adsorption for EE2 from a selected group of SILs provided by the research team. Followed by adsorption experiments comparing the best SIL with Activated Carbon.

| Reagent                        | Purity           | Supplier          |
|--------------------------------|------------------|-------------------|
| 3-chloropropyltrimethoxysilane | 98%              | Acros Organics    |
| Ethanol                        | Analytical Grade | Fisher Scientific |
| Ethynil Estradiol              | >98%             | Sigma             |
| Hydrochloric Acid              | 37%              | Sigma-Aldrich     |
| Methanol                       | HPLC grade       | Fisher Scientific |
| N,N-dimethyloctylamine         | 95%              | Aldrich           |
| Silica Gel (60Å)               | -                | Merck             |
| Sodium Hydroxide               | 98%              | Fisher            |
| Toluene                        | 99.80%           | Fisher Scientific |

Table 4-Materials used for the synthesis of [Si][N<sub>3118</sub>]Cl, with the respective degree of purity and supplier.

#### 2.2 Synthesis of Supported ionic liquids

The synthesis of [Si][N<sub>3118</sub>]Cl was preformed according to the protocol described by Bernardo et al. [36] Firstly, it is necessary to activate the silica, with this objective, HCl (37% m/m) is used to treat silica for a 24 h period. After this step, the silica was washed with distilled water, increasing its pH, and dried at 55 °C for another 24 h. The procedure to prepare the SILs can be described as a twostep synthesis. In the first step 60 mL of toluene and 5 mL of 3-chloropropyltrimethoxysiliane are added to 5 g of activated silica. The resulting suspension placed under reflux and agitation at 100 °C for 24 h. The resulting solid is subsequently filtrated and washed with 100 mL of toluene, 200 mL of ethanol:water 1:1 (v/v), 500 mL of water and 100 mL of methanol. The material obtained is dried at 60 °C for 24 h and denominated [Si][C<sub>3</sub>]Cl.

In the second step 5 mL of N,N-dimethyloctylamine are added to a round bottom flask containing 50 mL of toluene and 5,0 g of [Si][C<sub>3</sub>]Cl. The suspension is then placed under reflux and agitation for 24 h. The resulting solid is subsequently filtrated and washed with 100 mL of toluene, 300 mL of ethanol, 300 mL of water and 150 mL of methanol. The material obtained is dried at 50 °C for 24 h and denominated [Si][N<sub>3118</sub>]Cl(Figure 5).



*Figure 5- Synthesis route to prepare [Si][N3118]Cl.* 

#### 2.3 Characterization of [Si][N<sub>3118</sub>]Cl

#### 2.3.1 Elemental analysis

The carbon, hydrogen and nitrogen content of this SIL was determined using the equipment TruSpec 630-200-200, with a 2 mg sample, a combustion furnace temperature of 1075°C and afterburner temperature of 850°C.

The detection method for carbon and hydrogen was infrared absorption and for nitrogen was used thermal conductivity.

#### 2.3.2 Point of zero charge (PZC)

The point of zero charge was determined by the measurements of zeta potential of aqueous suspensions of the material in a wide range of pH values. To adjust the pH aqueous solutions of NaOH and HCl 0.01 M were used.

These results were acquired by the equipment Malvern Zetasizer Nano ZS (Malvern Instruments Ltd. Malvern) at room temperature (25 °C) and using an appropriate cell to perform this experiment.

#### 2.4 Methods of quantification

The method used for the quantification of EE2 (40% methanol:water solutions (40% v/v)) was UV-Vis spectroscopy, using Shimadzu UV-1800, Pharma-Spec UV-Vis and 1x1cm

quartz cells. Firstly, a calibration curve was assessed at the maximum absorbance wavelength of EE2, which is 281 nm (Figure A1).

#### 2.5 SILs screening

The first step is to identify the SIL with higher adsorption capacity for EE2 adsorption from the group of SILs being tested by the research team. With this objective, samples of 25mg of each SIL ([Si][C<sub>3</sub>]Cl, [Si][C<sub>3</sub>C<sub>1</sub>Im]Cl, [Si][N<sub>3114</sub>]Cl, [Si][N<sub>3116</sub>]Cl, [Si][N<sub>3118</sub>]Cl, [Si][N<sub>3222</sub>]Cl, [Si][N<sub>3444</sub>]Cl, Si[N<sub>3666</sub>]Cl, [Si][N<sub>3888</sub>]Cl, [Si][N<sub>311</sub>Bz]Cl), were placed in contact with 5mL of a methanol (40% v/v) solution of EE2 (0.4 M) and stirred at 25 °C for 60 minutes. 4 mL of each suspension were centrifuged at 13000 rpm for 2 minutes and the supernatant EE2 concentration was determined using UV-Vis spectroscopy and a calibration curve. Scheme of different SILs structures in figure B2.

#### 2.6 Adsorption experiments

Having found the best SIL for EE2 adsorption, it became interesting to compare  $[Si][N_{3118}]Cl$  adsorption kinetics and isotherms to those of one of the most popular adsorbents and benchmark, Activated Carbon (AC).

It is important to note that all the EE2 solutions used in this work were prepared in a 40% v/v methanol aqueous solution since EE2 solubility in water is very low which impairs the possibility of quantification of EE2 solutions via UV-Vis spectroscopy.

Additionally, EE2 solutions were prepared with much higher concentrations than those found in aquatic systems, this was needed to ensure a higher absorption at UV-Vis range and assure a minimal measurement error.

The amount of contaminant adsorbed per unit mass of adsorbent in equilibrium was expressed by the following equation:

$$qe = \frac{CO - Ce}{m} \times V$$

Where C0 is the initial concentration of contaminant in solution (mg/L), Ce is the equilibrium concentration of the contaminant (mg/L), V is the volume of the aqueous solution (L) and m is the mass of adsorbent used (g).

Additionally, the adsorption efficiency (AE%) was expressed as the percentage of removed adsorbate:

$$AE\% = \frac{C0 - Ce}{C0} \times 100$$

This parameter does not account for the adsorbent mass and as such can only be used as a reference.

#### 2.6.1 Adsorption kinetics

Kinetics become a key factor when analyzing commercial use of an adsorbent. In order to evaluate the materials  $[Si][N_{3118}]Cl$  and Activated Carbon adsorption kinetics a similar process to the one used in the screening was used. In this case multiple suspensions of EE2 in methanol aqueous solutions (0.2M) with either 25 mg of  $[Si][N_{3118}Cl]$  or AC were prepared and contact time was the variable.

Initially the time interval chosen was 60 minutes which was enough for EE2 to establish a convincing plateau, however, in Activated Carbon case samples were measured in an 8 h time frame.

Two different kinetic models were used the pseudo-first-order and the pseudo-second order. The pseudo-first-order equation of Langergren [38] assumes that the rate of change of solute uptake with time is directly proportional to difference in saturation concentration and the amount of solid uptake with time, being expressed by the following equation:

$$\log(qe - qt) = \log(qe) - \frac{k1}{2.303}t$$

With qt being the amount adsorbed at time t and  $k_1$  is the rate constant of first order (min<sup>-1</sup>).

The pseudo-second order equation of Ho and Mckay[39], assumes that the rate-limiting step is chemical sorption or chemisorption, being expressed by the following equation:

$$\frac{dqt}{dq} = k2(qe - qt)^2$$

With k<sub>2</sub> being the second-order sorption rate constant (g/mg min).

The program GraphPad Prism 9 was used to represent the experimental data and subsequently to fit the kinetic models to the experimental data.

#### 2.6.2 Adsorption isotherms

In order to study the variance in maximum  $q_e$  with adsorbate concentration, 5 mL EE2 solutions with concentration ranging from 0,4 M to 0,05 M were mixed with 15 mg of

 $[Si][N_{3118}]Cl$  and Activated Carbon. The suspensions were stirred until the equilibrium time determine by the kinetic studies and the final EE2 concentration was measured following the procedure in section 2.5.

Isotherm models can be used to study the relation between adsorbate concentration and adsorbent adsorption capability.

The Langmuir isotherm model assumes a homogeneous adsorption surface with a fixed number of accessible sites[40], being expressed by the following equation:

$$qe = qm \frac{bCe}{1 + bCe}$$

The constants  $q_m$  and b represent the maximum adsorption capacity and energy adsorption, respectively.

The Freundlich isotherm model, mostly used to represent nonlinear adsorption, assumes that multiply adsorbate layers can form[41], increasing adsorption capability with adsorbate concentration. This exponential equation is expressed as:

$$qe = Kf \times Ce^{1/n}$$

K<sub>F</sub> is the Freundlich equilibrium constant (mg/g), and n is the Freundlich exponent.

The SIPS model is a combination of the Langmuir and Freundlich models[41], resembles Freundlich model at low concentrations and Langmuir model at higher concentrations, being expressed by the following equation:

$$qe = \frac{Qmax \times Ks \times Ce^{1/n}}{1 + Ks \times Ce^{1/n}}$$

Ks is the Sips equilibrium constant, Qmax is the Sips maximum adsorption capacity (mg/g) and n is the exponent that describes the surface heterogeneity.

GraphPad Prism 9 was used to chart the experimental data and subsequently to fit isotherm models.

#### 2.6.3 Column adsorption

From the batch tests described in 2.6.2 it was not possible to determine the [Si][N<sub>3118</sub>]Cl maximum EE2 adsorption capacity (maximum mass of contaminant (in mg) per mass of adsorbent (in g)), a different method was employed.

For that 300 mg of  $[Si][N_{3118}]Cl$  were compacted between two permeable plaques in a Solid Phase Extraction (SPE) cartridge and a peristaltic pump was connected to ensure a constant flow. The column was continuously filled with a methanol aqueous solution of EE2 (0.4g/L) and 10mL aliquots of the eluted were quantified with UV-Vis spectroscopy, the procedure was stopped when the eluted aliquot concentration equaled the initial solution concentration.

[Si][N<sub>3118</sub>]Cl maximum EE2 adsorption capacity was determined by calculating the mass of EE2 retained in the column and dividing by the SIL mass.

# 3 Results and discussion

#### 3.1 Material characterization

3.1.1 Elemental analysis

The [Si][N<sub>3118</sub>]Cl elemental analysis results indicate 10.92%C, 2.33 %H and 0.79 %N content in mass percentage.

#### 3.1.2 Point of zero charge (PZC)

Figure 7 shows the zeta potential measurements used to find the PZC of [Si][N<sub>3118</sub>]Cl.



Figure 6-Zeta potential in function of pH for [Si][N<sub>3118</sub>]Cl.

[Si][N<sub>3118</sub>]Cl PZC was determined as 10.79, this means that this SIL will remain positively charged in the vast majority of real world or industrial conditions, as such, pH is not the most important variable to control when using this adsorbent and when electrostatic interaction between adsorbent and adsorbate may be present.

#### 3.2 SIL screening

Figure 6 plots the qe results of the tested SILs.



Figure 7-Comparasion of different SILs EE2 adsorption potential.

From the analysis of figure 5 it is clear that these adsorbents removal efficiencies for EE2 are relatively low and that  $[Si][N_{3118}]Cl q_e$  is the highest in the chosen conditions, inclusively producing better results than Activated Carbon. Results in table A1.

[Si][N<sub>3118</sub>]Cl octyl group likely promotes hydrophobic interactions with EE2, however, [Si][N<sub>3888</sub>]Cl didn't preform as well as [Si][N<sub>3118</sub>]Cl despite having more octyl groups, this is likely because the added octyl groups condition the adsorption by steric hindrance.

Additionally, the q<sub>e</sub> results of some adsorbents returned negative which stem from both zero adsorption and the margin of error of the procedure.

In light of this results, [Si][N<sub>3118</sub>]Cl was the only SIL further studied for EE2 removal in this work.

#### 3.3 Adsorption Kinetics



Conditions:  $w_{SIL} = 25 \text{ mg of SIL} | C_0 = 200 \text{ mg/L} | V = 5 \text{ mL} | t = 0 - 480 \text{ min}$ 

Figure 8- Fit of Langergren and Ho models to  $Si[N_{3118}]Cl$  and Activated Charcoal qe in function of time experimental data.

| Table 5- Langergr | en and Ho models | kinetic parameters. |
|-------------------|------------------|---------------------|
|-------------------|------------------|---------------------|

| SIL                        |                    |                        |                |                         |                        |                | Equilibrium time |
|----------------------------|--------------------|------------------------|----------------|-------------------------|------------------------|----------------|------------------|
|                            | Pseudo first-order |                        |                | Pseudo second-order     |                        |                | (min)            |
|                            | q <sub>t</sub> /   | k <sub>1</sub> / min-1 | R <sup>2</sup> | q <sub>t</sub> / mg.g-1 | k <sub>2</sub> / min-1 | R <sup>2</sup> |                  |
|                            | mg.g-1             |                        |                |                         |                        |                |                  |
| [Si][N <sub>3118</sub> ]Cl | 11.69              | 1.298                  | 0.893          | 11.95                   | 0.267                  | 0.747          | 5                |
| AC                         | 27.82              | 0.010                  | 0.965          | 33.57                   | 0.00035                | 0.981          | 360              |

It was possible to fit both the Langergren and Ho models to the experimental data using GraphPad and the curves follow the expected behavior although the  $R^2$  values are not ideal due to the variation between repetitions of the procedure. Experimental data in table C1.

Additionally, the analysis of Figure 8 and Table 5 reveals that Activated Carbon has a higher qt than the studied SIL, however, EE2 adsorption by  $Si[N_{3118}Cl]$  is much faster, reaching equilibrium at around 5 minutes. With Activated Carbon the EE2 adsorption only surpassing  $Si[N_{3118}Cl]$  adsorption after 40 minutes, reaching equilibrium at the 360-minute mark.

The differences in adsorption rate can be a determinant factor when adapting these adsorbents to large scale uses since these uses would likely involve continuous flow.

#### 3.4 Adsorption Isotherms



Conditions:  $w_{SIL}$  = 15 mg of SIL |  $C_0$  = 50 - 460 mg/L | V = 5mL | t = 20 and 480 min

*Figure 9- Fit of Langmuir and Freunlich models to Si*[*N*<sub>3118</sub>*Cl*] *and Activated Charcoal qe in function of EE2* concentration experimental data.

| Table 6-Langmuir and | Freundlich | isotherm parameter. | s. |
|----------------------|------------|---------------------|----|
|----------------------|------------|---------------------|----|

| SIL                        | F                      | reundlich |                | Langmuir                 |           |                | SIPS                     |                        |   |                | qe          |
|----------------------------|------------------------|-----------|----------------|--------------------------|-----------|----------------|--------------------------|------------------------|---|----------------|-------------|
|                            | K <sub>F</sub><br>mg/g | n         | R <sup>2</sup> | q <sub>max</sub><br>mg/g | B<br>L/mg | R <sup>2</sup> | q <sub>max</sub><br>mg/g | K <sub>s</sub><br>L/mg | n | R <sup>2</sup> | max<br>mg/g |
| [Si][N <sub>3118</sub> ]Cl | 0.0632                 | 0.9227    | 0.995          |                          | No fit    |                |                          | No fit                 |   |                | -           |
| AC                         | 8.426                  | 3.110     | 0.926          | No fit                   |           |                |                          | No fit                 |   |                | 52.41       |

The Freundlich isotherm model produced the best fit for a EE2 adsorption isotherm, GraphPad was able to fit the Langmuir model to the experimental data but unable to find good fit parameters, additionally, the program was unable to fit properly the SIPS model to the experimental data in table C2.

It was possible to determine Activated Carbon maximum  $q_e$  which is 52.41 mg/g, contrarily, [Si][N<sub>3118</sub>]Cl isotherm followed a linear pattern and did not return a maximum  $q_e$  value. The maximum  $q_e$  for EE2 using this SIL is 44.80 mg/g and was determined with the procedure described in section 2.6.3. Experimental data in table C3.

The material [Si][ $N_{3118}$ ]Cl maximum q<sub>e</sub> is 15% inferior to that of Activated Carbon while reaching equilibrium in 1/72 of the time.

Although the results are promising, EE2 is found in Nature/WWTPs at ng/L levels and in urine at  $\mu$ g/L levels. These values are orders of magnitude lower than those used in this work. On the same note, the EE2 solutions were prepared with 40% methanol in water.

This medium has vastly different properties to those of systems were EE2 removal should be evaluated, as such, the extrapolation of these results to real world scenarios is not straightforward.

# 4 Conclusion and future work

From a set of ten SILs, namely [Si][C<sub>3</sub>]Cl, [Si][C<sub>3</sub>C<sub>1</sub>Im]Cl, [Si][N<sub>3114</sub>]Cl, [Si][N<sub>3116</sub>]Cl, [Si][N<sub>3118</sub>]Cl, [Si][N<sub>3222</sub>]Cl, [Si][N<sub>3444</sub>]Cl, Si[N<sub>3666</sub>]Cl, [Si][N<sub>3888</sub>]Cl, [Si][N<sub>311</sub>Bz]Cl, [Si][N<sub>3118</sub>]Cl emerged has the best SIL for EE2 removal. The comparison of [Si][N<sub>3118</sub>]Cl and Activated Carbon EE2 adsorption kinetics showed that the use of SILs provides a speed advantage when compared to this popular adsorbent.

The maximum experimental  $q_e$  for [Si][N<sub>3118</sub>]Cl was 44.81 mg/g and the maximum experimental  $q_e$  for Activated Carbon was 52.41 mg/g. Additionally, the Freundlich isotherm model provided the best fit for EE2 adsorption experimental data.

Furthermore, we can conclude that different techniques are needed to study EE2 adsorption in matrices where EE2 contamination is a concern. EE2 low solubility and UV-Vis spectroscopy relative low sensibility greatly impair the obtention of meaningful results.

In future work, it would be interesting to evaluate how EE2 adsorption behavior changes in lower methanol ratios, similarly, the usage of a technique with more sensitivity like High performance Liquid Chromatography could allow the study of EE2 adsorption at more realistic concentrations.

Additionally, since EE2 removal directly in toilets might be the most effective method of decreasing the contamination in the environment, the study of EE2 adsorption in urine samples would further evaluate the viability of this method.

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# 6 Annex





Figure A1-Calibration curve of Ethinyl Estradiol in 60:40 water: methanol.

### 6.2 Screening

| SIL                                      | Ce (g/L) | %AE   | qe (mg <sub>EE2</sub> /g <sub>SIL</sub> ) |
|--|----------|-------|---|
| Silica Gel                               | 0.4124   | -3.40 | -2.73                                     |
| A. Carbon                                | 0.2840   | 28.80 | 22.79                                     |
| Si[C <sub>3</sub> ]Cl                    | 0.3428   | 14.05 | 10.85                                     |
| [Si][C <sub>1</sub> C <sub>3</sub> Im]Cl | 0.4071   | -2.08 | -1.67                                     |
| [Si][N <sub>3114</sub> ]Cl               | 0.4016   | -0.69 | -0.55                                     |
| Si[N <sub>3116</sub> ]Cl                 | 0.3141   | 21.23 | 16.68                                     |
| Si[N <sub>3118</sub> ]Cl                 | 0.2520   | 36.80 | 29.91                                     |
| [Si][N <sub>3222</sub> ]Cl               | 0.3312   | 16.95 | 12.71                                     |
| [Si][N <sub>3444</sub> ]Cl               | 0.3038   | 23.82 | 18.81                                     |
| Si[N <sub>3666</sub> ]Cl                 | 0.3171   | 20.48 | 16.24                                     |
| [Si][N <sub>3888</sub> ]Cl               | 0.2950   | 26.02 | 20.99                                     |
| [Si][N <sub>311</sub> Bz]Cl              | 0.3282   | 17.71 | 13.58                                     |

Table B1- Different adsorbents screening results.



Figure B2-Sructures of different SILs.

# 6.3 Adsorption studies

## 6.3.1 Adsorption kinetics

Table C1-Adsorption efficiency (%AE), equilibrium concentration of EE2 after adsorption (Ce) and concentration of EE2 in solid phase  $(q_e)$  at different contact times using  $[Si][N_{3118}]Cl$  and Activated Carbon.

| [Si][N <sub>3118</sub> ]Cl |          |       | Activated Carbon                          |            |          |       |  |
|----------------------------|----------|-------|---|------------|----------|-------|--|
| time (min)                 | Ce (g/L) | %AE   | qe (mg <sub>EE2</sub> /g <sub>SIL</sub> ) | time (min) | Ce (g/L) | %AE   | qe (mg <sub>EE2</sub> /g <sub>AC</sub> ) |
| 1                          | 0.1665   | 18.05 | 7.28                                      | 1          | 0.1935   | 4.78  | 1.93                                     |
| 2                          | 0.1545   | 23.95 | 10.08                                     | 2          | 0.1924   | 5.32  | 2.15                                     |
| 5                          | 0.1456   | 28.36 | 11.81                                     | 5          | 0.1833   | 9.81  | 3.93                                     |
| 10                         | 0.1448   | 28.73 | 12.04                                     | 10         | 0.1804   | 11.21 | 4.40                                     |
| 20                         | 0.1489   | 26.71 | 10.81                                     | 20         | 0.1648   | 18.88 | 7.43                                     |
| 40                         | 0.1443   | 28.98 | 11.38                                     | 40         | 0.1406   | 30.79 | 12.15                                    |
| 60                         | 0.1468   | 27.78 | 11.69                                     | 60         | 0.1299   | 36.07 | 14.09                                    |
| 1                          | 0.1545   | 23.95 | 9.93                                      | 5          | 0.1906   | 6.22  | 2.42                                     |
| 2                          | 0.1465   | 27.91 | 11.14                                     | 10         | 0.1871   | 7.91  | 3.19                                     |
| 5                          | 0.1424   | 29.93 | 12.47                                     | 30         | 0.1672   | 17.72 | 7.25                                     |
| 10                         | 0.1416   | 30.34 | 12.50                                     | 60         | 0.1394   | 31.41 | 12.23                                    |
| 20                         | 0.1405   | 30.87 | 12.38                                     | 120        | 0.1105   | 45.63 | 18.15                                    |
| 40                         | 0.1460   | 28.15 | 10.90                                     | 180        | 0.0983   | 51.61 | 21.06                                    |
| 60                         | 0.1471   | 27.62 | 11.17                                     | 240        | 0.0900   | 55.73 | 23.59                                    |
|                            |          |       |   | 360        | 0.0493   | 75.72 | 29.59                                    |
|                            |          |       |   | 480        | 0.0617   | 69.66 | 28.54                                    |

#### 6.3.2 Adsorption isotherms

Table C2-Adsorption efficiency (%AE), equilibrium concentration of EE2 after adsorption (Ce) and concentration of EE2 in solid phase  $(q_e)$  with different initial concentrations (Ci) using [Si][N<sub>3118</sub>]Cl and Activated Carbon.

|          | [Si][N <sub>3118</sub> ]Cl |           |   |       | Activated Ca | rbon                                     |
|----------|----------------------------|-----------|---|-------|--------------|--|
| Ci(mg/L) | %AE                        | Ce (mg/L) | qe (mg <sub>EE2</sub> /g <sub>SIL</sub> ) | %AE   | Ce (mg/L)    | qe (mg <sub>EE2</sub> /g <sub>AC</sub> ) |
| 400      | 23.71                      | 346.62    | 35.67                                     | 35.99 | 290.83       | 52.41                                    |
| 350      | 23.77                      | 300.38    | 30.60                                     | 38.35 | 242.92       | 48.43                                    |
| 300      | 23.38                      | 259.67    | 26.24                                     | 44.17 | 189.22       | 51.62                                    |
| 250      | 24.12                      | 217.12    | 22.27                                     | 42.71 | 163.93       | 42.43                                    |
| 200      | 21.80                      | 177.33    | 16.15                                     | 46.88 | 120.45       | 34.51                                    |
| 100      | 24.72                      | 85.19     | 9.14                                      | 65.80 | 38.70        | 24.82                                    |
| 50       | 17.93                      | 45.23     | 3.17                                      | 91.49 | 4.69         | 17.63                                    |

#### 6.3.3 Column adsorption

Table C3-Results used to calculate  $[Si][N_{3118}]Cl$  maximum  $q_e$ .

| V <sub>eluted</sub> (mL) | Ce (g/L)             | m <sub>EE2</sub> adsorbed(mg)                    |
|--------------------------|----------------------|--|
| 10                       | 0.04691              | 3.863  |
| 10                       | 0.08276              | 3.505  |
| 11                       | 0.21779              | 2.370  |
| 10                       | 0.30541              | 1.278  |
| 10                       | 0.36974              | 0.635  |
| 10                       | 0.38197              | 0.513  |
| 10                       | 0.39202              | 0.412  |
| 10                       | 0.39989              | 0.333  |
| 10                       | 0.41279              | 0.204  |
| 10                       | 0.41279              | 0.204  |
| 10                       | 0.41547              | 0.178  |
| Ci (g/L)                 | m <sub>SIL</sub> (g) | SIL max qe(mg <sub>EE2</sub> /g <sub>SIL</sub> ) |
| 0.43323                  | 301.2                | 44.81  |