Universidade de Aveiro

Departamento de Química

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MARIA JOÃO GONÇALVES DOS SANTOS

REMOÇÃO DE CITOSTÁTICOS UTILIZANDO MATERIAIS FUNCIONALIZADOS COM LÍQUIDOS IÓNICOS

CYTOSTATIC REMOVAL USING MATERIALS FUNCTIONALIZED WITH IONIC LIQUIDS

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Bioquímica realizada sob a orientação científica da Doutora Mara Guadalupe Freire Martins, Investigadora Coordenadora do Departamento de Química da Universidade de Aveiro, e coorientação da Doutora Márcia Carvalho Neves investigadora do Departamento de Química da Universidade de Aveiro.

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À minha Dulcinha…

o júri

presidente

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palavras-chave Citostáticos, contaminação ambiental, líquidos iónicos suportados, extração em fase sólida.

resumo

A incidência e a prevalência do cancro têm vindo a aumentar rapidamente ao longo dos anos e estima-se que se atinjam 22,2 milhões de novos casos em 2030. A quimioterapia é a abordagem de tratamento mais utilizada, empregando fármacos da família dos citostáticos que destroem as células cancerígenas. No entanto, estes fármacos induzem efeitos genotóxicos, mutagénicos e carcinogénicos nos humanos e animais e estes acabam por contaminar águas residuais hospitalares e domésticas através da urina e fezes de pacientes oncológicos. A maioria das estações municipais de tratamento de águas residuais não foi projetada para eliminar contaminantes emergentes, especialmente fármacos, resultando na contaminação de rios, estuários, lençóis de água e até de água potável. O principal objetivo desta dissertação é estudar uma nova tecnologia capaz de remover fármacos antineoplásicos de soluções aquosas, para que mais tarde possam ser aplicados para a remoção dos mesmos da urina, evitando a entrada de compostos tóxicos e seus metabolitos nas águas residuais.

Este trabalho consistiu na síntese e caracterização de líquidos iónicos suportados em partículas de sílica, nomeadamente, [Si][C_3C_1im]Cl, [Si][N_{3114}]Cl, [Si][N_{3222}]Cl, [Si][N_{3444}]Cl e [Si][N_{3888}]Cl, e na sua posterior aplicação na adsorção de um citostático, a ciclofosfamida. De forma a otimizar o procedimento experimental estudos de adsorção foram também levados a cabo com um pesticida, o imidacloprid. A análise dos dados experimentais resultantes dos estudos de adsorção foi feita a partir do ajuste de equações de modelos cinéticos e de isotérmicas.

A caracterização dos liquídos iónicos suportados demonstrou que a sua síntese ocorreu com sucesso. No estudo de adsorção do imidacloprid o modelo cinético que apresentou um melhor ajuste aos dados experimentais foi o de pseudo segunda-ordem e a isotérmica de Langmuir. No caso da ciclofosfamida, o modelo cinético que apresentou um melhor ajuste foi o modelo de Elovich para o [Si][N_{3222}]Cl e [Si][N_{3888}]Cl mas utilizando o [Si][N_{3444}]Cl é o modelo de pseudo segunda-ordem. Nas isotérmicas de adsorção o modelo SIPS apresenta um melhor ajuste para [Si][N_{3222}]Cl e [Si][N_{3888}]Cl e o modelo Freundlich para o [Si][N_{3444}]Cl. Para a ciclofosfamida o material que apresenta melhores resultados é o [Si][N_{3888}]Cl, atingindo um q_e de 67,6 mg/g.

Este trabalho alerta para um problema, a contaminação do meio ambiente por medicamentos antineoplásicos, e descreve uma possível solução para esta adversidade, a remoção de fármacos utilizando líquidos iónicos suportados.

keywords Cytostatics, environmental contamination, supported ionic liquids, solid-phase extraction

abstract

Cancer incidence and prevalence are growing rapidly and is estimated to reach 22.2 million new cancer cases by 2030. Chemotherapy is the most common treatment, which uses cytostatic drugs that destroy cancer cells. However, these drugs are reported to exert genotoxic, mutagenic and carcinogenic effects in humans and animals, and they end up contaminating hospital and household wastewaters via urine and faeces of oncologic patients. Most of the municipal wastewater treatment plant are not designed to eliminate emerging contaminants, specially pharmaceuticals, resulting in the contaminations of rivers, estuaries, groundwater and even drinking water. The main objective of this dissertation is to study a new technology to remove anticancer drugs from aqueous solutions, so it can later be applied to remove cytostatics from urine, avoiding the entrance of these toxic compounds and their metabolites into wastewater.

This work consisted in the synthesis and characterization of ionic liquids supported on silica particles, namely, [Si][C_3C_1im]Cl, [Si][N_{3114}]Cl, [Si][N_{3222}]Cl, [Si][N_{3444}]Cl and [Si][N_{3888}]Cl, and its subsequent application in the adsorption of a cytostatic, cyclophosphamide. In order to optimize the experimental procedure adsorption studies were also carried out with a pesticide, imidacloprid. The analysis of the experimental data resulting from the adsorption studies was made by adjusting equations of kinetics and isotherm models.

The characterization of the supported ionic liquids denoted a successful synthesis. In the imidacloprid adsorption study the kinetic model that presented the best fit to the experimental data was the pseudo second-order model and the Langmuir isotherm. For cyclophosphamide, the best fitted kinetic model was the Elovich model for [Si][N_{3222}]Cl and [Si][N_{3888}]Cl and using [Si][N_{3444}]Cl was the pseudo second-order model. In adsorption isotherms the SIPS model had a better fit for [Si][N_{3222}]Cl and [Si][N_{3888}]Cl and the Freundlich model for [Si][N_{3444}]Cl. The best performing material was [Si][N_{3888}]Cl, reaching a q_e of 67.6 mg/g.

This work raises the awareness of a problem, i.e. the contamination of the environment with anticancer drugs, and describes a possible solution to this issue, the use of supported ionic liquids to remove pharmaceuticals.

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List of abbreviations

%AE – Adsorption efficiency

5-FU – 5-fluorouracil

A – pore surface area

ABS – Aqueous Biphasic System

ATC – Anatomical Therapeutic Chemical

ATR-FTIR – Attenuated Total Reflectance – Fourier-Transform Infrared Spectroscopy

BA – Bonding Amount

BET – Brunauer-Emmett-Teller

BJH – Barrett-Joyner-Halenda

C_0 – Initial concentration

C_e – Equilibrium concentration

C_t – Concentration at t time

CP – Cyclophosphamide

CPMA – Cross Polarization Magic Angle Rotation

D_p – Pore size diameter

EMA – European Medical Agency

FDA – US Food and Drug Administration

HPLC-DAD – High Performance Liquid Chromatography with Diode-Array Detector

IARC – International Agency for Research on Cancer

IFO – Ifosfamide

IL – Ionic Liquid

k_1 – Rate constant of first order

k_2 – Rate constant of second order

K_F – Freundlich equilibrium constant

K_S – Sips equilibrium constant

PEC – Predicted Environmental Concentration

PZC – Point of Zero Charge

q_e – Concentration of adsorbate in solid phase in equilibrium

q_t – Concentration of adsorbate in solid phase at t time

$q_{máx}$ – Maximum adsorption capacity

S_{BET} – Surface Area

SEM – Scanning Electron Microscope

SIL – Supported Ionic Liquid

SPE – Solid-Phase Extraction

WHO – World Health Organization

WWTP – Wastewater Treatment Plant

1. Introduction

1.1. Cancer overview

According to the World Health Organization (WHO) cancer is a generic term to describe a large group of diseases that are capable to affect almost any part of the body.[1] It begins with an induced change in a single normal cell which makes it "neoplastic".[2] Tumours mature from benign to malign lesions by gaining multiple mutations over time.[3] DNA damage is a serious problem faced by aerobic organisms. Alteration and loss of DNA bases can alter the coding proteins leading to mutations, which is a powerful cause of human disease.[4] The mutated cell has a growth advantage when compared to the adjacent normal cells, and their proliferation initiates immediately or after a latent time. The fully developed tumour has an aberrant metabolic behaviour and develops specific antigenic properties. However, there are some exceptions for this unicellular concept, namely tumours of viral aetiology and neoplasms with a strong hereditary component, which increase the susceptibility of cells to neoplastic change.[2]

Cancer has many traits, as summarized in Figure 1, and the most fundamental one is the ability of cells to proliferate indefinitely. Growth-promoting signals are produced and released from normal tissues in order to control the cell growth and division cycle, thus maintaining the homeostasis of cell number and normal tissue architecture and function. Cancer cells can acquire the capacity to preserve proliferative signalling by producing growth factor ligands themselves or by sending signals to stimulate normal cells that are in the vicinity.[5] Tumour cells can also gain resistance to cell death by apoptosis, resulting in over proliferation and/or decreased removal of cells. To maintain this unlimited replicative potential, cancer cells express functional telomerase activity, a specialized DNA polymerase that adds telomere repeat segments to the ends of the chromosomes. Therefore, it can counter the progressive telomere destruction that would otherwise occur in its absence, correlating with the resistance to induce both senescence (gradual deterioration of functional characteristics) and apoptosis. Cancer cells lose their internal growth clock and, as a result, they multiply quickly and do not mature in a suitable way.[5]

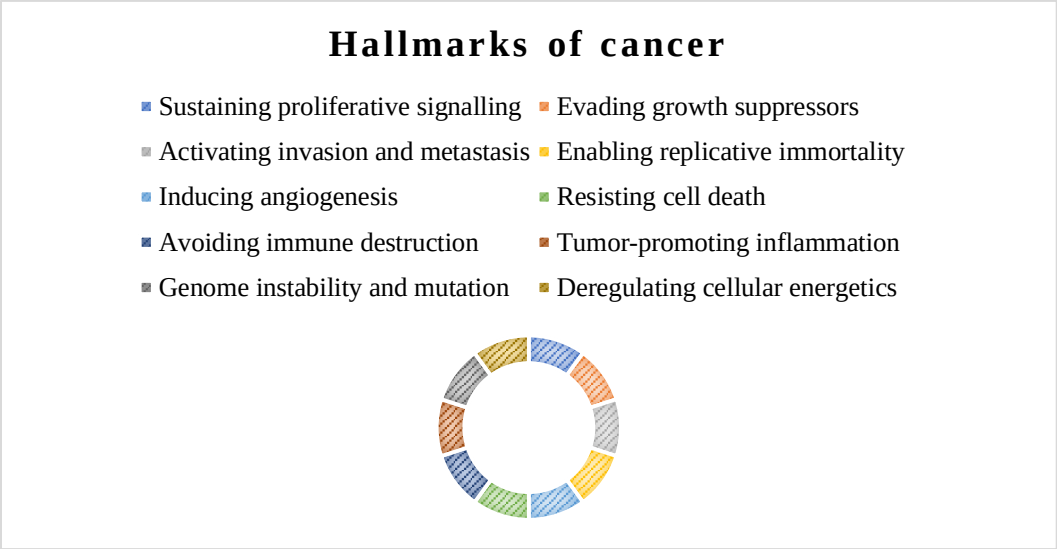


Figure 1: Hallmarks of cancer (adapted from Hanahan et al l[5]).

1.1.1. Incidence and prevalence

Cancer is anticipated to be the leading cause of death in every country of the world in the 21st century, surpassing strokes and coronary heart disease in many countries. Cancer incidence and mortality are growing rapidly for many reasons, including ageing population and changes in the prevalence and distributions of the main risk factors for cancer, multiple of which are related with socioeconomic growth.[6]

In the Global Cancer Statistics of 2018[7], the incidence and mortality of 36 cancers in 185 countries was evaluated, and it was estimated that there will be 18.1 million new cases and 9.6 million cancer deaths worldwide in 2018. Approximately one half of the cases in the world will occur in Asia, which is expected since close to 60% of the global population is located in Asia. Europe accounts for 23.4% of the total cancer cases and 20.3% rate of cancer deaths, which is alarming since it only represents 9% of the global population[7]. When combining both sexes, the most common diagnosed cancer is lung cancer, with 11.6% of the total cases, and is also the leading cause of cancer death, with 18.4% of the total cancer deaths. Figure 2 summarizes the cancer types distribution in men and women. When separating by sexes, lung cancer is the most commonly diagnosed cancer in man, but among females the most common and leading cause of cancer death is breast cancer. Worldwide, men have a 20% higher incidence rate of cancer than woman, and an astounding 50% higher

death rates for all cancers combined. Some types of cancers are scattered around the globe, whereas others tend to cluster in certain specific regions.[7]

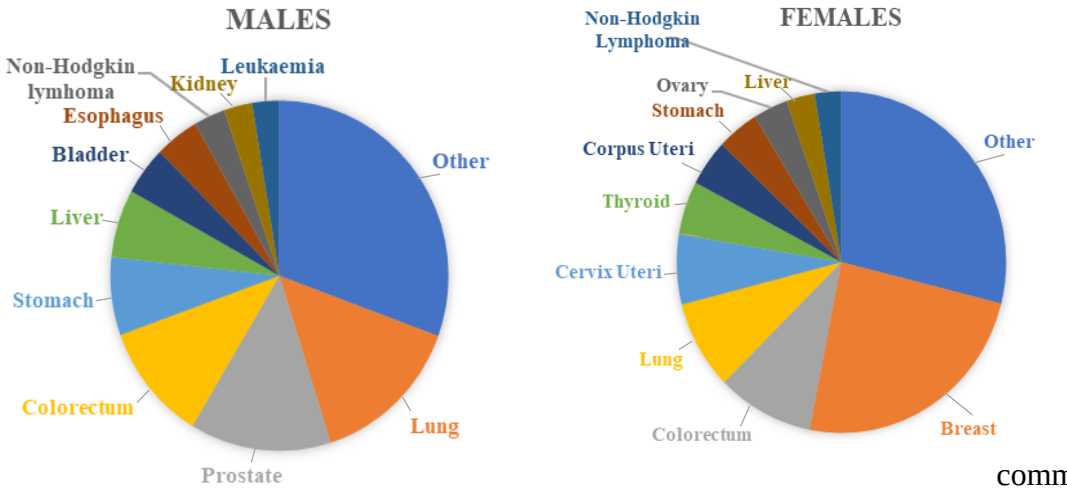


Figure 2: Most common types of cancer in males and females in 2018 (adapted from Bray et al.[7]).

Figure 3 shows the prevalence and incidence of cancer over the years. In 2008 12.7 million new cancer cases and 7.6 million cancer deaths occurred.[8] In 2012, there were 14.1 million new cases and 8.2 million death.[9] Unfortunately, the mortality and number of new cases have been increasing over the years, and estimates predict that it will continue to rise, reaching 22.2 new cancer cases in 2030.[10]

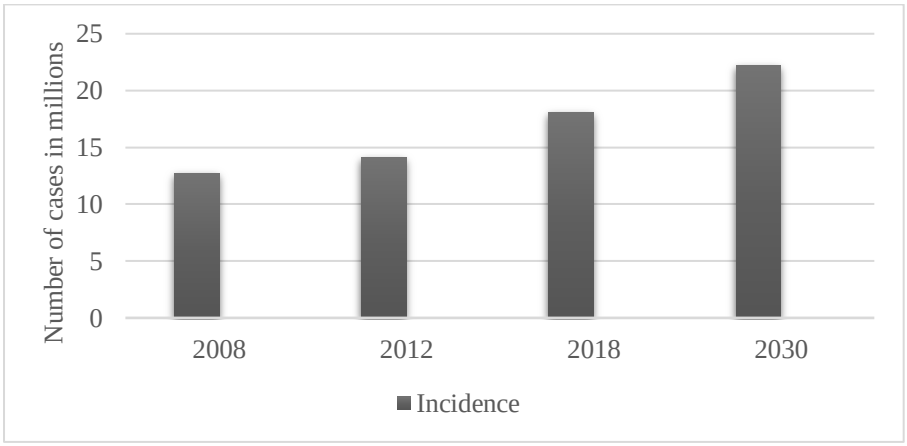


Figure 3: Incidence of cancer over the years (adapted from Bray et al[7]).

1.1.2. Anticancer drugs: cytostatics

The treatment for cancer is selected depending on the type, position and grade. It can involve surgery, intended for the removal of the tumour mass, radiotherapy and systemic therapy, which includes hormonal and targeted therapy, and chemotherapy, as depicted in Figure 4.[11] Chemotherapy is the most typical treatment approach. Anticancer drugs, also known as cytostatics or antineoplastic agents, are used to destroy cancer cells. These drugs can be broadly classified into two categories based on their mechanism of action: cytotoxic and targeted agents.[12] All cytotoxic chemotherapy drugs act by disturbing the cell cycle through targeting components of the mitotic and/or DNA replication pathways. The targeted agents stop the growth and spread of cancer by interacting with molecular targets that are involved in the pathways relevant to cancer growth, progression and spread.[12] Both of these types of drugs can be administered alone or combined to optimize their end result.[11] Chemotherapy is administered by various routes, including intravenous, intramuscular and oral, as shown in Figure 4. The chosen route depends on the pharmacological characteristics of the drug in vivo, such as absorption, metabolism and half-time life. There is in fact a significant group of chemotherapeutic drugs that are administered intravenously because they exhibit poor oral bioavailability.[13]

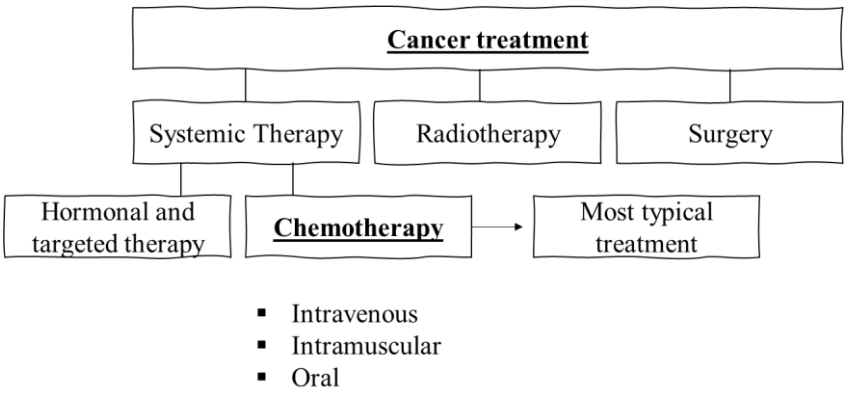


Figure 4: Types of cancer treatment.

Oral anticancer drugs were first approved by the US Food and Drug Administration (FDA) in the early 1950s, suffering a quick growth in the early 2000s, reached by the use of intravenous chemotherapy. Taking into account the inconvenience of infusion, including

pain, anxiety and inpatient status, the oral chemotherapy has the advantages of easy administration and improved quality of life.[14,15] Other advantages include flexibility for timing and location of administration (e.g. in the patients' own houses), and reduced use of healthcare resources. Besides these accessibilities, several problems arise with the use of oral chemotherapy, namely: many agents interact with other prescription and non-prescription drugs as well as with food; other drugs should be taken with food to reduce gastrointestinal irritation; dysphagia, nausea and vomiting can cause missed doses; and over-adherence increases risk for life-threatening toxicities and treatment intolerance.[16,17] Although there is a significant number of disadvantages, most of them are associated with all types of chemotherapy and are not specific of oral treatment.

The Anatomical Therapeutic Chemical (ATC) by WHO classified anticancer drugs under class L, which belongs to antineoplastic and immunomodulating agents. In this category there are four main groups, subdivided as in Table 1.[18] This list is periodically revised in order to include new compounds.[18]

Table 1: Classification of anticancer drugs by ATC.

Group	Subgroup
L01 - Antineoplastic agents	L01A - Alkylating agents
	L01B - Antimetabolites
	L01C - Plant alkaloids and natural substances
	L01D - Cytotoxic antibiotics and related substances
	L01X - Other antineoplastic agents
L02 - Endocrine therapy	L02A - Hormones
	L02B – Hormones antagonists
L03 - Immunostimulants	
L04 - Immunosuppressants	

The first use of chemotherapy for the treatment of cancer dates to 1943, during World War I.[19] It was recorded a reduction in both bone marrow and lymph nodes in soldiers exposed to the mustard gas.[19] When these results were published in 1946, the support for the synthesis and testing of these compounds blew up. In fact, it was the first evidence of drugs

that could cure cancer, specially lymphomas and leukemias.[20] Cytostatic drugs used in chemotherapy promote cell death through a variety of mechanisms, either by directly intruding with DNA (e.g. alkylating agents), or by targeting the main proteins required for cell division, for instance, by interfering with the synthesis of key co-factors and DNA/RNA protein precursors (e.g. antimetabolites), by interfering with other cellular structures and processes, or by inhibiting the growth/anti-death signal (e.g. tyrosine kinase inhibitors). All processes induce apoptosis or necrosis[11].

Cyclophosphamide (CP), an oxazaphosphorine compound, was introduced in clinical cancer chemotherapy in the 40's and since then it has been the most broadly used alkylating agent in the treatment of a variety of solid tumours, such as lung cancer[21] and breast cancer[22], and haematological malignancies, e.g. leukemias[23,24]. Despite not being a first line therapy, CP has also been prescribed as an immunosuppressive drug, often combined with other pharmaceuticals, to treat multiple autoimmune diseases, for instance rheumatoid arthritis[25,26] and systemic lupus erythematosus[27,28]. The anticancer effect of oxazaphosphorines results from the DNA crosslink formation between the alkyl groups and specific nucleophilic groups of DNA molecules, such as guanine residues. These drugs interfere with DNA replication by establishing intrastrand and interstrand DNA crosslinks[29].

CP is however an inactive prodrug that depends upon enzymatic activation to exert its function. The hepatic cytochrome P-450 system generates aldophosphamide, an unstable transport precursor, which later decomposes into two compounds accountable for the cytotoxic characteristics of the CP. After this metabolic activation, these alkylating species are translocated into the nucleus through passive diffusion or by using a transporter-mediated process[30]. Since oxazaphosphorines can damage DNA molecules all through any phase of the cell cycle, they do not have specificity for a cell-cycle phase[29].

An additional oxazaphosphorine commonly used in cancer treatment is ifosfamide (IFO). It can act as a single agent or combined with several other cytotoxic agents[31,32]. The tumour cell killing effect of ifosfamide results from DNA crosslink via the same mechanism as the one described for CP.[29] These two pharmaceuticals are an example of alkylating antineoplastic drugs. An example of an antimetabolite antineoplastic drug is fluoropyrimidine 5-fluorouracil (5-FU).[33] It can enter the cell using the same transport mechanisms as uracil.[34] Once there it converts to numerous active metabolites that disrupt

RNA synthesis. 5-FU inhibits thymidylate synthase, an enzyme that catalyses the reductive methylation of deoxyuridine monophosphate, necessary for DNA replication and repair.[35] It can also be incorporated within the DNA molecule, instead of thymidine during DNA synthesis. Therefore, it is vital that cancer cells are exposed to the 5-FU metabolites throughout S-phase, the synthesis phase. The structure of these three anticancer drugs are provided in Figure 5.

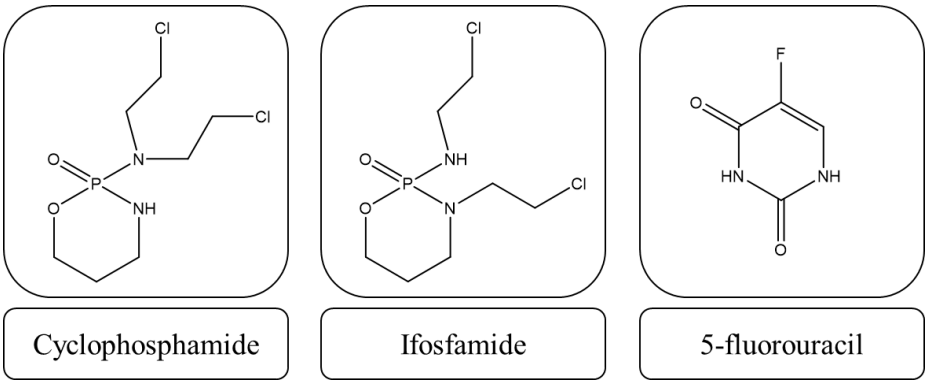


Figure 5: Chemical structures of cyclophosphamide, ifosfamide and 5-fluorouacil.

After administration, most of these drugs are only partly metabolized in the body and are excreted by urine and faeces. These drugs and respective metabolites can therefore enter hospital and household wastewater via the urine and faeces of patients undertaking chemotherapy. Metabolism of anticancer compounds is complex and can lead to the formation of multiple pharmacologically active and inactive metabolites, although the anticancer drug metabolism is well documented.[36] The majority of cytostatic drugs are highly soluble in water (10–50 g/L at 20°C–25°C), which from the medical viewpoint is extremely desirable.[37] Therefore, many anticancer drugs have high urinary excretion rates in their unchanged forms (up to 90%). Having in consideration their extremely low vapor pressure, the majority of cytostatic drugs, after being excreted from the human body, will contaminant the environment through water cycles and soil.[37]

It is estimated that about 75% of cytostatics are administered in outpatient departments[38,39] so Besse et al.[36] predicted the environmental concentrations of multiple cytostatics drugs in France, concluding that the major contribution of antineoplastic drugs in the aquatic environment was not from the hospitals effluents, but from domestic effluents,

which is linked with the increased home treatments.[36] The pathways for anticancer drugs to enter the aquatic environment is displayed in Figure 6.

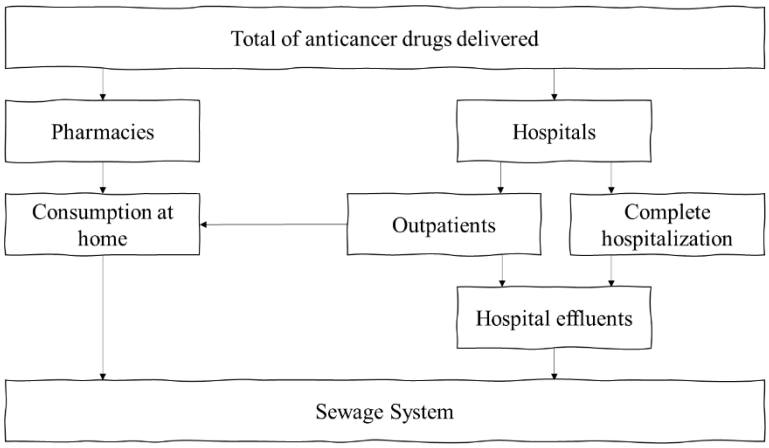


Figure 6: Pathways for anticancer drugs to enter the aquatic environment (adapted from Besse et al.[36]).

Conventional chemotherapy, even if it is targeted in the direction of certain macromolecules or enzymes, still has the trouble to distinguish tumour cells from normal cells that proliferate promptly (e.g. cells from the gastrointestinal tract and bone marrow). This leads to plentiful toxic side effects, like bone marrow suppression, gastrointestinal tract lesions, hair loss, nausea and the development of clinical resistance.[40] They can also result in genotoxic effects in non-tumour cells and lead to genetic alterations in normal cells and/or secondary tumours in case of cancer patients.[40] The International Agency for Research on Cancer (IARC) by WHO has evaluated the carcinogenic risks to humans of multiple antineoplastic drugs, and cytostatics are reported to exert genotoxic, mutagenic and carcinogenic effects on humans and animals.[41–43] In the most recent review involving pharmaceuticals, IARC re-evaluated 20 agents or combined therapies categorized as carcinogenic to humans, and these include antineoplastic agents.[44] This may lead to secondary tumours in cancer patients who were treated with these pharmaceuticals and primary tumours in subjects treated with the drugs to solve other diseases.[41–44] And further they are the only pharmaceuticals products being classified as “hazardous” under the Waste Framework Directive of the European Commission.[45]

As discussed above, cytostatic agents have been identified as genotoxic substances. Therefore, the contact with these drugs raises apprehensions regarding possible toxic effects on healthcare professionals (nurses, pharmacists, physicians, etc), especially for those who are chronically exposed to these substances. Workers interact with these drugs at multiple points, such as, delivery, preparation of infusions, transport, preparation of syringes, etc.[46] In the 90's it has been reported acute adverse health effects in healthcare professionals. Long-term side effects include negative impact on the reproductive system, and spontaneous abortion and malformation.[47] The genotoxic effects typically do not depend on a minimum dosage; hence, it is important to minimize the exposure to healthcare employees as much as possible. To accomplish this, several guidelines and regulation for safe handling of cytotoxic drugs have been published over the years.[48,49] Currently, due to the progress of the technical protection resources available, such as biological safety cabinets, the most problematic route of exposure is through skin absorption rather than inhalation or ingestion.[50] As dermal route embodies the most significant point of access into the body, surface contamination in the working area plays a key role in occupational exposure.[51] In order to guarantee the most comprehensive protection possible, protective actions must be taken into account, combining technical mechanisms and personal protective equipment (protective clothing). Simple rules must be followed, as summarized in Figure 7.

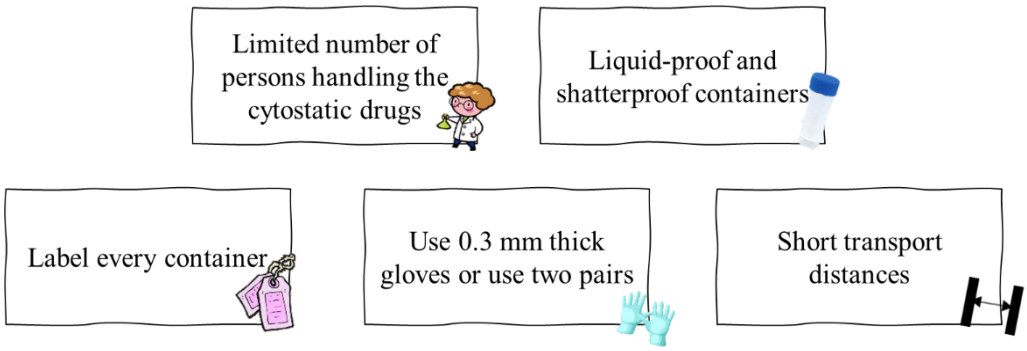


Figure 7: Rules that must be followed when handling cytostatics.

As shown in Figure 7, the number of persons handling cytostatic drugs must be limited, transport distances should be kept as short as possible, and liquid-proof, shatterproof and easy-to-clean containers (e.g., plastic boxes) should be used. Furthermore, all containers should be labelled. The glove material should be to at least 0.3 mm thick or two pairs of

gloves should be worn. They should be immediately discarded in case of visible contamination or damage. The cytostatic waste must be disposed of as hazardous waste by incineration.[52]

1.2. Contamination of water streams with emerging contaminants

The revolutionized development of resources and technologies has produced a large quantity of chemicals and compounds, which consequently increased the number of compounds that are nowadays identified as holding potential environmental threats to living beings.[53] The need to access to clean drinking water has been more difficult as a result of the release of anthropogenic contaminants into the environment. Emerging contaminants are used in everyday products, such as personal care products, plasticizers, pharmaceuticals and pesticides.[54]

1.2.1. Pharmaceuticals

Pharmaceuticals are essential for the maintenance of public health and improvement of quality of life, which makes them part of a progressively growing industry.[55] About 3000 different substances are used in human medicine, but also a large number of pharmaceuticals are used in veterinary medicine, among them are antibiotics and inflammatory drugs.[56,57]

From 1949 to 2014, a total of 150 pharmaceuticals for cancer treatment have been approved by FDA, with indication for at least one type of cancer.[58] The number of accepted drugs in cancer treatment had a gradual increase, from 1991-2014, where 116 new drugs were approved.[58] The use of anticancer drugs is expected to increase even more because the average life expectancy is increasing and the probability to have cancer increases with age; new developed drugs have less unwanted side effects; the treatment itself lasts longer as survival time increases; antineoplastic drugs have been used to treat others pathologies; and there is a trend to increasingly apply anticancer drugs in veterinary medicine.[59] For example,

the sales data by International Marketing Statistics Health Germany showed that the use of imatinib, a medicine to treat cancer, increased by 76% from 2006 to 2012.[59]

In Portugal, since 2012, there is a progressive increase in the number of sold pharmaceuticals.[60] In 2017, each Portuguese used, on average, 1 package of medicine, almost half were for citizens over 80 years-old and almost 60% for women. The most purchased non-prescription medicine in 2017 belongs to the class of analgesics and antipyretics, with paracetamol being the top-selling active substance.[60] Based on the packaging declared to VALORMED and using the market data provided by companies in the medicinal product sector, it was possible to calculate the total weight of pharmaceuticals placed on the market, with 8.371t in 2017.[61] Last year, Infarmed approved 60 new medicines, of these 49 are for hospital use and 11 for outpatients. Most of the approved drugs are for oncology (lung cancer, melanoma, myeloma and colorectal cancer).[60]

Pharmaceuticals are excreted after administration in their native form or as metabolites by humans and animal, entering aquatic systems via different ways, such as urine, faeces and also through disposal of out of date medicine.[62] Groundwater contamination by pharmaceutical ingredients (analgesic, antibiotics, antidepressants, antidiabetics, contraceptives, growth regulators, impotence drugs, painkillers and tranquilizers) is an environmental issue of widespread concern.[63] Pharmaceuticals are categorized as emerging contaminants due to their negative effects on aquatic flora and fauna. This problem has drawn more attention due to an increased ability to detect them with advanced analytical techniques over the last 20 years.[55] These emerging contaminants can not only affect fauna and flora, but also reach drinking water and be harmful to humans, as summarized in Figure 8.

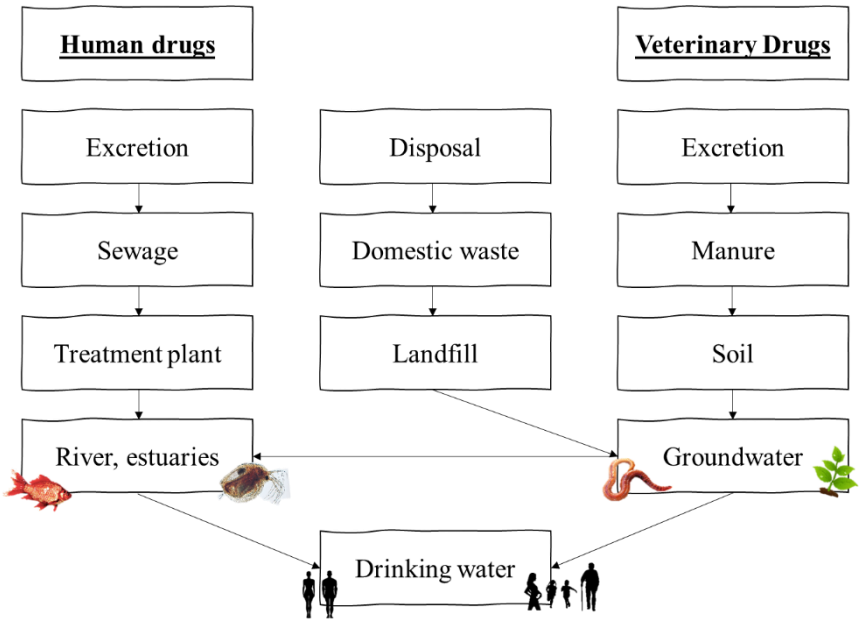


Figure 8: Fate of pharmaceuticals in the environment (adapted from Khetan et al.[62]).

Pharmaceuticals that are not readily degraded or removed in sewage treatment plants are discharged in treated effluents, resulting in the contaminations of rivers, estuaries and groundwater and drinking water (fig. 8).[57] Therefore, the need to find a solution for the contamination of wastewaters with pharmaceuticals is obvious. A class of pharmaceuticals widely used in medicine are antibiotics, and they have been detected in aquatic environments such as lakes, rivers, water reservoirs, wastewater influents, groundwater and even in drinking water.[64] In 2018, Tran et al.[65] studied the occurrence of several emerging contaminants from different geographical regions, and antibiotics were detected in the effluents at concentrations close or exceeding the predicted no-effect concentrations for resistance selection. In Spain 84 samples from 7 different wastewater treatment plants were analysed to assess the occurrence of 73 pharmaceuticals, the results indicated that the treatment applied was unable to completely remove most of the pharmaceuticals, being widespread pollutants in the aquatic environment.[66] When comparing between regions, most of the emerging contaminants tend to have higher concentrations in influents of Asian countries then those in Europe and North America countries.[65]

Anticancer drugs are used in lower amounts compared to other drugs, like painkillers or antibiotics. However, they are highly active compounds since they exert action on the structure and function of DNA[38], and are considered dangerous environmental contaminants.[36,67] A few cytotoxic drugs have been detected in the aquatic environment, at the ng/L range.[36] Studies have reported incidence of anticancer drugs in hospital and municipal effluents in China,[68,69] Spain,[70,71] Germany,[72,73] France,[74] Austria,[75,76] and Switzerland.[77] Even though the concentrations found are considerate low, a matter has been brought to light by a work in Spain[78] that showed that pharmaceutical compounds are ubiquitous in the marine environment. Pollution hotspots can be found mostly in enclosed or semi-enclosed water bodies, such as bays and estuaries that are impacted by direct and/or undirect sewage discharges.[78] For this reason, even if only few cytotoxic drugs have been detected in water samples there might be some areas with higher concentrations.

As a result of the discharge of Wastewater Treatment Plant (WWTP) effluents, soil can be also contaminated. It depends on several factors, such as physicochemical properties of the pollutants, the type of wastewater treatment technology implemented, and climatic conditions. As many pharmaceuticals are ionizable, soil hydraulic properties and environmental conditions also strongly influence their transport. For instance, the occurrence of 5 pharmaceuticals (salicylic acid, ibuprofen, caffeine, naproxen and carbamazepine) has been reported in volcanic sandy loam soil irrigated with treated wastewater in New Zealand.[79] In China, the contamination of irrigated soils by bisphenol-A, triclocarban, triclosan, 4-nonylphenol, salicylic acid, oxytetracycline, tetracycline, trimethoprim and primidone as also been reported.[80]

Pharmaceuticals are intended to act on specific metabolic and molecular pathways in humans and animals. The high polarity and low volatility of most of them means that they are likely to be transported to water compartments. The risk assessment towards aquatic organisms is particularly important as they are exposed to contaminants via wastewater residues their whole life.[57] Crane et al.[81] showed that human pharmaceuticals have the potential to affect aquatic organisms adversely such as microorganisms, algae and higher plants, invertebrates and fish and amphibians[81]. On the other hand, although the pharmaceutical concentrations measured in water are much lower than the concentrations who are able to affect humans, long-term exposure to drugs leads to health concerns.[62]

Some drugs are persistent, and others are pseudo-persistent. Even though they degrade in the environment at reasonable rates, they are continuously being discharged by ongoing widespread use. Most drug compounds are soluble in water, but about 30% display a non-polar character, leading to bioaccumulation by entering cells and moving up food chains.[62] For instance, a well-known destructive effect of pharmaceuticals for living species is the feminization of male fishes.[82] It is now widely accepted that aquatic vertebrates are highly sensitive to endocrine modulation, especially through exposure to steroid oestrogens excreted by women either naturally or as a result of oral contraception.[82] An important issue regarding the contamination of water with antibiotics is the possible development of resistance by bacteria.[83,84]

The licensing of cytotoxic drugs is supervised by the European Medical Agency (EMA), which started in 1995 in Europe. Its mission is to protect and promote public and animal health by evaluating and supervising the use of drugs in medicine for humans and animals. The prioritizing of pharmaceuticals can be based on risk quotients. To construct such risk ratios, predicted environmental concentration (PEC) and predicted no effect concentration values are required. EMA has established that 10 ng/L PEC for an individual drug should be a trigger value for further environmental risk assessment.[85]

Many pharmaceuticals may pose serious risks to different organisms as described above, but the key concern of this work is the impact of anticancer drugs in wastewaters. In Table 2 are presented multiple studies carried out in various types of organisms that suffered different kinds of changes after exposure to anticancer drugs, like DNA damage, growth impairment, oxidative stress, etc.[86–95]

Table 2: Consequences of exposure of various types of organisms to anticancer drugs.

Drug	Organism	Changes observed after exposure	Ref
Cisplatin	Protozoans, *Tetrahymena pyriformis*	Deformed cells. Non-specific esterase activities were modified. Growth impairment	86
Cisplatin, 5-FU, Capecitabine, Cisplatin, Doxorubicin, Etoposide, Imatinib	Microcrustaceans, *Daphnia Magna* and *Ceriodaphnia Dubia*	All drugs induced DNA damage	96
Cisplatin	Polychaete, *Nereis diversicolor*	Behavioural impairments, neurotoxicity, oxidative stress and lipid peroxidation	87
CP and its metabolites	Heterotrophic micro-organisms in membrane bioreactor system	No immediate response. At a long-term exposure an increase in the endogenous respiration was observed. Chemical stress causes diversion of carbon and/or energy from growth to adaptive responses and protection	88
CP	Proliferative cells of polychaetes, *Nereis diversicolor*	DNA damage and interference with the antioxidant status	89
Endoxifen and 4-hydroxy-tamoxifen (two metabolites of tamoxifen)	Microcrustacean, *Daphnia Pulex*	Induce reproductive and survival effects. For endoxifen, the concentration of 202,4 µg/L was lethal in 100% of the daphnids after 4 days of exposure	90
Imatinib mesylate, cisplatinum, etoposide	Crustacean, *Daphnia Magna*	DNA damage caused a dysregulation responsible for effects to the neurological system, development and overall growth	91
Imatinib	Rotifer, *Brachionus Calyciflorus*	A feeding depression was observed. The activity of acetylcholine esterase was inhibited, and the levels of reactive oxygen species increased	92
Mixture of Ciprofloxacin, CP and Tamoxifen	Human hepatoma cell line	Individual drug does not induce any DNA breaks on hepatic cells, whereas a mixture leads to a dose dependent increase of DNA breaks	93
Mixture of 5-FU, Cisplatin, Etoposide and Imatinib	Green alga *Pseudokirchneriella subcapitata* and Cyanobacterium *Synechococcus leopoliensis*	The toxicities of the binary mixtures tested showed synergistic but also antagonistic effects	94

Besides the risk assessment of individual compounds, it is important to evaluate whether the combinations of drugs have a negative impact. As showed in Table 2 the last two studies[93,94] were performed with a mixture of anticancer drugs, and the synergistic effects of mixtures of drugs observed on both studies highlights the importance of investigating the ecotoxicological effects of contaminants at low concentrations and in mixtures.[93,94]

In summary, numerous negative effects on aquatic life exposed to anticancer drugs are observed. However, a significant difference between humans and aquatic wildlife, in addition to the species itself, is that the water humans consume goes through water purification technologies.[97,98] Still, some of the water-soluble cytostatic drugs, such as cyclophosphamide, may be present in drinking water.[99] Although these drugs are still below levels of concern to human[100], exposure to them in the embryogenesis period, which occurs in the first trimester of pregnancy, could lead to a variety of malformations.[101] A significant inhibition of human embryonic cells growth in vitro in presence of cyclophosphamide has also been reported.[102] Besides, multi-generation exposures can result in accumulation of mutations, changes in the genetic diversity and gene flow.[45]

1.2.2. Pesticides

Pesticides have been widely used over the years and were frequently thought to be safe as they present higher affinity for insects rather than vertebrates. The first neonicotinoid commercially available was imidacloprid, and it has been used since the 90s. Neonicotinoids are nicotinic acetylcholine receptor agonists and bind strongly to nicotinic acetylcholine receptor in the central nervous system of insects, competing with the natural neurotransmitter acetylcholine. At low concentrations they cause nervous stimulation but at higher concentrations they can cause death.[103] They are selectively more toxic to insects than vertebrates.[104] They can be classified as N-nitroguanidines (for example, imidacloprid), nitromethylenes (for example, nitenpyram) and N-cyanoamidines (for example thiacloprid).[105] The structures of these pesticides are displayed in figure 9.

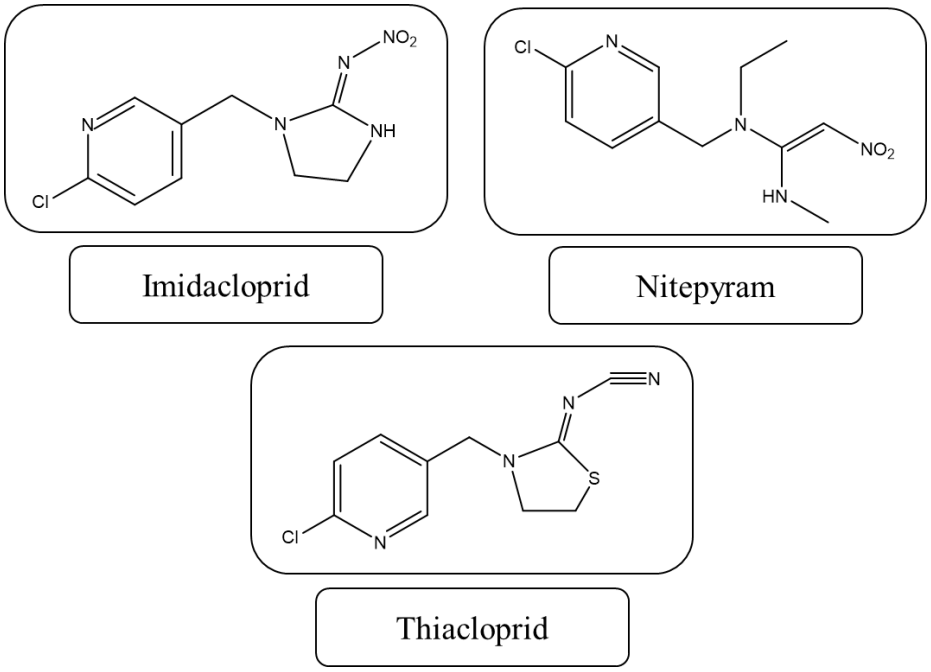


Figure 9: Chemical structures of Imidacloprid, Nitepyram and Thiacloprid.

These compounds are water soluble and are absorbed by plants through its roots or leaves and then are scattered throughout the tissues so they can protect all parts of the plants.[104] They can be used as foliar spays, bait formulations or be applied as soil drench or in irrigation water. Since they present low toxicity to vertebrates, have flexible use and systemic activity this led to neonicotinoids being the most extensively pesticide used in the world.[104]

The undisciplined discharged of pesticides into the environment contributes to its accumulation in the aquatic areas, with potential toxicological effects on aquatic and human ecosystems if the water is used for public and food consumption.[106] In Portugal few studies have reported the presence of pesticides in biota,[106] but they are present around the world. In Canada[107] four pesticides were measured at detectable levels across sampling year, season and site from Red River. Atrazine, thiamethoxan and clothianidin were present in all samples, and imidacloprid was detected in >90% of the samples. The maximums concentrations of thiamethoxan, clothianidin and imidacloprid ranged from 14.1-31.7 ng/L, and over a two-year study it showed a mean concentration of < 8 ng/L.[107] The Environmental Protection Agency of the United States established a benchmark for aquatic life of 385 ng/L for acute effects and 10 ng/L for chronic effects of imidacloprid for invertebrates[108] and a study performed in Florida showed imidacloprid concentrations ranging from 2.1 to 520

ng/L.[109] In Canada[110] some neonicotinoids were even detected in treated drinking water, in the low ng/L range.[110]

There have been a raising concern for their harmful impacts on non-target species, like birds and honeybees.[111–113] Even with the presence of legislative limits to reduce the pesticides use[111], seed-eating vertebrates can be exposed to lethal doses by consuming treated seeds, or by being exposed to environmental medias like soils, water and plants.[104,114] The concern of the impact of pesticides for pollinators has begun a few years ago, they can be exposed to them by consuming the nectar or pollen of flowers treated with pesticides or be exposed through the dust deriving out of seed coatings.[104] Budge et al[115] could even find a correlation between colony loss of honey bees and the usage pattern of imidacloprid in the United Kingdom.[115] Aquatic insects are also an example of a non-target organism that has been negatively impacted, with insects belonging to Ephemeroptera, Trichoptera and Diptera orders being the most sensitive, while those belonging to Crustacea are more tolerant. Neonicotinoids can employ adverse effects on survival, growth, mobility and behaviour at concentration at or below 1 µg/L for acute exposures and 0.1 µg/L for chronic exposure.[116] Seed-eating birds can also be affected, there is evidence that seeds treated with imidacloprid can cause impaired conditions, migration delays and improper migratory direction, all together these effects could lead to increased risk of mortality or lost breeding opportunity of songbirds.[117] So we can conclude that besides pharmaceuticals, pesticides are emerging contaminants that pose a major risk for the environment and should also be a concern.

1.2.3. Wastewater treatment systems

The conventional wastewater treatment systems comprise two or three treatment steps. In these stages there are various biological and physicochemical procedures, as showed in Figure 9.[118]

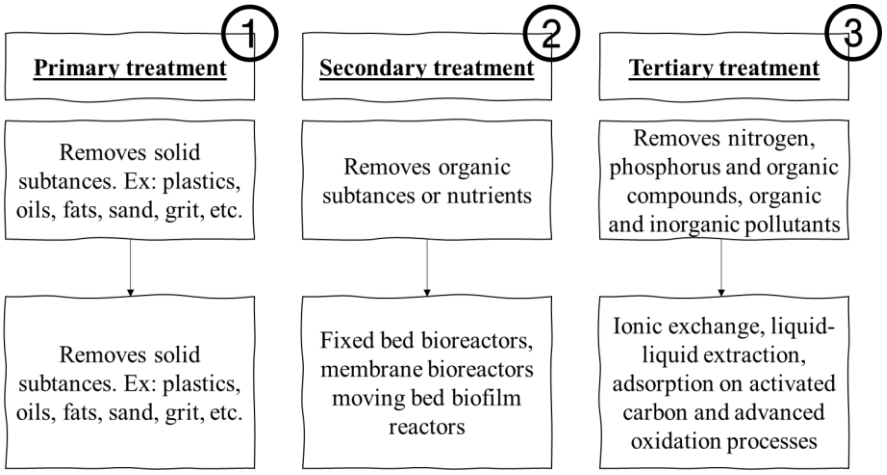


Figure 10: Conventional wastewater treatment systems.

The primary treatment is responsible to remove solid waste substances of wastewater, such as settleable solids, plastics, oils and fats, sand and grit, etc., which is accomplished mechanically by filtration and sedimentation. Many hydrophobic contaminants tend to heavily adsorb to the primary sludge and are partially removed after the primary treatment.[118] The secondary treatment normally depends on the biological (aerobic and anaerobic) degradation of organic substances or nutrients.[118] The techniques used are, for example, fixed bed bioreactors, membrane bioreactors, moving bed biofilm reactor, etc. The most well-known technique is the use of activated sludges, where the organic substances and nitrogen are removed.[118] Lastly, the tertiary treatment includes biological systems to remove nitrogen based compounds, ionic exchange to remove ions, chemical precipitation to remove phosphorus based compounds, distillation to remove volatile organic compounds, liquid-liquid extraction, adsorption with activated carbon to remove organic and inorganic pollutants, and advanced oxidation processes.[119]

One of the most important elimination processes is adsorption using activated carbon. The high removal efficiency of activated carbon is due to dispersive interactions, formation of a donor-acceptor complex between the carbonyl type surface group and the organic pollutant compounds, and establishment of hydrogen-bonds interactions. The properties of the adsorbent, such as specific surface area, permeability, polarity etc., and the characteristics of the compound, such as size, shape, hydrophobicity, charge, etc., influence the removal efficiency. The main advantage of using this process to remove pharmaceuticals is that it does not generate toxic or pharmacologically active products.[119] The technologies based on the advanced oxidation processes are very effective in the oxidation of multiple organic and inorganic compound. The base of these processes is the generation of free radicals that can attack organic molecules. Drinking water treatment primarily relies upon adsorptive and oxidative processes to remove or transform organic materials.[119] In addition, WWTP disinfection of the effluent can be done by ultra-violet (UV) irradiation or chlorination before discharge. The use of chlorine is still the most used method for disinfecting drinking waters.[119]

The primary objective of most municipal WWTP is to remove organic nutrients, such as organic carbonaceous, nitrogenous and phosphorus substances from water. They are not necessarily designed to eliminate emerging contaminants, particularly persistent and toxic ones, such as pharmaceuticals and pesticides since they are present at very low concentrations.[65] In USA a study[120] showed that some pesticides, including imidacloprid, persist through the conventional wastewater treatment and is entering water bodies and potentially harming sensitive aquatic invertebrates.[120] And in Bucharest only 22.4% of imidacloprid detected in influent waters was removed after WWTP.[121]

There are however four possible approaches to eliminate pharmaceuticals pollutants[122], which are represented in Figure 10.

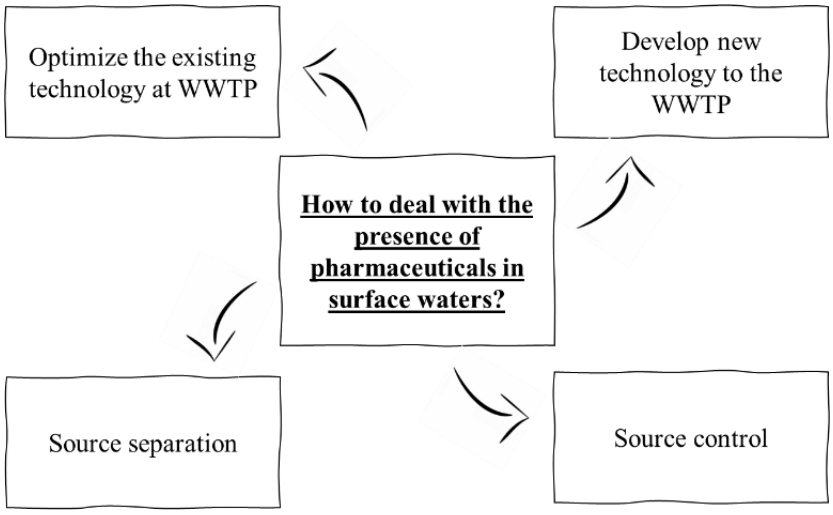


Figura 11: Possible approaches to eliminate pharmaceuticals from surface waters.

Source separation means avoiding the entry of toxic compounds and their metabolites into wastewater, and this might be a solution to the contamination of aquatic environment with pharmaceuticals. A method could be urine source separation, which could prevent hazardous micropollutants from inflowing the wastewater stream. It would be easier if drug metabolites in urine, which often are bioactive and toxic to the environment, could be prevented from entering waterways, since urine only represents 1% of the volume of the total domestic wastewater[123], being the main target of research of the current work.

1.3. Removal of pharmaceuticals from aqueous media

The characteristics of pharmaceuticals differentiate them from conventional industrial chemical contaminants, and therefore the WWTP fails to remove them completely from wastewater. They have heterogenous composition, being formed by chemically complex molecules that vary extensively in molecular weight, structure and shape. They are polar or non-polar molecules, most of the times with ionizable groups. There are some pharmaceuticals, such as erythromycin, cyclophosphamide, naproxen, and sulfamethoxazole that can persist in the environment for more than a year, and others, e.g. clofibric acid, that can persist for multiple years. In surface water, there are a few natural important elimination processes, like biodegradation, adsorption and photodegradation. Nevertheless,

pharmaceuticals have a general designed resistance to biodegradation.[124] Furthermore, most of these emerging contaminants are not completely eliminated in WWTPs, so they remain in effluents contaminating surface and ground waters, which are the foremost source of drinking water. In Table 3 is displayed some types of processes used in WWTPs aiming the removal of pharmaceuticals.

Table 3: Removal of pharmaceuticals with multiple types of processes.

Process used	Pharmaceutical removed	Ref
Chlorination	Acetaminophen	125
	Fluoroquinolone antibiotics	126
	Antibiotics (sulfonamides, macrolides and carbadox)	127
Activated carbon adsorption	Naproxen, carbamazepine and nonylphenol	128
Coagulation and granular activated carbon filtration	Tetracycline antibiotics	129
	Clofibric acid, diclofenac, fenoprofen, gemfibrozil, ibuprofen, indomethacin, ketoprofen, naproxen and propyphenazone	130
Ozone treatment	Clofibric acid, ibuprofen, diclofenac	131
	Carbamazepine, clofibric acid, diclofenac, sulfamethoxazole, ofloxacin and propranolol.	132
Zerovalent iron powder	Amoxicillin and ampicillin	133

As described in the previous section, chlorination is used for disinfection of wastewater effluents, and the pharmaceuticals that contain amine groups undergo a rapid reaction to form chlorinated compounds.[134] Some pharmaceuticals, such as acetaminophen, sulfamethoxazole, diclofenac and metoprolol, oxidize during the process of chlorination with substitution of one chlorine atom.[135] With this treatment numerous sub products can be formed, which are toxic compounds.[125,135] Some authors concluded that the removal of pharmaceuticals by adsorption into suspended solids is of high efficiency when dealing with hydrophobic compounds and compounds with positively charged functional groups (e.g. amines).[136] According to the literature, activated carbons generally demonstrate a high capacity to adsorb pharmaceuticals.[128–130] However, Simazaki et al.[130] showed that the removal of pharmaceuticals by powered activated carbon is not efficient due to competitive adsorption between pharmaceuticals when tested in a mixture.[130] Ozone treatment of WWTPs is supposed to reduce concentrations of many pharmaceuticals below detection limits. This treatment would be useful in cases where the wastewater used poses

ecotoxicological risks, such as irrigation in agriculture or drinking water.[137] Compounds that are susceptible to oxidation frequently contain heteroatoms with lone pairs of electrons (oxygen, nitrogen, sulphur), suggesting that pharmaceuticals should be susceptible to oxidative treatment.[62] Comparing the different water treatment processes for their potential to eliminate pharmaceuticals, it has been demonstrated that among the conventional drinking water treatment processes, ozonation and filtration through granular activated carbon are the most effective in removing some drugs.[97] New techniques have been developed, for example, the use of zerovalent iron powder to remove antibiotics from water samples.[133]

The removal of cyclophosphamide in a membrane bioreactor system, at concentrations close to those detected in hospital sewage, was already proposed, achieving a removal efficiency of 80%.[138] Sludge adsorption might also play a relevant role in the process of CP removal but is very time consuming.[138] Other study[139] evaluated the removal of 5-FU with a solid phase extraction method, which showed to be effective only in low-volume simple matrix samples, being inappropriate to be applied to complex environmental samples.[139] Granular activated carbon filtration has also been used to remove neutral cyclophosphamide, almost completely, from pre-treated surface water.[140] In summary, although several approaches have been proposed to remove cytostatic drugs from water, their presence in tap water was already reported,[99] being expected to become a more significant problem in the future with the continuous increase of cancer incidence and prevalence. Therefore, it is of outmost relevance to identify possible alternatives to mitigate environmental pollution by cytostatics.

1.3.1. Supported ionic-liquid-materials

Ionic liquids (ILs) are liquid molten salts, composed of inorganic or organic anions and organic cations. They do not form an ordered crystal structure, so they can be liquid at room temperature.[141] The first ionic liquid, $[C_2H_5NH_3][NO_3]$, was synthesized by Walden[142] via the neutralization of ethylamine with concentrated HNO_3, in 1914, while testing new explosives. ILs can also be named "designer solvents" since there is a high number of possible cation/anion combinations, allowing the manipulation of their physicochemical properties, including their extraction potential.[143] They can selectively interact with different types of solutes and solvents, have excellent chemical, thermal and electrochemical stabilities, are not flammable, have negligible volatility and have an excellent solvation ability for a wide range of compounds and materials.[144] In Figure 11 are displayed some of the chemical structures of cations and anions present in the most common ILs.

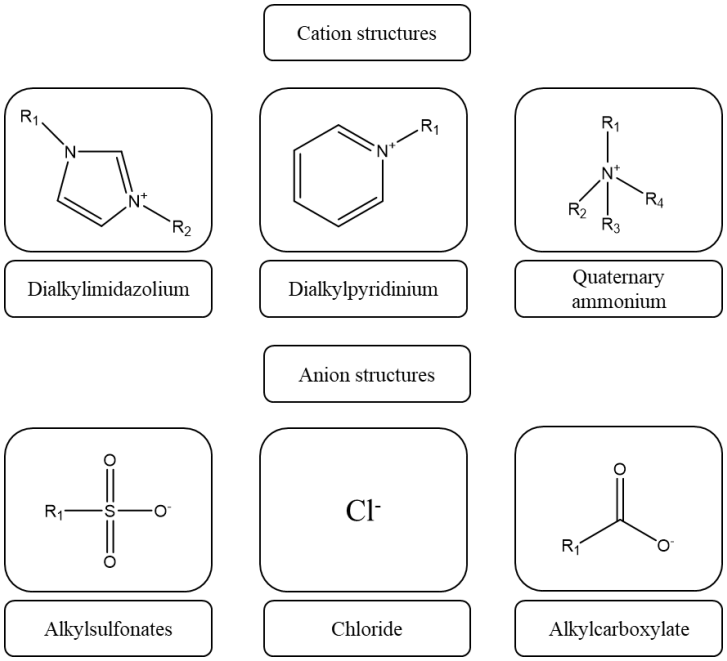


Figure 12: Chemical structure of cations and anions present in common ionic liquids.

In the previous chapters it was highlighted that the presence of pharmaceuticals in the environment is a matter of concern and new solutions must be developed. ILs and their use in several techniques offer several advantages, and the use of IL-based aqueous biphasic systems (ABS) to remove pharmaceutical contaminants from wastewater has already been

proposed. ABS were designed to extract non-steroidal anti-inflammatory drugs from aqueous streams and high levels of ibuprofen and diclofenac removal (>90%) were reached.[145] Other ILs were employed in ABS for the removal of antibiotics, with the intention of introducing this method in the final stages of a WWTP. In this case an extraction efficiency of fluoroquinolones up to 97% was obtained in a single-step.[146]

Other studies applied IL-based ABS to extract pharmaceuticals, although with a different goal, namely to recover drugs from pharmaceutical wastes.[147] The studied systems revealed a good aptitude to recover paracetamol from pharmaceutical wastes, with extraction efficiencies ranging from 80% up to 100%.[147] The authors demonstrated that ammonium-based ABS are enhanced alternatives for the extraction of paracetamol from a solid state pharmaceutical matrix.[147] Few years later, an additional study followed with the goal of extracting and recovering an antidepressant from pharmaceutical expired pills through the application of IL-based ABS.[148] The isolation of amitriptyline hydrochloride from the main contaminants present in pharmaceutical wastes was successfully achieved.[148]

ILs have taken a step forward and have been immobilized onto silica particles or polymeric supports, known as supported ionic liquid (SILs), resulting in new sorbents to be applied as stationary phases in solid-phase extraction (SPE).[149] Silica is a commonly used adsorbent because it provides some advantages such as simple handling, kinetically fast, mechanically, thermally and chemically stable even under different conditions. But the main one is that it has a large surface enabling to act as an effective adsorbent for various types of organic and inorganic pollutants.

For the synthesis of SIL materials ILs can be fixed on the surface of silica by physisorption or chemisorption. In physisorption a thin film of ionic liquid is dispersed on a porous solid, so the distinctive physicochemical characteristics of ILs are transferred to the surface of a solid material by binding the fluid to the surface. In chemisorption ILs are immobilize on surfaces by covalent binding of a monolayer of IL onto a support. In this case, the IL becomes part of the material, so it loses its properties.[150] However this cannot be easily accomplished only using native silica, it is frequently required to modify the silica, adding more functional groups with the purpose of improving selectivity and capability of adsorption.[151] Once the IL is immobilized onto a surface it loses its liquid state; however, properties, such as the designer ability are maintained.[152] SILs have attracted much attention because they are very stable, are low cost, the production is simple and have strong

mechanical property, when comparing with other materials, in particular, activated carbon, films and graphene.[153]

The first step to anchor ILs to silica surface is to activate the silica particles using an acidic aqueous solution (usually nitric acid or hydrochloric acid) to enhance the content of silanol groups on the silica surface and to eliminate impurities.[150] Then, one option, is to carry out the reaction in toluene between these activated silica particles with one silane-coupling agent, for example, 3-chloropropyltrimethoxysilane, leading to chloropropyl silica. Then, it can react with a cation source, for example, with 1-methylimidazole, since usually imidazolium-based ILs are a preferred choice, resulting in a SIL denominated propylmethylimidazolium chloride ([Si][C_3C_1im]Cl).[150] The chloride anion of these SILs can undergo ion-exchange leading to other desired surface-confined ILs.[150] For this work this technique was the one selected, although there is another possible way to perform the synthesis, by reacting the silane-coupling agent and the imidazole first and then the resulting IL can react with the activated silica particles.[150,154]

The first study on IL-modified silica sorbents for SPE was published in 2009 by Tian et al.[155] The obtained ionic-liquid-modified particles, N-methylimidazolium modified silica, with chloride as counterion, was used to extract three tanshinone compounds from *Salvia Miltiorrhiza Bunge*. A comparison with a commercial silica cartridge was carried out, showing higher recovery values using SILs due to the stronger interactions of the IL-modified silica material with tanshinones.[155] In 2010, the application of SILs was investigated for the SPE of 12 sulfonylurea herbicides from both environmental and soil samples.[156] This method was also compared with a commercial sorbent. The prepared sorbent was successfully applied for the extraction of the target sulfonylurea herbicides. The average recovery for each analyte ranged from 53.8% to 118.2% for water samples, and from 60.9% to 121.3% for the soil samples. The SIL cartridges showed higher selectivity for the herbicides than the commercial one.[156] Another possible application of the SILs is the SPE of organophosphate pesticides from water samples. The prepared SIL was dispersed in the sample solution containing the analytes.[157] The modified sorbent exhibited high efficiency in the removal of four pesticides from three different water samples (tap, river, and well water).[157] Liao et al.[158] developed an approach that combined solid-phase microextraction with liquid desorption for the determination of trace levels of seven steroid sex hormones in

water and urine samples.[158] This method was successfully applied with recoveries ranging from 75.6% to 116%[158] being able to develop a material to remove organic, inorganic and microbial contaminants simultaneously from water offers significant advantages over conventional purification methods. Herrmann et al.[159] proposed a SIL composite able to remove toxic heavy metals, nuclear waste, organic aromatics and microbes from the water. This multi-functional material opens paths for pollutant adsorption and water purification.[159]

Most of the compounds extracted using SILs are aromatic and contain hydroxyl groups.[150] These comprise compounds such as tanshinone,[155] sulfonylurea herbicides,[156] organophosphate pesticides,[157] steroid sex hormones,[158] phenolic acids and flavonoids,[160] and naphthenic acid.[161] Therefore, in SILs where imidazolium-based groups are immobilized onto silica there are different interaction mechanisms that can rise with the target compounds, such as anion-exchange, and hydrogen-bonding, electrostatic and π-π interactions.[150] To increase selectivity and recovery of compounds, the functionalization of the IL should take into account the specific chemical properties of the target compound and the main contaminants present in the medium to optimize the process performance.[149]

In conclusion, it was raised awareness to a significant future problem, the contamination of the environment with anticancer drugs. Considering the possible solutions to this issue, the development of effective sorbents for these drugs removals appears as the most promising one. To accomplish this, supported ionic liquids can be considered as improved sorbents, with interesting properties that can be tailored to enhance the adsorption of target pharmaceuticals. Contrarily to most common approaches regarding water treatment, in this work it is envisaged to identify efficient SILs able to be used in the preparation of a device that could be delivered to cancer patients to remove cytostatics at the entrance point, i.e. from urine.

1.4. Objectives

Based on the exposed, the goal of this work is to synthesize supported ionic liquids that are able to adsorb a specific cytostatic from aqueous solutions, in order to, in the future, develop a device that can be used to remove anticancer drugs from urine of oncologic patients, avoiding the entrance of toxic compounds into wastewater. It is expected to be the most viable approach since urine only represents 1% of the volume of the total domestic wastewater.[123]

The main objectives of this work are to synthetize the supporting ionic liquids and do its characterization by using multiple techniques. Then apply these to adsorption experiments in aqueous solutions with a chosen cytostatic, cyclophosphamide. But, despite the primary focus of this work is to study a new technology to remove anticancer drugs from urine, cytostatics are reported to exert genotoxic, mutagenic and carcinogenic effects in humans and animals. So, before starting working with CP, for safety reasons, the methods of adsorption were optimized by using aqueous solutions of other type of emerging contaminant, more specifically the pesticide imidacloprid. Adsorption kinetics and isotherms experiments will be studied for both imidacloprid and cyclophosphamide.

2. Experimental section

2.1 Materials

The experimental part of this work was divided in two main phases, firstly the synthesis of SILs and its characterization and then adsorption experiments were performed using aqueous solution of pesticide imidacloprid and cytostatic cyclophosphamide. All the materials used in both parts are listed in table 4, along with the information of their degree of purity and its respective supplier.

Table 4: Materials used for the synthesis of SILs and preparation of solutions, with the respective degree of purity and supplier.

Reagent	**Purity**	**Supplier**
1-methylimidazole	99%	Acros Organics
3-chloropropyltrimethoxysilane	98%	Acros Organics
Cyclophosphamide	97%	Acros Organics
Ethanol	Analytical Reagent Grade	Fischer Scientific
Hydrochloric Acid	37%	Sigma-Aldrich
Imidacloprid	99,9 %	Sigma-Aldrich
Methanol	HPLC grade	CHEM-LAB
N,N-Dimethylbutylamine	99%	Aldrich
Silica Gel (60Å)	-	Merck
Sodium Hydroxide	98%	JMGS
Toluene	99.8%	Carlos Erba
Tributylamine	99%	Acros Organics
Triethylamine	HPLC grade	Fisher Chemical
Trioctylamine	<98%	Fluka

2.2. Methods of quantification

The prepared aqueous solutions of the pesticide imidacloprid were quantified by UV-Vis spectroscopy, using Shimadzu UV-1800, Pharma-Spec UV-Vis, and quartz cells with 1x1cm dimensions. Firstly, a calibration curve was assessed at the maximum absorbance wavelength of imidacloprid, which is 270 nm (Figure 27 in annex).

For the quantification of aqueous solutions of cyclophosphamide it was adopted a different approach for the quantification of this pharmaceutical in solution: high performance liquid chromatography with diode-array detector (HPLC-DAD). The equipment used was Shimadzu, Prominence Modular HPLC, with a Reprosil C-18 analytical column (with porous spherical silica of 25 µm and pore diameter of 100Å and 250 × 4.6 mm in size) of GmbH. The temperature of the column was set at 25ºC. The mobile phase consisted of a mixture of acetonitrile (A) and water (B) in a ratio of 20:80 (v/v). Elution was performed at a flow rate of 1.0 mL/min and the injection volume was 20 µL. The wavelength for the quantification of cyclophosphamide was set to 197 nm, which was used to establish a calibration curve (Figure 28 in annex).

2.3. Synthesis of supported ionic liquids

The first step for the synthesis of supported ionic liquids consists in taking silica gel with pore size of 60 Å (0.2-0.5 mm) and adding hydrochloric acid for 24h. This pre-treatment allows the activation of the silica, prior to its functionalization with ionic liquids. Before it can be used, the silica must be washed with water until the pH increases and equals the pH of the distillery water, and it is dried at 55 ºC for 24h. Then 5.0 g of the activated silica and 60 mL of toluene are placed in a round bottom flask with a reflux condenser and 5 mL of 3-chloropropyltrimethoxysilane is added. The suspension prepared is refluxed under magnetic stirring at around 100ºC for 24h. Subsequently, the pale yellow solid resulting from this reaction is filtrated and washed by several solvents and mixtures in the following order: 100 mL of toluene, 200 mL of ethanol:water 1:1 (v/v), 500 mL of water and lastly 100 mL of methanol. The material produced is dried in an oven at 50 ºC for 24h and denominated [Si][C_3]Cl. For the second part of the functionalization, the process is the same, 5.0 g of

[Si][C_3]Cl, 50 mL of toluene and 5 mL of 1-methylimidazole are put in a round bottom flask and refluxed under magnetic stirring for 24h. Thereupon the material is filtrated and washed with 100 mL of toluene, 350 mL of methanol, 350 mL of water, 150 mL of methanol and finally dried at 50ºC for 24h. The SIL obtained is based on the IL propylmethylimidazolium chloride ([Si][C_3C_1im]Cl) and the scheme of its synthesis is shown in figure 13.

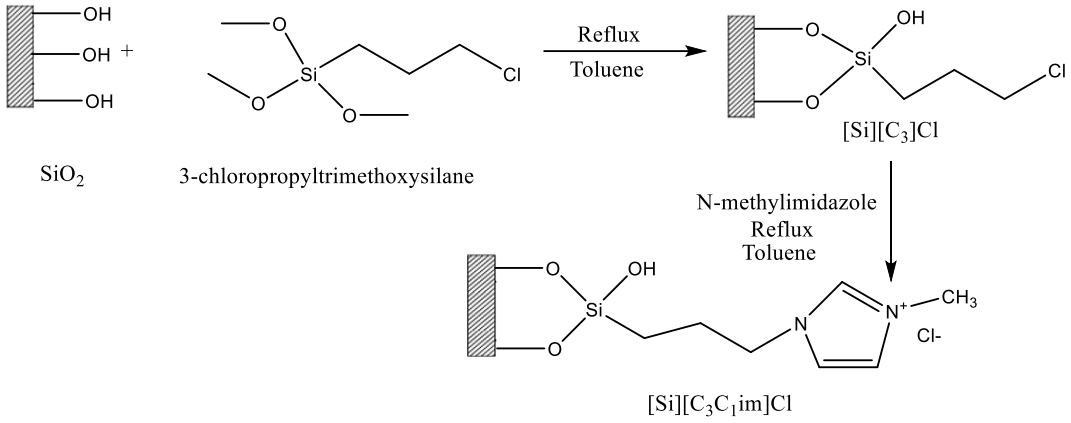
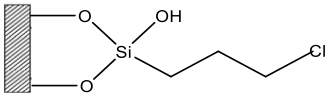
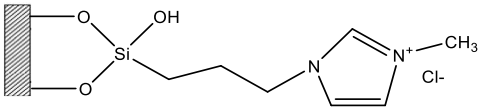
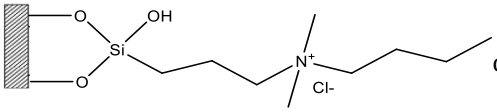
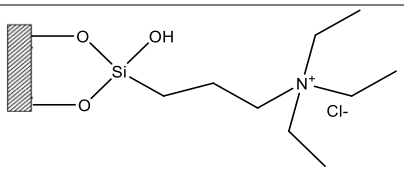
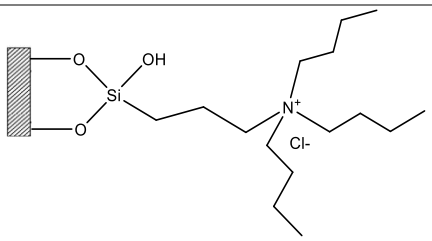
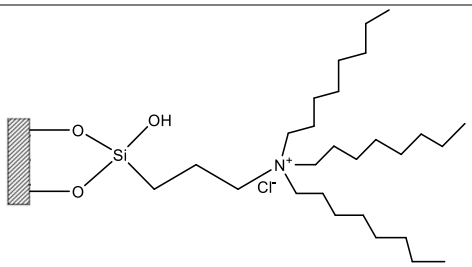


Figure 13: Scheme for the synthesis of supported ionic liquids.

The synthesis was repeated using different cation sources and all the resulting SILs are displayed in table 5. As depicted, the volume added of cation source was 5 mL for all of the SILs, and since they all have different molecular weight and densities (table 17 in annex) the amount added in each synthesis was different which is also shown in table 5.

Table 5: Supported ionic liquids synthesized and corresponding abbreviation, cation source and number of mols used in each synthesis.

Abbreviation	Structure of materials	Cation source	n (mol)
[Si][C_3]Cl		-	-
[Si][C_3C_1im]Cl		1-methylimidazole	0.061
[Si][N_{3114}]Cl		N,N-dimethylbutylamine	0.035

[Si][N_{3222}]Cl		Triethylamine	0.036
[Si][N_{3444}]Cl		Tributylamine	0.021
[Si][N_{3888}]Cl		Trioctylamine	0.011

2.4. Characterization of the supported ionic liquids

2.4.1. Elemental analysis

The content of carbon, hydrogen and nitrogen of the SILs was determined by elemental analysis using the equipment Truspec 630-200-200, with a sample of around 2 mg, combustion furnace temperature of 1075 ºC and afterburner temperature of 850 ºC.

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

2.4.2. Attenuated total reflectance – Fourier-transform infrared spectroscopy (ATR-FTIR)

Infrared spectroscopy was assessed by ATR-FTIR analysis. This technique was executed using a spectrophotometer of FTIR (Perkin Elmer FT-IRSystem Spectrum BX) and a solid sample of each SIL, at 25 ºC and between 4000-400 cm^{-1}. The samples were scanned 128 times in a resolution of 8.0 and interval of 2.0.

2.4.3. Solid state ^{13}C Nuclear Magnetic Resonance (^{13}C CPMAS)

For the nuclear magnetic resonance of ^{13}C in the solid state it was used a Bruker Avance III – 400 MHz (model DSX) spectrometer, and the spectra was recorded at 9,7 T with 4 nm VTN probe and cross polarization magic angle rotation (CPMAS) at 100.6 MHz, at room temperature.

2.4.4. Specific surface area and pore structure

This characterization method was executed by nitrogen adsorption and using a surface area analyser Micromeritics Gemini V-2380. The Brunaver-Emmet-Teller (BET) method was used to estimate the surface area (S_{BET}). The Barret-Joyner-Halenda (BJH) model was used to determine the pore surface (A) and pore volume (V).

2.4.5. Point of zero charge (PZC)

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

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

2.4.6. Scanning electron microscope (SEM)

The microscope used for SEM was the Hitashi SU-70, equipped with EDX Bruker, model Quantax 400. A sample of each SIL was placed in a piece of carbon tape and to further increase the conductivity a thin carbon film was deposited.

2.5. Adsorption experiments

Adsorption is the aggregation of matter from a gas or liquid to the surface of an adsorbent and it depends on the existence of a force field at the surface of the solid.[162]

There are two classes of adsorption, physical and chemical adsorption (chemisorption) according to the nature of the surface forces.[163] In the physical adsorption the forces mainly involved are dispersion-repulsion forces (Van der Waals forces) complemented by various electrostatic contributions which are crucial for polar adsorbents. The forces of chemisorption are stronger and involve electron transfer or electron sharing, forming a chemical bond. Therefore, chemisorption is highly specific, and the adsorption energies are typically greater than those for physical adsorption.[162]

Chemisorption is limited to a monolayer coverage of the surface while, in physical adsorption, multilayer adsorption is possible.[162] The capacity for physical adsorption matches the specific micropore volume. Van der Waals are dominant in non-polar adsorbents and the affinity is determined by size and polarizability of the sorbate and dimensions of the pore. Non-polar adsorbents are often described as hydrophobic since they have low affinity for water and higher affinity for organic molecules.[162]

The adsorption of pollutants from aqueous solutions is one of the most applied techniques for wastewater treatment, being accepted as an efficient and economically feasible process for the elimination of toxic contaminants.[164] The most common adsorbents are activated carbon, molecular sieves, polymeric adsorbents and others. This technique has proven to have a lot of advantages such as low initial cost, simple designs, easy to operate, insensitivity to toxic substances and complete removal of contaminants.[164]

The challenge in the field of adsorption is to find the most promising type of adsorbent, but kinetics and isotherms models can help the pursue to find the more appropriate ones and to find reasonable explanation for the mechanisms behind the adsorption process.[165,166]

To determine which SIL has better removal capacity initial adsorption experiments were performed using 10 mL of aqueous solutions of imidacloprid or cyclophosphamide and 50 mg of each SIL. These suspensions were kept in agitation in an orbital shaker at 25 ºC, 150 rpm for an hour. Further experiments were carried out with the SIL´s that showed the best performances.

2.5.1. Adsorption kinetics

The study of adsorption kinetics is key in wastewater treatment since it provides relevant insights of the reaction pathways and respective mechanism, it characterizes the solute uptake rate and residence time of sorbate uptake at the solid-solution interface and it can also help to predict the size and capital cost of an actual adsorption system for industrial and commercial applications.[163,165]

There are three phases that occur during the adsorption process, the first one is the solute transfer from the solution to the external surface of the sorbent, secondly is the solute transfer from the surface to the intraparticle spaces and thirdly is the interactions of the solute with the available sites in the external and internal surfaces of the sorbent.[167] one or more of this phases are going to define the rate and the amount of solute that is adsorbed onto the sorbent.[168]

For pesticide, imidacloprid, it was prepared an aqueous solution with a concentration of 9 mg/L and it was distributed, 10 mL each, through multiples erlenmeyers containing 50 mg of the chosen SIL. These suspensions were kept in motion in an orbital shaker at 25 ºC and 150 rpm. Each erlenmeyer was withdraw according time, and the sample was transferred to eppendorfs to be centrifuged for 10 min at 12000 rpm for the total deposition of the solid. The concentration of imidacloprid in the supernatant was calculated through UV-Vis spectroscopy.

For the cytostatic, cyclophosphamide, the method used was the same, using an aqueous solution of 90 mg/L. After centrifugation the supernatant of each sample was filtered into vials to be quantified with HPLC-DAD. When handling the cyclophosphamide, plastic containers were used whenever possible and were discarded in red bags classified as type IV residues for incineration, along with the aqueous residues collected in the end of each experiment. Volumetric glass flasks were dipped in bleach for at least 24h before washing, as well as the vials used for HPLC. All the experiments were performed in a laminar flow chamber class II, using two pairs of gloves for the protection of the operator.

2.5.2. Adsorption isotherms

Adsorption isotherms are quantitative models used to characterize the retention or mobility of a substance from an aqueous media to a solid phase at a constant temperature and time of contact.[169,170] The adsorption equilibrium is reached when an adsorbate containing phase has been in contact with the adsorbent for enough time, which means the adsorbate concentration in the solution is in a dynamic balance with the solid interface.[170]

Both isotherms, for imidacloprid and for cyclophosphamide, were equally executed, the required time of contact for the adsorption equilibrium to be established was determined by analysing the adsorption kinetics performed before. The mass of the SILs was kept the same for all the erlenmeyers used, 25 mg for imidacloprid and 50 mg for cyclophosphamide. Solutions of different concentrations were prepared, ranging from 2 mg/L to 230 mg/L for imidacloprid and from 50 mg/L to 1500 mg/L for cyclophosphamide. The method applied was the same as depicted in the kinetics experiments, using 5 mL of each solution of imidacloprid and 10 mL of each solution of cyclophosphamide in separate batches containing the SILs. After the determined time of contact the samples were centrifuged for 10 min at 12000 rpm to precipitate the solid and the supernatant was used to measure the concentration of the compounds using the respective method of quantification. For the cytostatic the same handling rules were adopted, as previously described.

2.5.3. Data analysis of the results

For the purpose of calculating the adsorption capacity and performance of the materials, the amount of contaminant adsorbed per unit mass of adsorbent in equilibrium and at *t* time (q_e and q_t in milligrams per gram of adsorbent)[168] can be evaluated using the following equations:

$$q_e = \frac{C_0 - C_e}{m} \times V \quad (1)$$

$$q_t = \frac{C_0 - C_t}{m} \times V \quad (2)$$

Where C_0 is the initial concentration of contaminant in solution (mg/L), C_e is the equilibrium concentration of the contaminant (mg/L), C_t is the concentration at *t* time, *V* is the volume of the aqueous solution (L) and *m* is the mass of adsorbent used (g), assuming that the adsorbate, which has disappeared from the solution must be in the adsorbent.[171] To evaluate practical and engineering processes the units mg/g are the most commonly used in adsorption studies, but the parameter q_e and q_t can be expressed in different units depending on the purpose of the study.[171]

Another important parameter that can be calculated is the adsorption efficiency (AE%) expressed as the percentage of removed adsorbate:

$$AE\% = \frac{C_0 - C_e}{C_0} \times 100 \quad (3)$$

However, it must be carefully used as it is very approximate and can induce misleading conclusions about the adsorption performance. But it is helpful for a quick and very approximate screening of adsorbent materials.

Various kinetic models have described the reaction order of adsorption systems, which include pseudo-first-order equation of Langergren[172], based on solid capacity, it considers that the rate of sorbate assimilation with time is directly proportional to the variance in the saturation concentration and is expressed as:

$$\log (q_e - q_t) = \log (q_e) - \frac{k_1}{2.303} t \quad (4)$$

Where q_e and q_t are the amounts adsorbed onto the adsorbents (mg/g) at equilibrium and at time *t*, respectively. k_1 is the rate constant of first order (min^{-1}).

Pseudo-second-order equation of Ho and Mckay[165] is based on solid phase adsorption, it assumes that the rate limiting step is a chemical sorption, which involves valence forces or exchanged electron between the adsorbent and the adsorbate,[165,166] this chemisorption kinetic rate equation is expressed as:

$$\frac{dq_t}{dt} = k_2(q_e - q_t)^2 \quad (5)$$

The q_e and q_t are the same variables as present is pseudo-first-order and k_2 is the second-order sorption rate constant (g/mg min). This equation is based on adsorption capacity from concentration of solution and has been effectively applied to the adsorption of metal ions, dyes, herbicides, oils and organic substances from aqueous solutions.[173]

The Elovich's equation was initially used to describe the adsorption of gas into a solid phase but recently is has also been widely applied to the chemisorption of pollutants from aqueous solution. The equation can be expressed mathematically in the following way:

$$\frac{dq_t}{dt} = \alpha^{(-\beta q_t)} \quad (6)$$

Where q_e and q_t are the amounts of adsorbate uptake per mass of adsorbent at equilibrium and at *t* time (min), respectively, α is the initial rate constant (mg/g × min) and β is the desorption constant during the experiment (mg/g). The Elovich equation is generally applied to chemisorption kinetics, it has been found to cover slow adsorptions rates and is frequently valid for systems with heterogenous surfaces.[174]

In the literature a wide variety of isotherms adsorption models have been applied, they can be classified as irreversible isotherms and one-parameter isotherms, two-parameter

isotherms, three-parameter isotherms and more than three-parameter isotherms. Although adsorption isotherms are less helpful in elucidating adsorption mechanisms than adsorption kinetics they are very helpful to describe the relationship between the adsorbate concentration in solution and the adsorbent at a constant temperature and to design adsorption systems.[170]

The models commonly used for adsorption isotherms are Langmuir and Freundlich isotherms, they differ from each other because Langmuir model assumes a totally homogeneous adsorption surface whereas Freundlich isotherm is suitable for a heterogenous surface.[175,176]

The Langmuir isotherm model can be effectively applied to multiple adsorption processes, being the best-known isotherm describing sorption. It is depicted as:

$$q_e = q_m \frac{bC_e}{1+bC_e} \quad (7)$$

C_e is the equilibrium concentration in the aqueous solution (mg/L), q_e is the quantity of molecules adsorbed per gram of adsorbent at equilibrium (mg/g), q_m and b are the Langmuir constants which represent the maximum adsorption capacity and energy adsorption, respectively. This equation assumes that a fixed number of accessible sites are available on the adsorbent surface and they all have the same energy, it also assumes that the adsorption is reversible, that once an adsorbate lodges in a site no further adsorption can occur in that spot, there is no interaction between adsorbate species and the adsorbent gets saturated once a monolayer is formed on the surface.[169,174,177]

The Freundlich isotherm is commonly used for the adsorption of organic components in solution or highly interactive species on activated carbon and molecular sieves.[178,179] Is an exponential equation and it presumes that the concentration of adsorbate on the surface increases as the adsorbate concentration increases, assuming that multiple layers could occur instead of a single layer.[174]

$$q_e = K_F \times C_e^{1/n} \quad (8)$$

K_F is the Freundlich equilibrium constant (mg/g) and n is the Freundlich exponent (dimensionless). The *n* term can range from 0 to 1, and it indicates the distribution of binding affinity or binding energy on the adsorbent surface.[169]

Besides the more common isotherms other models could be applied to this work, for instance, a three-parameter Sips isotherm, is an empirical model that consists of the combination of the Langmuir and Freundlich isotherm models.[174] The Sips model is given as follow:

$$q_e = \frac{Q_{max} \times K_S \times C_e^{1/n}}{1 + K_S \times C_e^{1/n}} \quad (9)$$

K_S is the Sips equilibrium constant, Q_{max} is the Sips maximum adsorption capacity (mg/g) and *n* is the exponent that describes the surface heterogeneity. Sips model reduces to the Freundlich model at low adsorbate concentrations, and for high adsorbate concentrations it predicts a monolayer adsorption capacity characteristic of the Langmuir isotherm (1/n=1).[174]

The linearization of any model was not used because its misuse is probably the most common error in data analysis of adsorption studies. The main reason why linear equations are used is because of the possibility of performing less experimental points to be able to define a line (using nonlinear equations requires more experimental points to define the curve). Regardless, a big issue is that some points are discarded in order to increase the R^2 values and transforming a nonlinear model to a linearized one tends to change the error distribution, and thus distort the parameters of the equilibrium and kinetics adsorption models.[164,174] To compare the fits of different models the same experimental data, including the same number of experimental data points, must be used.[180]

For the nonlinear method, the software GraphPad Prims 8 was used for determining the kinetic and isotherm parameters.

To identify the best-fit model, it was calculated the coefficient of determination (R^2) for the nonlinear method.[164]

$$R^2 = 1 - \frac{\sum(q_{e,exp} - q_{e,cal})^2}{\sum(q_{e,exp} - q_{e,mean})^2} = \frac{\sum(q_{e,cal} - q_{e,mean})^2}{\sum(q_{e,cal} - q_{e,mean})^2 + \sum(q_{e,cal} - q_{e,exp})^2} \quad (10)$$

Where $q_{e,exp}$ (mg/g) is the amount of adsorbate uptake at equilibrium achieved during the experiment, $q_{e,cal}$ (mg/g) is the amount of adsorbate uptake achieved from the model used and $q_{e,mean}$ (mg/g) is the mean of the $q_{e,exp}$ values.

When two-parameter models are being compared with three-parameter models it is recommended to use $R^2_{adj.}$. This parameter is used to penalize the models with more parameters because higher number of parameters have the tendency to exhibit R^2 closer to one. Using the $R^2_{adj.}$ you know if the best fitting model is due to having more terms in the equation or if the equation is really close to the reality of the system.[174]

$$R^2_{adj} = 1 - (1 - R^2) \times \left(\frac{n_p - 1}{n_p - p - 1}\right) \quad (11)$$

n_p is the number of experiments performed, and p is the number of terms of each fitted model. When R^2 and R^2_{adj} are closer to 1 it means that the model has a better fit. However, to really know if the best fitting model is really suitable it is vital to interpret the values of the obtained parameters.[174]

3. Results and Discussion

3.1. Synthesis and characterization of SIL's

3.1.1. Elemental analysis

The percentages of carbon, hydrogen and nitrogen obtained by elemental analysis are displayed in table 6.

Table 6: Mass percentages of carbon, hydrogen and nitrogen

Sample	%C	%H	%N
[Si][C_3]Cl	4.637	1.394	0.000
[Si][C_3C_1im]Cl	8.382	2.120	2.841
[Si][N_{3114}]Cl	7.719	1.840	0.767
[Si][N_{3222}]Cl	7.289	1.509	0.263
[Si][N_{3444}]Cl	5.741	1.336	0.148
[Si][N_{3888}]Cl	6.899	1.335	0.071

From the elemental analysis we can conclude that all the SILs samples contain in their composition nitrogen. As expected **[Si][C_3]Cl** do not present a content in nitrogen.

These results prove that the immobilization of the ionic liquids in silica was successful achieved, because, as predicted the **[Si][C_3]Cl** don´t present a nitrogen content but after the introduction of the cation source a nitrogen content is observed. The higher content of nitrogen is for **[Si][C_3C_1im]Cl** which is consistent with the expected due to the fact that the imidazolium ring has two N atoms in its structure. The percentage of nitrogen in the others SIL's decreases has the length of alkyl chains increases. Another reason for the decrease percentage of nitrogen is due to the fact that the volume used of each amine was kept the same, 5 ml, and as previously explained in table 5 their densities are not the same and the molar mass increases has the length of the alkyl chain increases, the quantity of each amine introduced in the synthesis was not the same. Therefore, the extend of the functionalization could be lower in the SILs with bigger alkyl chain. In order to increase the functionalization degree, the amount (in mol) of amines introduced to the media of the synthesis should be kept the same, which means changing the volume added of each amine.

3.1.2. Attenuated total reflectance – Fourier-transform infrared spectroscopy ATR - FTIR

Infrared spectroscopy is a useful technique for the identification of chemical modifications and to identify the surface composition which is important to understand the adsorption process. The collected spectra of all SILs and silica are represented in figure 14.

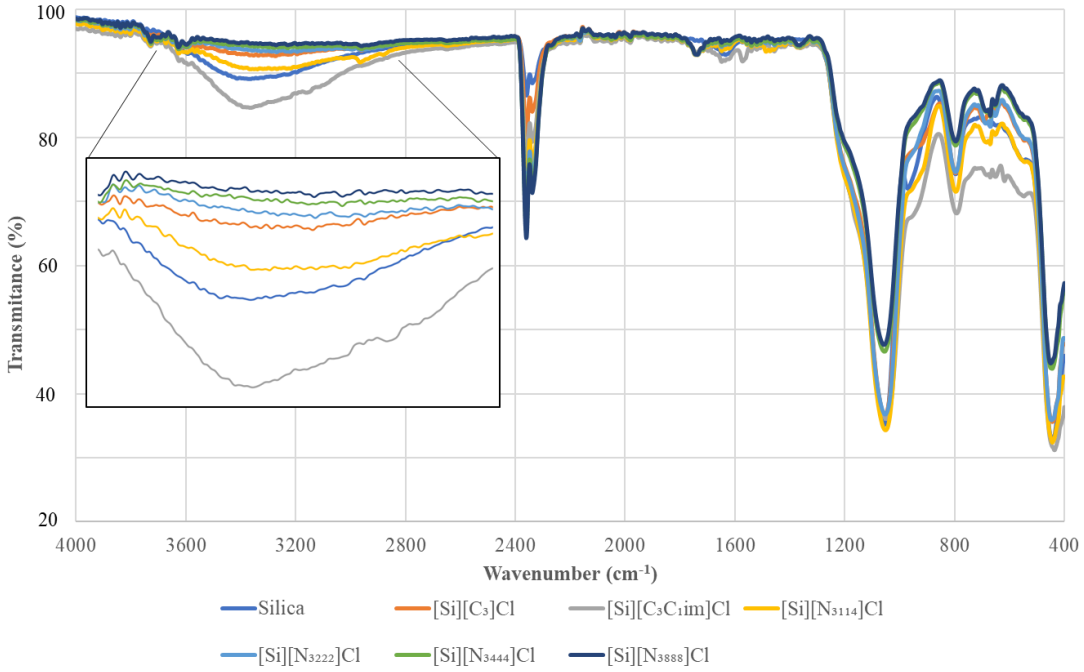


Figure 14: FTIR-ATR spectrum of the SILs.

The broad absorption band around 1100 cm^{-1} and around 800 cm^{-1} was attributed to Si-O-Si asymmetric and symmetric vibration respectively, this bond is presented in the matrix of the silica.[181]

The absorption band at around 3400 cm^{-1} corresponds to the silane, Si-OH group, for almost all the SILs and for **[Si][C_3]Cl** this peak is weaker than the one found in silica, indicating the successful bonding of the ionic liquid because it means they reacted effectively.[182] The exception is the **[Si][C_3C_1im]Cl**, it has a bigger peak, but it can be explained by disturbance of water. The absorbance around 1500 cm^{-1} correspond to the stretching vibration of C=N, C=C, C=H of imidazole ring.[154] All the aliphatic carbons should appear around 2900 and 3000 cm^{-1}, but it is not possible to distinguish between them because of the presence of water.[183]

3.1.3. Solid state ^{13}C Nuclear Magnetic Resonance (^{13}C CPMAS)

The ^{13}C CPMAS spectra are displayed in figure 15 but a more detail analysis was done for each SIL in figure 16-19.

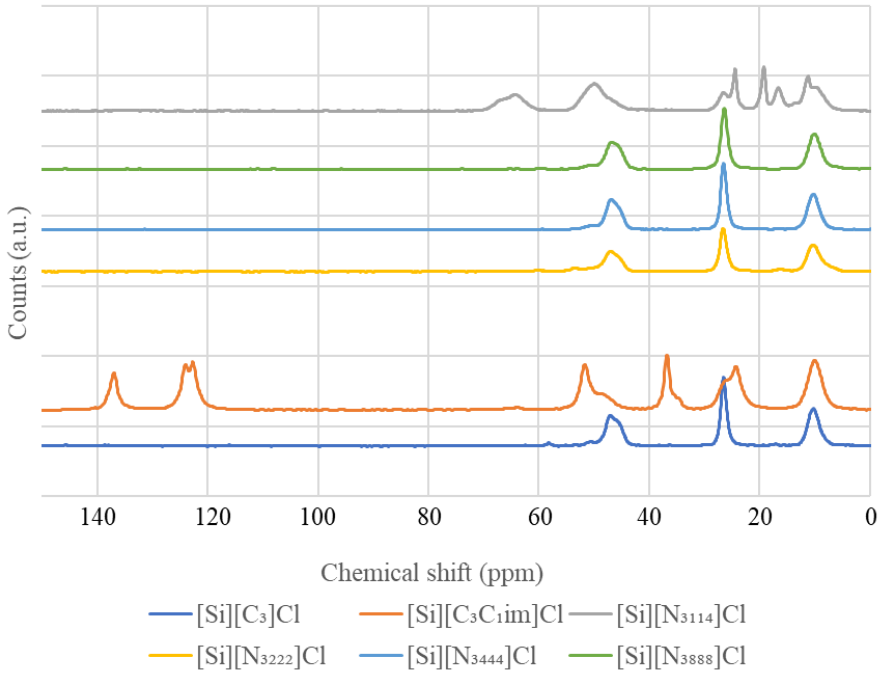


Figure 15: ^{13}C CPMAS spectra of the synthetized SILs.

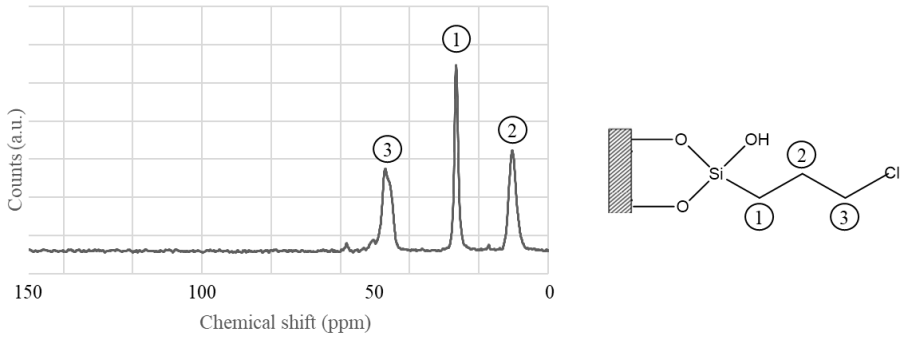


Figure 16: ^{13}C CPMAS spectra of [Si][C_3]Cl.

In figure 16 are the results for **[Si][C_3]Cl**. There are three peaks at 10, 27 and 47 ppm which correspond to the three carbons in the propyl chain.

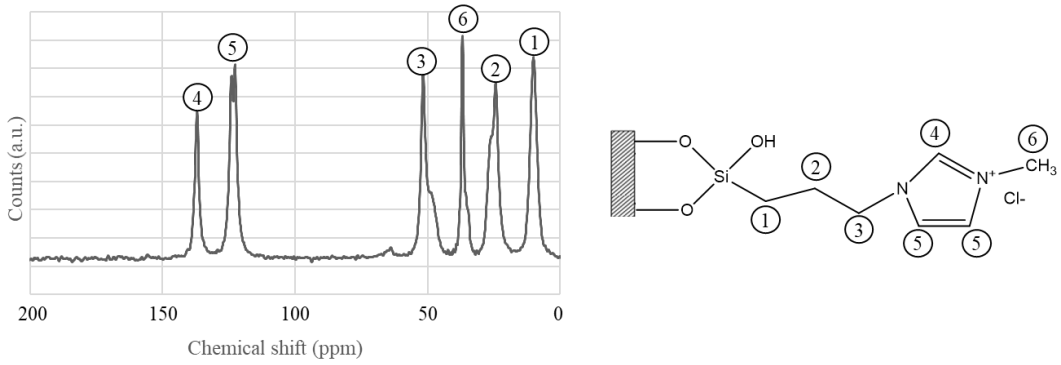


Figure 17: ^{13}C CPMAS spectra of [Si][C_3C_1im]Cl.

In figure 17, the spectra for **[Si][C_3C_1im]Cl**, we can identify the peaks from carbon 5 and 4 from the imidazolium ring at 122 and 137 ppm. The methyl chain, C6, is at 37 ppm. The peaks from the propyl chain, C1, C2 and C3 are illustrated at 10, 24 and 52 ppm.

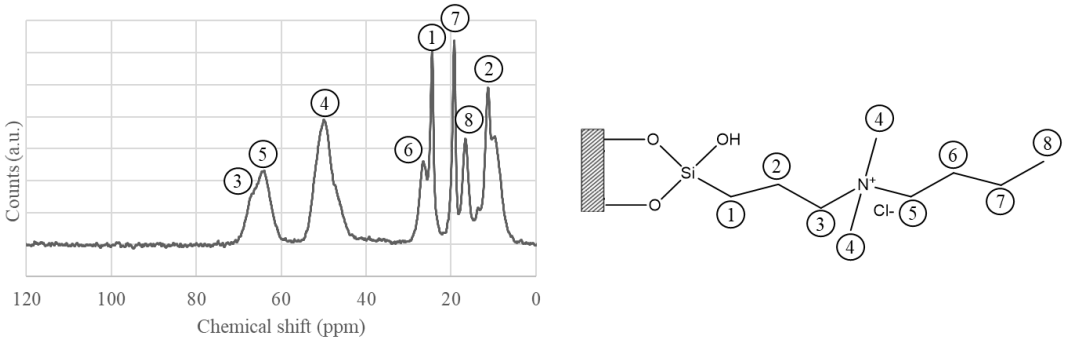


Figure 18: ^{13}C CPMAS spectra of [Si][N_{3114}]Cl.

In figure 18, the spectra for **[Si][N_{3114}]Cl**, the peaks from the propyl chain, C2, C1 and C3 are represented at 11, 24 and 67 ppm. The carbon from the methyl chain is at 50 ppm and lastly the carbons from the butyl chain, C8, C7, C6 and C5 are at 17, 19, 25 and 63 ppm.

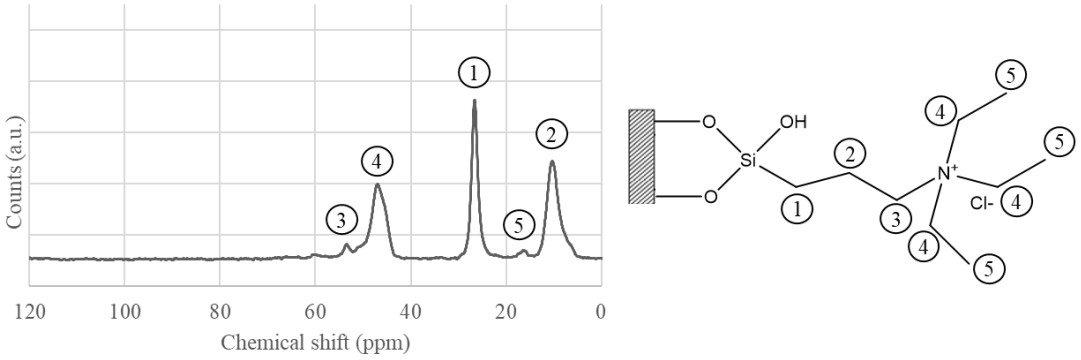


Figure 19: ^{13}C CPMAS spectra of [Si][N_{3222}]Cl.

In figure 19, the spectra for **[Si][N_{3222}]Cl**, we can identify the peaks from the propyl chain C2, C1 and C3, at 10, 27 and 54 ppm. Then the two other peaks correspond to the carbons of the ethyl chain, C5 and C4, at 17 and 47 ppm.

All these results are another evidence that the functionalization of the silica occurred, meaning that the ionic liquids are anchored to the silica.

The spectra from **[Si][N_{3444}]Cl** and **[Si][N_{3888}]Cl** do not show every carbon from the alkyl chains butyl and octyl, meaning the extent of the functionalization was low, enabling to confirm its functionalization. To do so it was necessary to resort to the others characterization techniques.

3.1.4. Specific surface area and pore structure

A pore system is described by the porosity itself, in particular, specific pore volume and pore size. The specific surface area includes the inner surface, which is the surface of the available pores, and the external surface, which characterizes the surface of the porous particle and depends mostly on the size of the particle.[184] In table 7 are presented the results of BET surface area, BJH pore surface area, BJH pore volume and pore size diameter of each SIL, silica and **[Si][C_3]Cl**.

Table 7: BET surface area (S_{BET}), BJH pore surface area (A), BJH pore volume (V), pore size diameter (D_p) of each SIL, silica and [Si][C_3]Cl.

	S_{BET} (m^2/g)	A (m^2/g)	V (cm^3/g)	D_p (Å)
Silica	435	570	0.7	49
[Si][C_3]Cl	323	327	0.3	39
[Si][C_3C_1im]Cl	185	256	0.3	45
[Si][N_{3114}]Cl	187	227	0.3	47
[Si][N_{3222}]Cl	318	427	0.5	47
[Si][N_{3444}]Cl	323	312	0.3	42
[Si][N_{3888}]Cl	319	302	0.3	44

The material with the higher pore size diameter and surface area is the silica, 49.485 Å and 434.545 m^2/g, respectively. The one with the lowest pore size diameter is [Si][C_3]Cl with 38.746 Å. All the synthetized SILs have pore volumes between 46.740 Å and 42.198 Å. The results for each SILs are similar, with the same order of magnitude, so this characteristic will not differentiate the adsorption performance between them.

Since this method provided the surface area of silica available to the functionalization with ionic liquids (434.545 m^2/g) it was possible to combine this data with the percentage of carbon and nitrogen found in **[Si][C_3]Cl** and in each SIL to calculate the bonding amount (BA) in mol/m^2 to the silica by using the following equations:

$$BA = \frac{\frac{\%C}{3 \times M(C)}}{S_{BET}} \quad (12)$$

This equation is used for the calculation of the bonding amount of **[Si][C_3]Cl**, %C was evaluated in table 6, M(C) is the molar weight of carbon, which is 12 g/mol, and is multiplicated by three to represent the three carbons present in the molecule, and S_{BET} is the surface area of silica.

$$BA = \frac{\frac{\%N}{2 \times M(N)}}{S_{BET}} \quad (13)$$

$$BA = \frac{\frac{\%N}{1 \times M(N)}}{S_{BET}} \quad (14)$$

The equation 13 is used for **[Si][C_3C_1im]Cl** because it has two atoms of nitrogen per molecule, equation 14 is applied to **[Si][N_{3114}]Cl**, **[Si][N_{3222}]Cl**, **[Si][N_{3444}]Cl** and **[Si][N_{3888}]Cl** because they only have one atom of nitrogen in their structure. %N of each one is presented in table 6, M(N) is the molar weight of nitrogen, which is 14 g/mol and S_{BET} is also the surface area of silica. These calculations are shown in table 8.

Table 8: Amount of IL bounded to the silica (µmol/m^2)

Material	Bonding amount (µmol/m^2)
[Si][C_3]Cl	2.96
[Si][C_3C_1im]Cl	2.33
[Si][N_{3114}]Cl	1.26
[Si][N_{3222}]Cl	0.43
[Si][N_{3444}]Cl	0.24
[Si][N_{3888}]Cl	0.12

From these results we can conclude that after introducing the cation source not all the amines reacted with the amount of **[Si][C_3]Cl** existed on the surface of silica, meaning that every SIL material not only have the IL anchored to the surface but also some **[Si][C_3]Cl**.

The most functionalized SIL is the **[Si][C_3C_1im]Cl** and, on the other hand, **[Si][N_{3222}]Cl**, **[Si][N_{3444}]Cl** and **[Si][N_{3888}]Cl** are poorly functionalized. An explanation could be because the number of mols of triethylamine, tributylamine and trioctylamine used in the synthesis was less than the number of mols used of 1-methylimidazole. One the other hand, by looking to the amount functionalized in **[Si][N_{3114}]Cl** and **[Si][N_{3222}]Cl** maybe adding the same amount of amine to the synthesis, as suggested before, will not change the extend of functionalization because the number of mols added for both were the same but **[Si][N_{3114}]Cl** has 1.26 µmol of nitrogen bonded per m^2 of silica and **[Si][N_{3222}]Cl** only has 0.43. Thus the poorly functionalization might be due to the stereochemistry of the molecules.

3.1.5. Point of zero charge.

Zeta potential measurements were used to study the particle surface charge of the materials. In figure 20 are displayed the graphic representation of the zeta potential in function of pH and in table 9 are displayed the point of zero charge of each SIL, silica and **[Si][C3]Cl**.

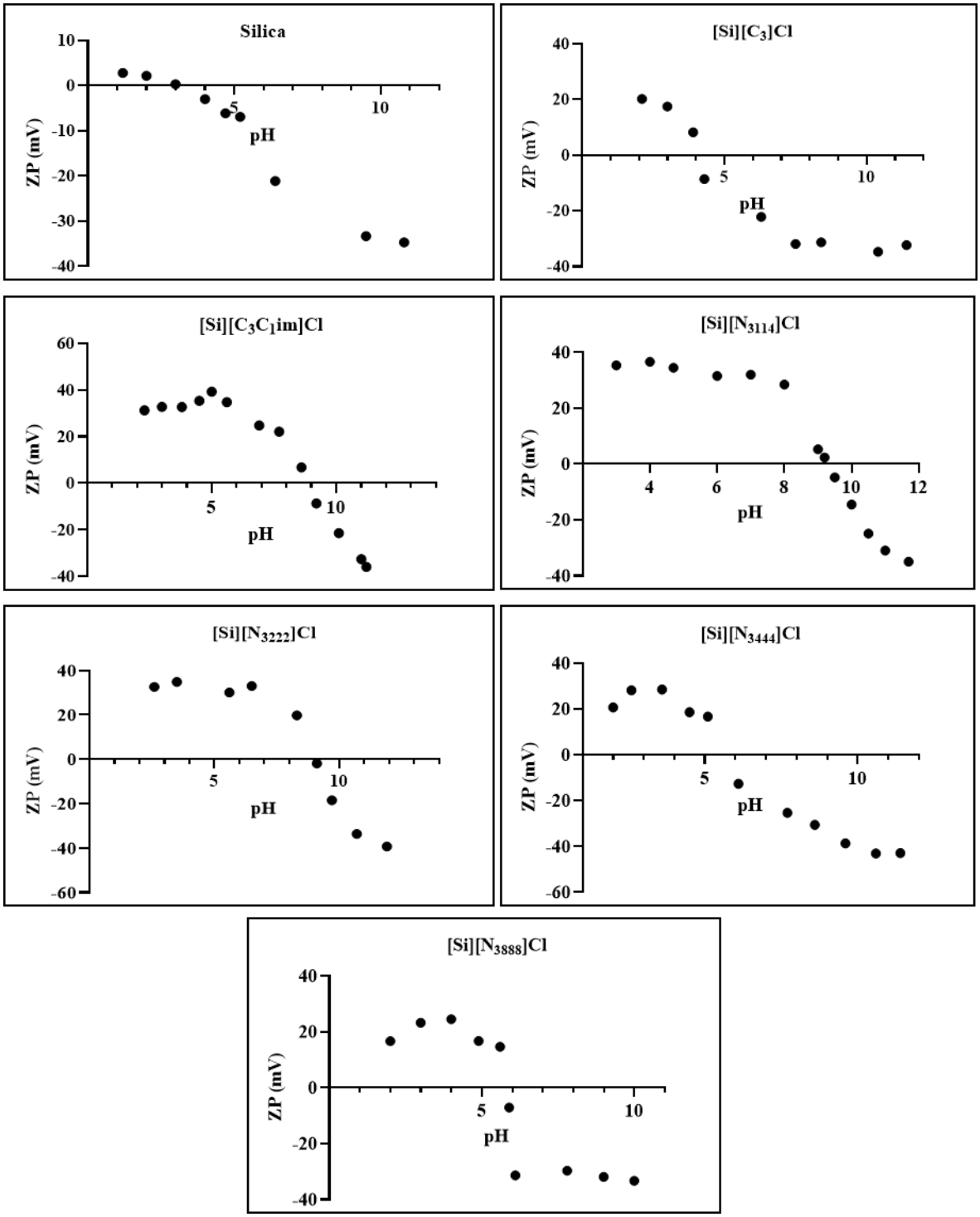


Figure 20: Zeta potential in function of pH for all SILs synthetized.

Table 9: Point of zero charge of each SIL.

Sample	PZC
Silica	3.4
[Si][C_3]Cl	4.2
[Si][C_3C_1im]Cl	8.9
[Si][N_{3114}]Cl	9.3
[Si][N_{3222}]Cl	9.0
[Si][N_{3444}]Cl	6.0
[Si][N_{3888}]Cl	5.5

The electrical state of an adsorbent's surface in solution can be characterized by the point of zero charge, that is defined as the solution conditions under which the surface charge density is equal to zero. This technique is frequently used to quantify surface charge and can be used to anticipate the coulombic interaction (opposite charges on the adsorbate and adsorbent induce coulombic attraction) between particle and ions.[169,185]

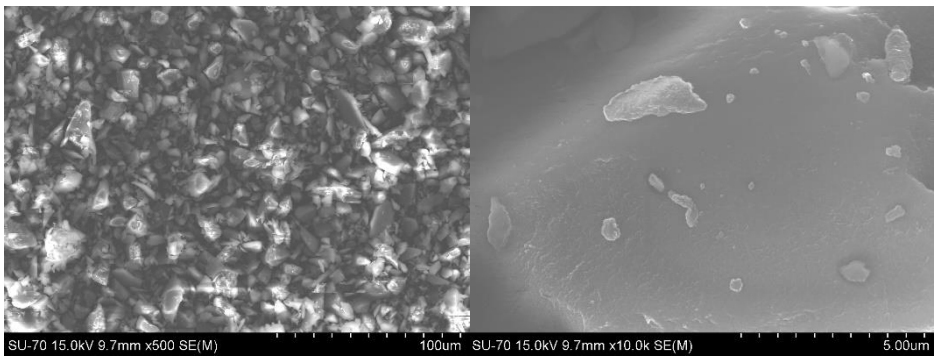
Amongst many physico-chemical parameters used to characterize the solid-liquid interface, the zeta potential is crucial to explain the adsorption mechanism from an electrostatic adsorption point of view.[186]

From our results we can observed that the value of PZC for silica conforms to what is found in the literature.[187] The PZC of all of the SILs synthesized is higher when comparing with silica and **[Si][C_3]Cl** ranging from 5.5 (**[Si][N_{3888}]Cl**) to 9.3 (**[Si][N_{3114}]Cl**), this indicates that the SILs surfaces are more positively charged. From a structure point of view these results are in agreement with the successfully functionalization of the silica, because the cations are present and on the surface of the silica.[188] The difference between the PZC values of SILs might be due to the differences of functionalization since the lower values of PZC correspond to the SILs with lower bonding amounts, therefore, with less cations on the surface.

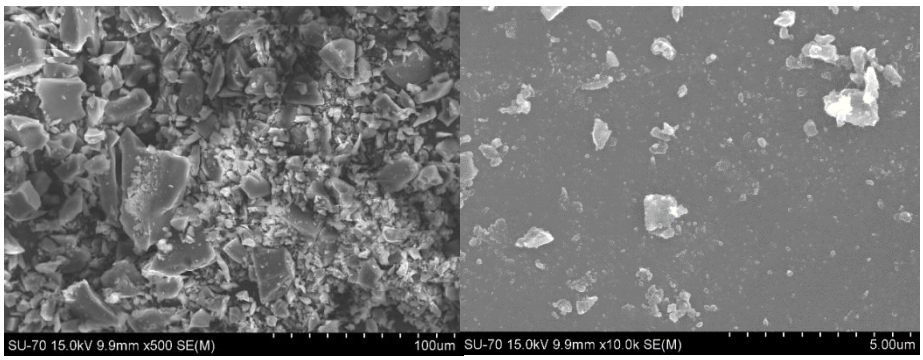
3.1.6. Scanning electron microscope (SEM)

The morphology of each SIL was characterized by SEM. In figure 21 are portrayed the SEM images of SILs in two different magnifications.

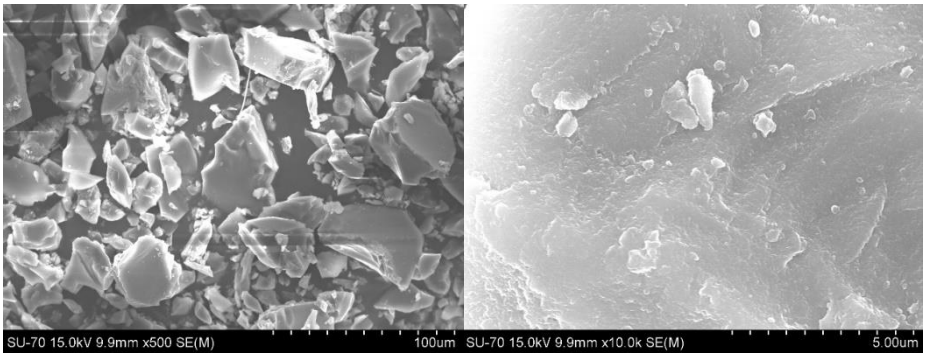
Silica



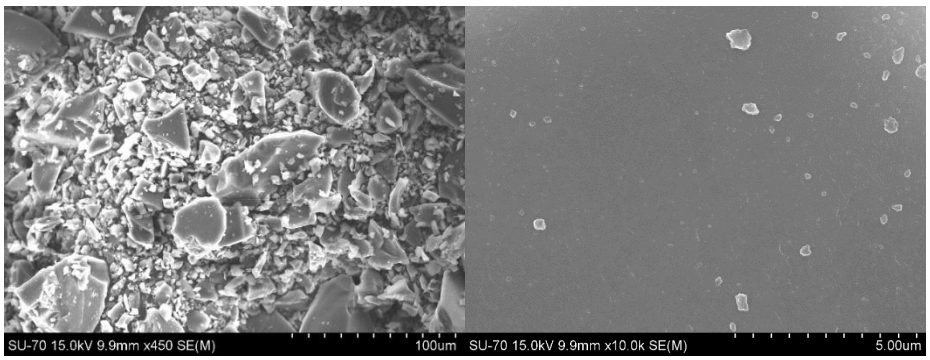
[Si][C_3C_1im]Cl



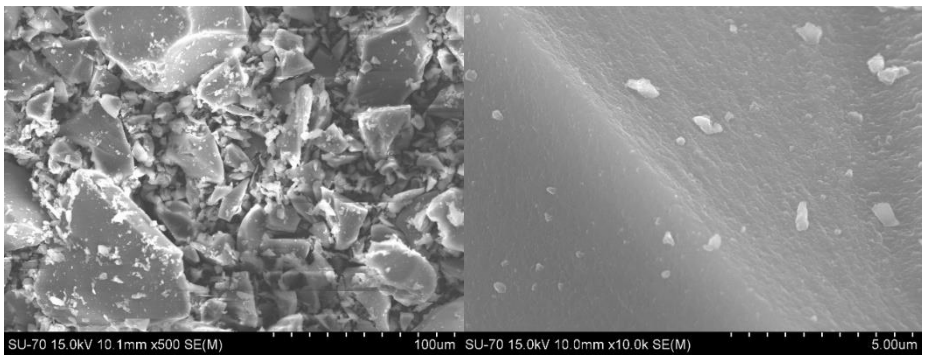
[Si][N_{3114}]Cl



[Si][N_{3222}]Cl



[Si][N_{3444}]Cl



[Si][N_{3888}]Cl

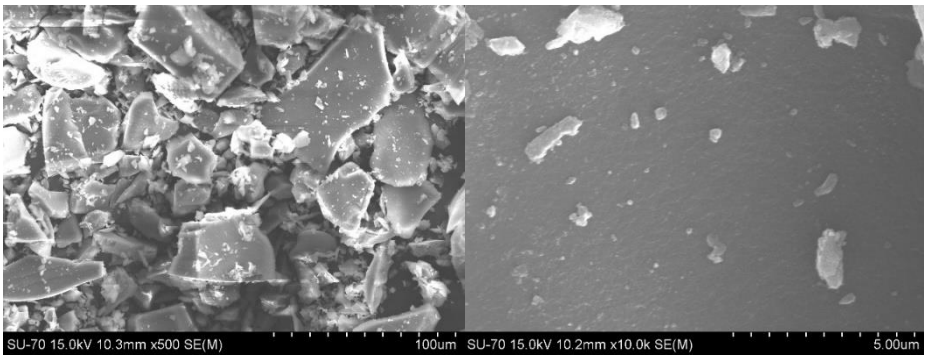


Figure 21: SEM images of the silica and synthesized SILs.

The surface structure of silica in comparison with each SIL is not significantly different, and a rugged layer can be seen in all of them, there is also no difference between SILs themselves. This is because during the functionalization of the silica only a few molecules are introduced to its surface which cannot be seen or distinguished between them by this technique.

3.2. Adsorption experiments of imidacloprid

Initial adsorption experiments for imidacloprid were performed for each SIL material using 50 mg, an aqueous solution of imidacloprid 9.4 mg/L and time of contact of 30 min to evaluate the SIL's with better performance. By looking at figure 22 and table 10 it can be determine that **[Si][N_{3888}]Cl**, **[Si][N_{3444}]Cl** and **[Si][C_3]Cl** showed the best removal efficiency, with q_e of 1.08, 0.94 and 0.86 mg/g, respectively. These were the SIL's chosen to perform kinetics and isotherms studies.

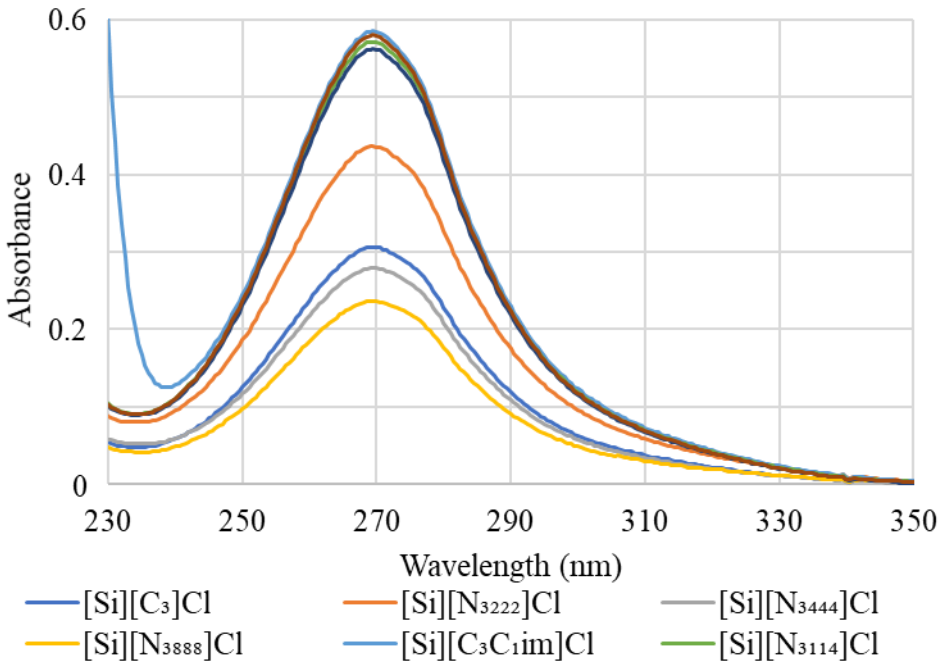


Figure 22: Initial adsorption experiments for imidacloprid with SILs.

Table 10: Values of C_e (mg/L), q_e (mg/g) and adsorption efficiency (%AE) corresponding to the initial studies of imidacloprid with SILs.

	C_e (mg/L)	q_e (mg/g)	%AE
Silica	9.10	0.06	3.02
[Si][C_3]Cl	5.09	0.86	45.7
[Si][C_3C_1im]Cl	9.46	0	0
[Si][N_{3144}]Cl	9.24	0.03	1.52
[Si][N_{3222}]Cl	7.14	0.45	23.9
[Si][N_{3444}]Cl	4.67	0.94	50.2
[Si][N_{3888}]Cl	4.00	1.08	57.4

To understand the adsorption process and the mechanism behind it it's necessary to have knowledge of the adsorbent and adsorbate itself. Since one of the SILs, **[Si][C_3C_1im]Cl**, has a imidazolium ring, π-π interaction could occur. It is a dipole interaction weaker than H-bonding and is a term used to interpret attractions between neutral organic molecules and electron rich π-systems (functional group with π-bonds such as C=C double bonds or aromatic rings) that can attract polar molecules and other π-systems. π-π interactions occur between opposite polarized arene systems with orientation of a parallel-planar fashion, that is called "stacking".[169] In these studies **[Si][C_3C_1im]Cl** did not any adsorption for this contaminant, so other types of interactions must be responsible for the adsorption process.

Other type of interaction that could occur is hydrogen bonding between a hydrogen donor and acceptor. In the imidacloprid molecule there is one hydrogen bonded to nitrogen, meaning it is more prompt to form hydrogen bonds.[169] But this characteristic cannot tell apart the adsorption efficiency of each SIL.

The other SILs, beside **[Si][C_3C_1im]Cl**, have carbon chains, so hydrophobic interactions can be the mainly ones involved. They occur due to the tendency of non-polar groups to aggregate in water with the intention of minimize their contact with water molecules. If hydrophobic interaction is the dominant mechanism, which it could be the case because the major difference between SILs are the length of the alkyl chains, adsorption of nonpolar contaminants on porous materials would be proportional to the octanol-water

distribution coefficient (K_{ow}), higher K_{ow} means stronger hydrophobic interactions.[169] The $LogK_{ow}$ corresponded for imidacloprid and each cation source are displayed in table 11.

Table 11: Values of $LogK_{ow}$ of imidacloprid and of the cation source of each SIL.

	$LogK_{ow}$
Imidacloprid	0.8
1-methylimidazole	-0.1
N,N-dimethylbutylamine	1.7
Triethylamine	1.4
Tributylamine	4.0
Trioctylamine	10.5

The most hydrophobic one is trioctylamine which is in agreement with the performance of **[Si][N_{3888}]Cl**, because it was the SIL that showed the better performance, followed by **[Si][N_{3444}]Cl.** 1-methylimidazole has a negative $LogK_{ow}$ value which is other reason why **[Si][C_3C_1im]Cl** did not showed adsorption for the compounds in study, because the possibility for hydrophobic interaction is low.

[Si][C_3]Cl has a primary alkyl halide meaning there is a partial positive charge on the carbon attached to the chlorine and a partial negative charge on the chlorine. Therefore the carbon is susceptible to be attacked by nucleophiles[189], like the nitrogen present in the pyridine or imidazole part of the structure of imidacloprid. This can explain why the adsorption with **[Si][C_3]Cl** worked.

3.2.1. Adsorption kinetics of imidacloprid

In the study of adsorption kinetics, the initial time periods are key in performing accurate modelling and conclusions.[190] Depending on the affinity between the adsorbent and adsorbate the adsorption rate in the beginning can be very fast. When there is low activation energy required, more than half of organic contaminants can be removed in less than 5 min or even within 1 minute of contact with the adsorbent.[191–193] As time progresses, the amount of adsorbate uptake per mass of adsorbent at any time *t* reaches an equilibrium (q_e), thus, plotting the kinetic data it is anticipated that a straight line is produced, independent of the kinetic order by which the system reaches equilibrium.

The graphic representation of the experimental values of q_e as of function of time for the pesticide imidacloprid with **[Si][N_{3444}]Cl** and **[Si][N_{3888}]Cl**, **[Si][C_3]Cl** is showed in figure 23. In each graphic there is also the representation of adsorption kinetics models, more specifically, pseudo first-order, pseudo second-order and the Elovich models. The software GraphPad Prims 8 was used for determining the kinetic parameters and R^2, which are displayed in table 12.

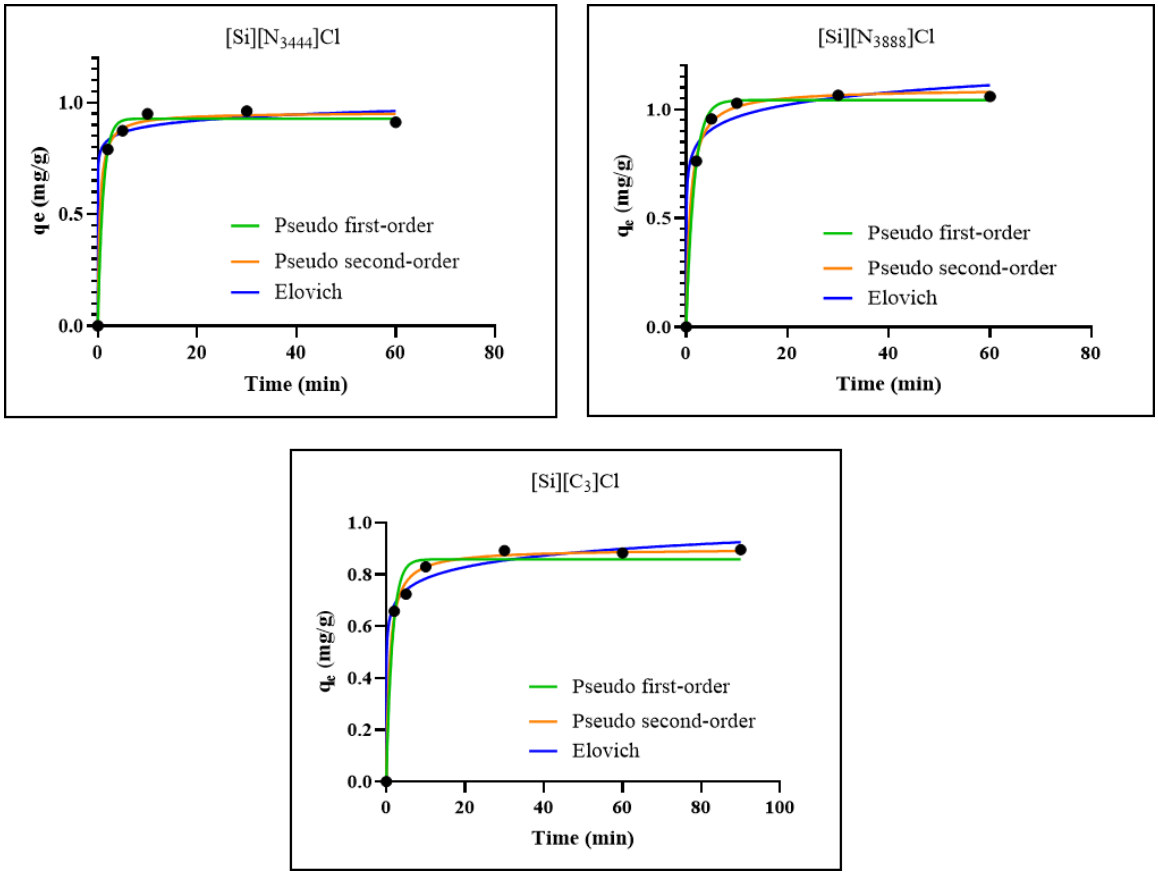


Figure 23: Experimental values of q_e (mg/g) of imidacloprid as a function of time (min) and representation of pseudo first-order, pseudo second-order and Elovich kinetic models for **[Si][N$_{3444}$]Cl** and **[Si][N$_{3888}$]Cl**, **[Si][C$_3$]Cl**.

Table 12: Kinetic parameters for the pseudo first-order, pseudo second-order and Elovich models and their correlation coefficients.

		[Si][N$_{3444}$]Cl	**[Si][N$_{3888}$]Cl**	**[Si][C$_3$]Cl**
	$q_{e\ exp}$ (mg g^{-1})	0.9625	1.066	0.8955
Pseudo first-order	q_e (mg g^{-1})	0.9288	1.043	0.8582
	k_1 (min^{-1})	0.9275	0.6289	0.6414
	R^2	**0.9943**	**0.9965**	**0.9752**
Pseudo second-order	q_e (mg g^{-1})	0.9563	1.094	0.8980
	k_2 (g mg^{-1} min^{-1})	2.561	1.114	1.332
	R^2	**0.9959**	**0.9987**	**0.9950**
Elovich	α (mg g^{-1} min^{-1})	4.501×10^7	1081	1360
	β (g mg^{-1})	25.94	12.22	15.65
	R^2	**0.9882**	**0.9834**	**0.9912**

The adsorption of imidacloprid is fast, reaching an equilibrium after 10 minutes of contact and the kinetic model that best fitted the experimental values is the pseudo second-order model for the three SILs with R^2 of, 0.9959, 0.9987 and 0.9950 for **[Si][N_{3444}]Cl**, **[Si][N_{3888}]Cl** and **[Si][C_3]Cl** respectively. It is also noticeable a decrease in the value of q_e over time for **[Si][N_{3444}]Cl** and **[Si][N_{3888}]Cl**, meaning the opposite mechanism occurred, desorption. To ensure that the system reached an equilibrium and maximum adsorption, the time of contact chosen for the isotherm studies was 30 min.

Multiple studies have been carried out for the removal of imidacloprid from aqueous solutions by adsorption with different types of adsorbents, and the best fitted kinetic model found was also the pseudo second-order. Keshvardoostchokami et al[194] used silver graphene oxide nanocomposites, Zahoor et al[195] used both powdered activated carbon and magnetic activated carbon and Daneshvar et al[196] used granular activated carbon. Even though pseudo second order is related to chemisorption, these findings are not enough to conclude that, only to know at what time the equilibrium is reached.

3.2.2. Adsorption isotherms of imidacloprid

The graphic representation of the experimental values of q_e as of function C_e for the pesticide imidacloprid with [Si][N_{3444}]Cl, [Si][N_{3888}]Cl and [Si][C_3]Cl are showed in figure 24. In each graphic there is also the representation of adsorption isotherm models, more specifically, Langmuir, Freundlich and SIPS models. The software GraphPad Prims 8 was used for determining the isotherms parameters, R^2 and $R^2_{adj.}$, which are displayed in table 13.

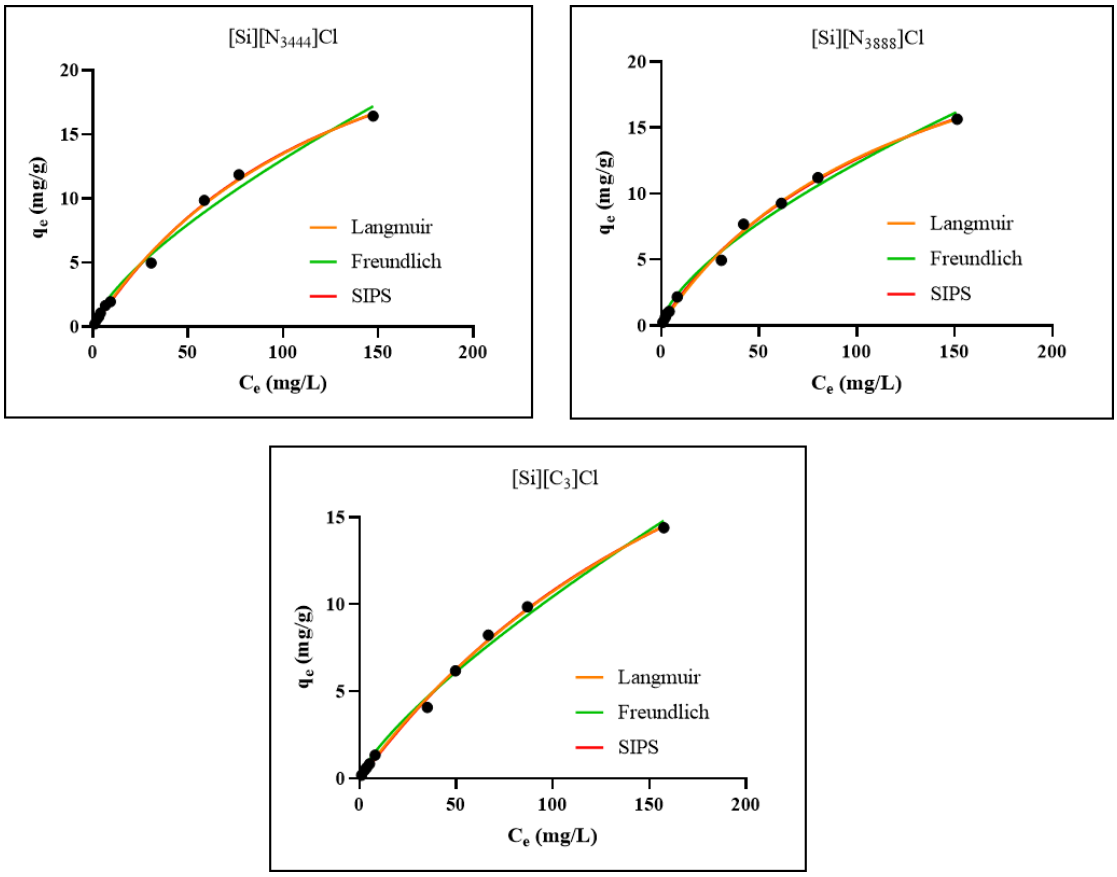


Figure 24: Experimental values of q_e (mg/g) of imidacloprid as a function of C_e (mg/L) and representation of Langmuir, Freundlich and SIPS isotherm models for [Si][N_{3444}]Cl, [Si][N_{3888}]Cl and [Si][C_3]Cl.

Table 13: Constants calculated from the Langmuir, Freundlich and SIPS models and their correlation coefficients for imidacloprid with [Si][C_3]Cl, [Si][N_{3444}]Cl and [Si][N_{3888}]Cl.

		[Si][N_{3444}]Cl	**[Si][N_{3888}]Cl**	**[Si][C_3]Cl**
Langmuir	$q_{máx}$ (mg g^{-1})	32.65	28.73	37.29
	b (L mg^{-1})	7.041×10^{-3}	7.906×10^{-3}	4.040×10^{-3}
	R^2	0.9963	0.9962	0.9977
	R^2_{adj}	**0.9959**	**0.9957**	**0.9974**
Freundlich	k_f (mg g^{-1})	0.4969	0.5763	0.3019
	n	1.409	1.505	1.300
	R^2	0.9880	0.9910	0.9940
	R^2_{adj}	**0.9865**	**0.9898**	**0.9933**
SIPS	$q_{máx}$ (mg g^{-1})	30.10	33.29	33.24
	K_s (L mg^{-1})	8.255×10^{-3}	5.856×10^{-3}	4.956×10^{-3}
	1/n	1.047	0.9236	1.046
	R^2	0.9964	0.9965	0.9978
	R^2_{adj}	**0.9954**	**0.9955**	**0.9971**

By analysing the R^2_{adj} of the three isotherms, the best fitted model is the Langmuir with R^2_{adj} of 0.9959, 0.9957 and 0.9974 for **[Si][N_{3444}]Cl**, **[Si][N_{3888}]Cl** and **[Si][C_3]Cl** respectively. The SIPS model also has a similar R^2_{adj} but the 1/n is approximately 1 so it means it takes the form of the Langmuir isotherm. The best predicted $q_{máx}$ in the Langmuir model was 37.29 mg/g for **[Si][C_3]Cl** but the best experimental q_e was 16.4 mg/g for **[Si][N_{3444}]Cl**. The better correlation with the Langmuir isotherm reflects a monolayer adsorption, which indicates a homogeneous distribution of adsorption sites on the adsorbate surface and a single layer of pesticide was formed.

Comparing with the adsorption of the same pesticide imidacloprid, Keshvardoostchokami et al[194] used silver graphene oxide nanocomposite and reached an experimental q_e of 18 mg/g, which is close to the results showed in this work, but the best fitted model was Freundlich.[194]

3.3. Adsorption experiments of cyclophosphamide

Initial adsorption experiments were also performed for the adsorption of cyclophosphamide, for each SIL material, including silica and **[Si][C$_3$]Cl**. It was used 50 mg of each material, an aqueous solution of cyclophosphamide at a concentration of 118 mg/L and time of contact of 60 min to evaluate the SIL's with better performance in the removal of this compound. By looking at table 14 we can conclude that **[Si][N$_{3888}$]Cl**, **[Si][N$_{3444}$]Cl** and **[Si][N$_{3222}$]Cl** showed the best removal efficiency from all SILs, with q_e of 11.1, 6.1 and 3.4 mg/g, respectively. These were the SIL's chosen to perform kinetics and isotherms studies.

Table 14: Values of C_e (mg/L), q_e (mg/g) and adsorption efficiency (%AE) corresponding to the initial studies of cyclophosphamide with each SIL.

	C_e (mg/L)	**q_e (mg/g)**	**% AE**
Silica	119.2	0	0
[Si][C$_3$]Cl	95.7	4.5	19.0
[Si][C$_3$C$_1$im]Cl	123.7	0	0
[Si][N$_{3114}$]Cl	105.8	2.5	10.6
[Si][N$_{3222}$]Cl	101.5	3.4	14.2
[Si][N$_{3444}$]Cl	87.9	6.1	25.7
[Si][N$_{3888}$]Cl	62.9	11.1	46.8

Similarly, as it was exposed on the explanation of imidacloprid adsorption process, the **[Si][C$_3$C$_1$im]Cl** did not adsorb cyclophosphamide, meaning there are no π-π interactions involved. The molecule of cyclophosphamide does have a hydrogen bonded to nitrogen that might play an important role in forming hydrogen bonds. From the results shown, once again, the SILs that showed a better performance were the ones that have alkyl chains, concluding that hydrophobic interactions are key in this adsorption process, meaning a physical adsorption is expected.

3.3.1. Adsorption kinetics of cyclophosphamide

The graphic representation of the experimental values of q_e as function of time for the cytostatic cyclophosphamide with **[Si][N$_{3222}$]Cl**, **[Si][N$_{3444}$]Cl** and **[Si][N$_{3888}$]Cl** are showed in figure 25. In each graphic there is also the representation of adsorption kinetics models, more specifically, pseudo first-order, pseudo second-order and the Elovich models. The software GraphPad Prims 8 was used for determining the kinetic parameters and R^2, which are displayed in table 15.

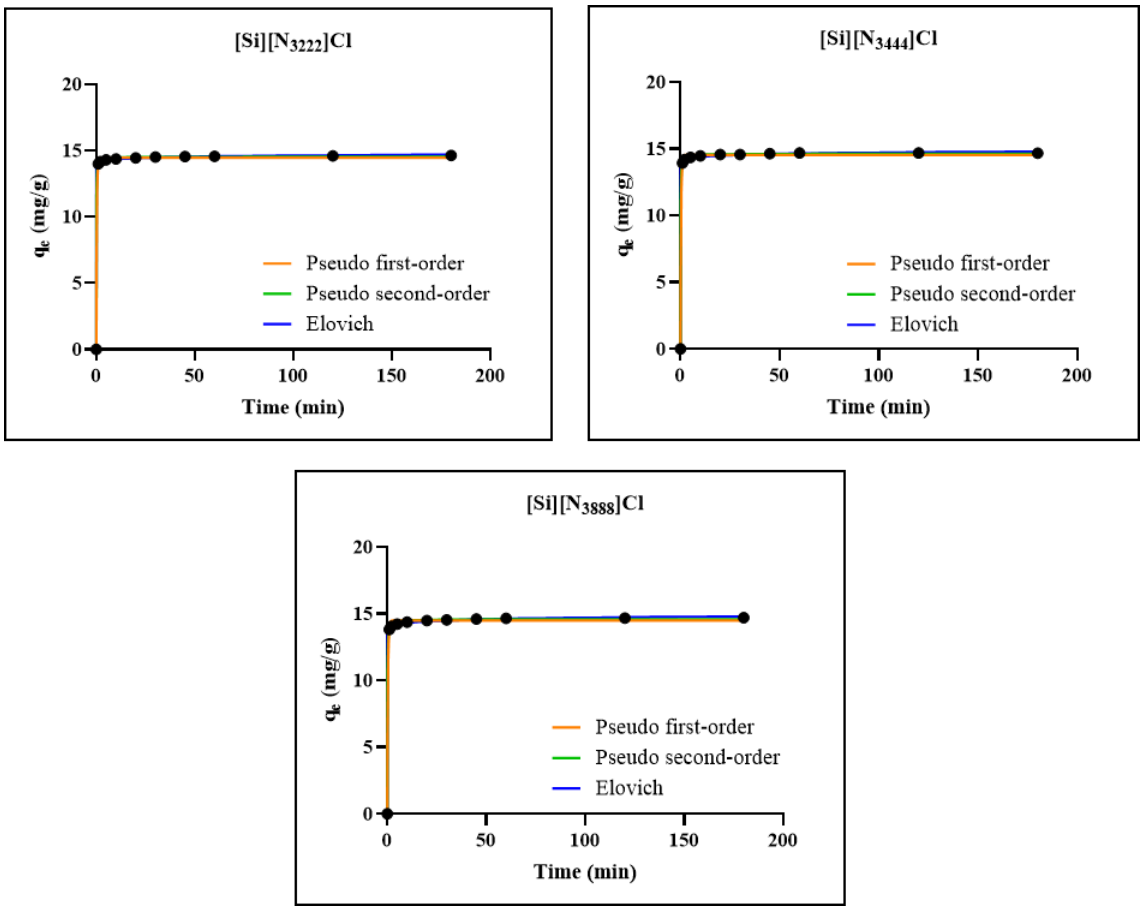


Figure 25: Experimental values of q_e (mg/g) of cyclophosphamide as a function of time (min) and representation of pseudo first-order, pseudo second-order kinetic and Elovich models for [Si][N$_{3222}$]Cl, [Si][N$_{3444}$]Cl and [Si][N$_{3888}$]Cl.

Table 15: Kinetic parameters for the pseudo first-order, pseudo second-order and Elovich models and their correlation coefficients.

		[Si][N_{3222}]Cl	**[Si][N_{3444}]Cl**	**[Si][N_{3888}]Cl**
	$q_{e\ exp}$ (mg g^{-1})	14.63	14.69	14.70
Pseudo first-order	q_e (mg g^{-1})	14.46	14.55	14.49
	k_1 (min^{-1})	3.385	3.121	3.020
	R^2	**0.9908**	**0.9989**	**0.9981**
Pseudo second-order	q_e (mg g^{-1})	14.53	14.63	14.59
	k_2 (g mg^{-1} min^{-1})	1.582	1.268	1.094
	R^2	**0.9997**	**0.9998**	**0.9995**
Elovich	α (mg g^{-1} min^{-1})	2.059×10^{50}	7.770×10^{43}	2.318×10^{35}
	β (g mg^{-1})	8.390	7.316	5.873
	R^2	**0.9999**	**0.9997**	**0.9998**

The adsorption of cyclophosphamide was fast, reaching an equilibrium after 45 min of contact but in one minute most of the cyclophosphamide was adsorb, however, unlike imidacloprid no significant desorption was observed, so the time of contact was set to 120 min for the isotherm studies. The kinetic model that best fitted the experimental values was the Elovich model for **[Si][N_{3222}]Cl** and **[Si][N_{3888}]Cl** with R^2 of 0.9999 and 0.9998, respectively and for **[Si][N_{3444}]Cl** the model that best fitted was the pseudo second-order model, with R^2 of 0.9997. The experimental q_e was 14.63, 14.69 and 14.70 mg/g for **[Si][N_{3222}]Cl, [Si][N_{3444}]Cl** and **[Si][N_{3888}]Cl,** respectively, so experimental and estimated values by both pseudo first-order and pseudo second-order models were similar for the three SILs indicating that they are a good fit.

Both models, the pseudo second-order and the Elovich model are associated with a chemical adsorption which could conclude that valence forces are involved through sharing or exchange of electrons between the adsorbent and the adsorbate, but this can only the proven by using other analytical techniques that are able to prove the formation of chemical bonds, only then it is acceptable to confirm whether the adsorption is a chemical or physical process.[174] The pseudo first order model has also a good fitting, so this experiment cannot be used to conclude about the adsorption process, but it is a prerequisite to perceive at what time an equilibrium is reached between the adsorbent and the adsorbate. To achieve a deeper comprehension of the adsorptive behaviour other approaches are necessary to elucidate the underlying mechanism of adsorption, for example adsorption thermodynamics.

3.3.2. Adsorption isotherms of cyclophosphamide

The graphic representation of the experimental values of q_e as of function of time for the cytostatic cyclophosphamide with **[Si][N_{3222}]Cl**, **[Si][N_{3444}]Cl** and **[Si][N_{3888}]Cl** are showed in figure 26. In each graphic there is also the representation of adsorption isotherms models, more specifically, Langmuir, Freundlich and SIPS models. The software GraphPad Prims 8 was used for determining the isotherms parameters, R^2 and $R^2_{adj.}$, which are displayed in table 16.

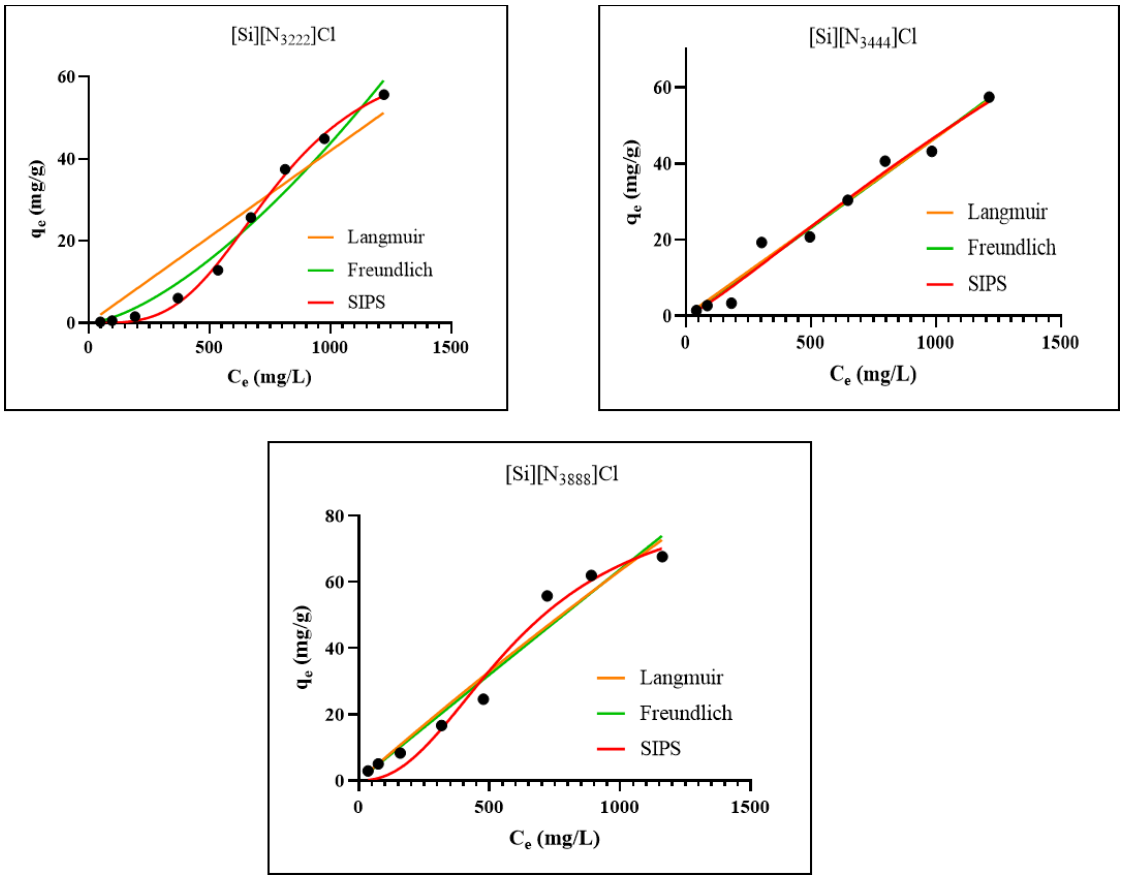


Figure 26: Experimental values of q_e (mg/g) of cyclophosphamide as a function of C_e (mg/L) and representation of Langmuir, Freundlich and SIPS isotherm models for [Si][N_{3222}]Cl, [Si][N_{3444}]Cl and [Si][N_{3888}]Cl.

Table 16: Constants calculated from the Langmuir, Freundlich and SIPS models and their correlation coefficients for cyclophosphamide with [Si][N_{3222}]Cl, [Si][N_{3444}]Cl and [Si][N_{3888}]Cl.

		[Si][N_{3222}]Cl	**[Si][N_{3444}]Cl**	**[Si][N_{3888}]Cl**
Langmuir	$q_{máx}$ (mg g^{-1})	2.806×10^{5}	4.085×10^{4}	829.6
	b (L mg^{-1})	1.496×10^{-7}	1.146×10^{-6}	8.272×10^{-5}
	R^2	0.9187	0.9749	0.9592
	R^2_{adj}	**0.9071**	**0.9713**	**0.9524**
Freundlich	k_f (mg g^{-1})	1.345×10^{-3}	3.998×10^{-2}	6.627×10^{-2}
	n	0.6648	0.9774	1.006
	R^2	0.9752	0.9751	0.9580
	R^2_{adj}	**0.9717**	**0.9715**	**0.9510**
SIPS	$q_{máx}$ (mg g^{-1})	67.06	217.9	86.98
	K_s (L mg^{-1})	1.288×10^{-3}	3.418×10^{-4}	1.615×10^{-3}
	1/n	3.437	1.199	2.266
	R^2	0.9974	0.9761	0.9764
	R^2_{adj}	**0.9966**	**0.9681**	**0.9670**

By analysing the R^2_{adj} of the three isotherms, the best fitted model is the SIPS one with R^2_{adj} of 0.9966 and 0.9670 for **[Si][N_{3222}]Cl** and **[Si][N_{3888}]Cl**, respectively and the Freundlich model for **[Si][N_{3444}]Cl** with a R^2_{adj} of 0.9715 but in this isotherm there is hardly any difference between the Langmuir and Freundlich model. By looking at table 16 the better predicted $q_{máx}$ in the SIPS model was for the **[Si][N_{3444}]Cl** but the best experimental $q_{máx}$ was 67.6 mg/g for **[Si][N_{3888}]Cl**. The SIPS model reflects an adsorption on heterogenous surfaces and at low concentrations it reduces to the Freundlich isotherm, on the other hand, at high concentration of adsorbate it predicts the Langmuir isotherm, meaning it was a mixed adsorption mechanisms, because it is composed of a non-ideal adsorption on heterogenous surfaces with multilayer sorption at low concentrations, while having a monolayer adsorption at high concentrations.[197] The obtained isotherm for **[Si][N_{3222}]Cl** and **[Si][N_{3888}]Cl** is a S-type shape[198], where it has a concave trend in the initial slope, suggesting an unfavourable sorption for lower concentrations. For higher concentrations, when the affinity to the surface increases an increment in the slope is observed. These findings denote the existence of a cooperative sorption, the sorption gets less difficult as more adsorbate gets incorporated, being an indicative of physical mechanism.[199]

With the purpose of developing a device to adsorb cytostatics from urine of oncologic patients, like cyclophosphamide, we can take into account that if a person takes 500 mg of CP per day, that only partly is metabolized in the body meaning 25% of CP is excreted through urine and that a person's average amount of urine per day is 2 L there is a concentration of 62.5 mg/L of CP in the urine.[52,200] Since **[Si][N_{3888}]Cl** can adsorb 67.6 mg of CP per gram of SIL, one gram of this SIL can treat 1.08L of urine.

4. Conclusion and future work

Considering all the perceived and discussed results from the characterization of SILs, we can conclude that the functionalization of silica with ionic liquids proved to be successful and they pose as versatile and promising materials for adsorption of contaminants from aqueous solutions, but the extend of functionalization is low, so SILs with higher bonding amount should be synthesized to further improve their performance. The supported ionic liquids with longer alkyl chains showed better performances for the adsorption of both imidacloprid and cyclophosphamide which concludes that hydrophobic interactions must play an important role in the adsorption process.

The adsorption kinetics showed that both contaminants are quickly adsorbed by the SILs, reaching an equilibrium in a few minutes. The adsorption kinetic model that best fitted the experimental data for imidacloprid was the pseudo second-order for the three SILs studied and for cyclophosphamide the best fitted model was the Elovich model for **[Si][N_{3222}]Cl** and **[Si][N_{3888}]Cl** and the pseudo second-order model for **[Si][N_{3444}]Cl**. These models are associated with chemical adsorption but we cannot confirm this fact without performing other analytical techniques should to prove the formation of chemical bonds.

The adsorption isotherm model that best fitted the results from imidacloprid was the Langmuir model with the best experimental q_e being 16.4 mg/g for **[Si][N_{3444}]Cl**. The adsorption isotherm model that best fitted the experimental data from cyclophosphamide was the SIPS model for **[Si][N_{3222}]Cl** and **[Si][N_{3888}]Cl**, respectively and the Freundlich model for **[Si][N_{3444}]Cl**. The best experimental q_e was 67.6 mg/g for **[Si][N_{3888}]Cl**, proving that supported ionic liquids can be applied as stationary phases in solid-phase extractions of cyclophosphamide in aqueous solutions.

For future work SILs with higher bonding amount should be synthesized, more adsorption experiments should be performed with other types of cytostatics and adsorption experiments in column could also be investigated to prove the saturation of the stationary phase. The SIL that showed the best performance (**[Si][N_{3888}]Cl**) in removing cyclophosphamide from aqueous solutions is the one with lower functionalization extent so its performance may be improved and may be applied for the adsorption of CP from samples of synthetic urine in order to get further to the developing of a device that can be used to remove anticancer drugs from urine of oncologic patients.

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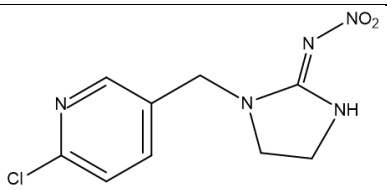
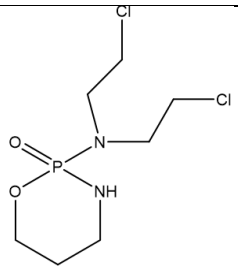
6. Annex

Table 17: Physicochemical properties of imidacloprid and cyclophosphamide.

Properties	Imidacloprid	Cyclophosphamide
Chemical structure		
Molecular formula	$C_9H_{10}ClN_5O_2$	$C_7H_{15}Cl_2N_2O_2P$
Molecular weight (g/mol)	255.66	261.08
CAS	105827-78-9	50-18-0
Water solubility	346 mg/L	1-5g/100mL
Melting point (°C)	144	48-49
pKa	5.28 9.39	12.78
LogKow	0.8	0.6

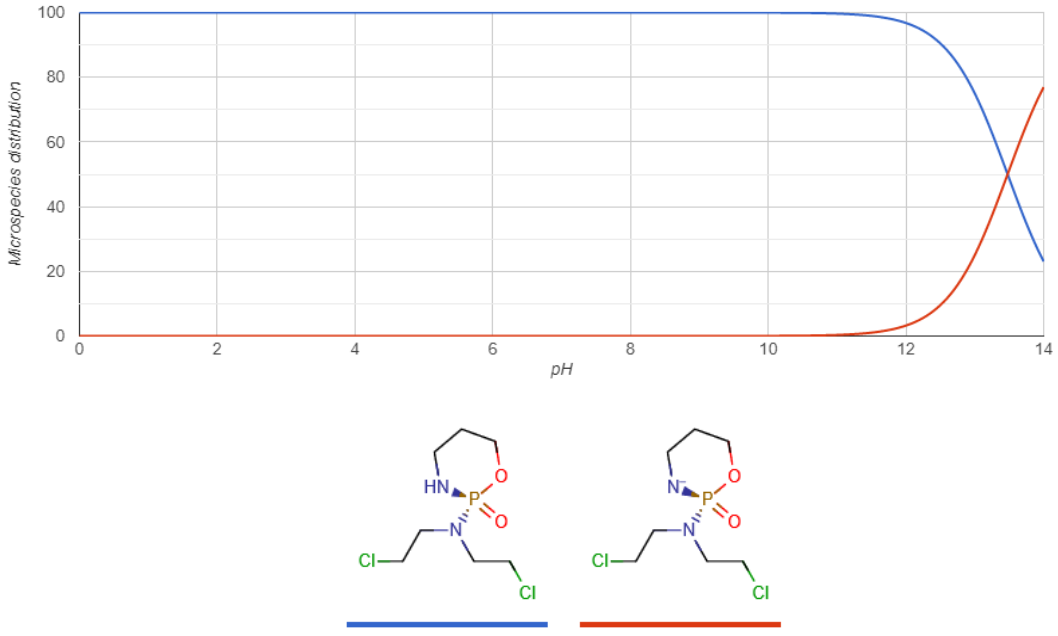


Figure 27: Speciation of cyclophosphamide with pH.

Table 18: Volume (V) used in the synthesis of each cation source, molar mass (M), density (d), weight/weight percentage (%(w/w)) and number of mols (n).

Cation source	V (ml)	M (g/mol)	d (g/ml)	%(w/w)	n (mol)
1-methylimidazole	5.0	83.1	1.03	0.99	0.061
N,N-dimethylbutylamine	5.0	101.19	0.721	0.99	0.035
Triethylamine	5.0	101.19	0.726	0.99	0.036
Tributylamine	5.0	185.35	0.778	0.99	0.021
Trioctylamine	5.0	353.67	0.811	0.98	0.011

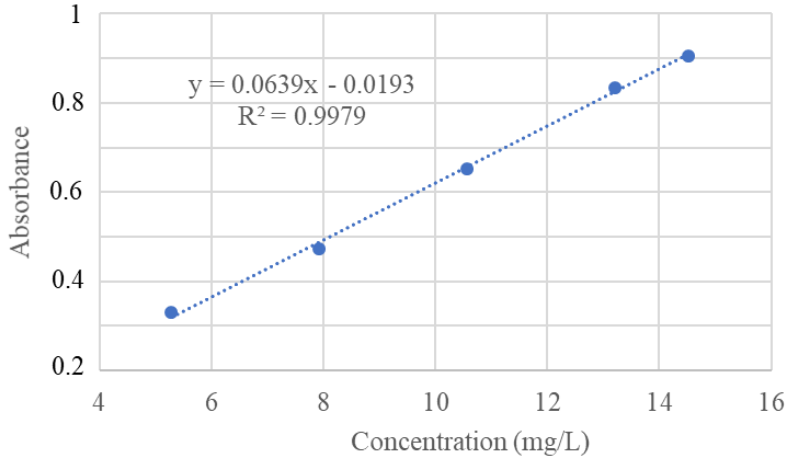


Figure 28: Calibration curve of imidacloprid.

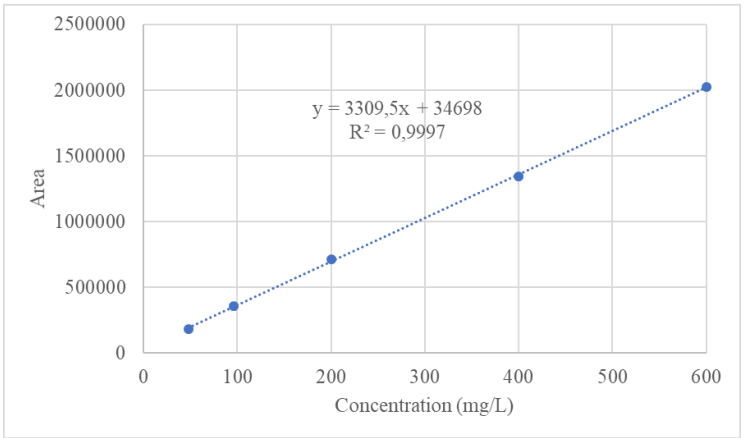


Figura 29: Calibration curve of cyclophosphamide.

Table 19: Adsorption efficiency (%AE), equilibrium concentration of imidacloprid after adsorption (C_e) and concentration of adsorbate in solid phase (q_e) using [Si][N_{3444}]Cl.

[Si][N_{3444}]Cl			
t (min)	**%AE**	**C_e (mg/L)**	**q_e (mg/g)**
2	42.3	5.39	0.79
5	46.8	4.97	0.87
10	50.9	4.59	0.95
30	51.5	4.53	0.96
60	48.8	4.78	0.91

Table 20: Adsorption efficiency (%AE), equilibrium concentration of imidacloprid after adsorption (C_e) and concentration of adsorbate in solid phase (q_e) using [Si][N_{3888}]Cl.

[Si][N_{3888}]Cl			
t (min)	**%AE**	**C_e (mg/L)**	**q_e (mg/g)**
2	40.8	5.53	0.76
5	51.2	4.56	0.96
10	55.0	4.20	1.03
30	57.1	4.01	1.07
60	56.7	4.04	1.06

Table 21: Adsorption efficiency (%AE), equilibrium concentration of imidacloprid after adsorption (C_e) and concentration of adsorbate in solid phase (q_e) using [Si][C_3]Cl.

[Si][C_3]Cl			
t (min)	**%AE**	**C_e (mg/L)**	**q_e (mg/g)**
2	34.4	6.20	0.66
5	37.9	5.87	0.72
10	43.5	5.34	0.83
30	46.8	5.03	0.89
60	46.4	5.08	0.88
90	47.0	5.01	0.90

Table 22: Adsorption efficiency (%AE), equilibrium concentration of cyclophosphamide after adsorption (C_e) and concentration of adsorbate in solid phase (q_e) using [Si][N_{3222}]Cl.

[Si][N_{3222}]Cl			
t (min)	**%AE**	**C_e (mg/L)**	**q_e (mg/g)**
1	76.5	21.2	14.0
2	78.6	19.2	14.2
5	79.4	18.6	14.3
10	79.0	18.9	14.4
20	78.7	19.2	14.4
30	80.3	17.7	14.5
45	80.4	17.6	14.5
60	81.5	16.6	14.6
120	81.7	16.5	14.6
180	81.8	16.4	14.6

Table 23: Adsorption efficiency (%AE), equilibrium concentration of cyclophosphamide after adsorption (C_e) and concentration of adsorbate in solid phase (q_e) using [Si][N_{3444}]Cl.

[Si][N_{3444}]Cl			
t (min)	**%AE**	**C_e (mg/L)**	**q_e (mg/g)**
1	79.0	18.9	13.9
2	81.3	16.9	14.2
5	80.1	17.9	14.4
10	80.4	17.6	14.5
20	80.8	17.3	14.6
30	82.6	15.7	14.6
45	80.2	17.8	14.6
60	81.1	17.0	14.7
120	82.2	16.0	14.7
180	82.5	15.8	14.7

Table 24: Adsorption efficiency (%AE), equilibrium concentration of cyclophosphamide after adsorption (C_e) and concentration of adsorbate in solid phase (q_e) using [Si][N_{3888}]Cl.

[Si][N_{3888}]Cl			
t (min)	**%AE**	**C_e (mg/L)**	**q_e (mg/g)**
1	78.0	19.8	13.8
2	79.3	18.6	14.1
5	78.7	19.1	14.2
10	80.2	17.8	14.4
20	81.4	16.7	14.5
30	81.1	17.0	14.5
45	81.8	16.4	14.6
60	82.3	16.0	14.7
120	80.7	17.4	14.7
180	83.3	15.0	14.7

Table 25: Equilibrium concentration of imidacloprid after adsorption (C_e) and concentration of adsorbate in solid phase (q_e) using [Si][N_{3444}]Cl, [Si][N_{3888}]Cl and [Si][C_3]Cl.

[Si][N_{3444}]Cl		[Si][N_{3888}]Cl		[Si][C_3]Cl	
C_e (mg/L)	**q_e (mg/g)**	**C_e (mg/L)**	**q_e (mg/g)**	**C_e (mg/L)**	**q_e (mg/g)**
1.1	0.2	1.0	0.2	1.2	0.2
2.2	0.6	2.2	0.6	2.8	0.5
3.2	0.8	2.7	0.9	3.9	0.6
4.1	1.1	4.2	1.1	5.2	0.8
6.6	1.7	8.3	2.2	8.1	1.3
9.2	2.0	30.9	4,9	35.2	4.1
30.8	5.0	42.2	7,7	49.7	6.2
58.6	9.9	61.5	9,3	66.7	8.2
76.9	11.9	80.2	11,2	86.9	9.9
147.3	16.4	151.3	15,6	157.5	14.4

Table 26: Equilibrium concentration of cyclophosphamide after adsorption (C_e) and concentration of adsorbate in solid phase (q_e) using [Si][N_{3222}]Cl, [Si][N_{3444}]Cl and [Si][N_{3888}]Cl.

[Si][N_{3222}]Cl		**[Si][N_{3444}]Cl**		**[Si][N_{3888}]Cl**	
C_e (mg/L)	**q_e (mg/g)**	**C_e (mg/L)**	**q_e (mg/g)**	**C_e (mg/L)**	**q_e (mg/g)**
49	0.3	43	1.4	36	2.9
97	0.6	86	2.7	75	5.0
192	1.6	183	3.4	159	8.3
370	6.1	304	19.3	317	16.6
536	12.9	496	20.7	477	24.5
671	25.7	648	30.3	721	55.8
813	37.5	797	40.7	890	62.0
975	44.9	984	43.2	1162	67.6
1222	55.7	1213	57.4		