Treatment of aqueous effluents contaminated with active pharmaceutical ingredients

Hugo Ferrão Dias de Almeida

Dissertation presented to obtain the Ph.D degree in Sustainable Chemistry

Instituto de Tecnologia Química e Biológica António Xavier | Universidade Nova de Lisboa

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I declare that the work presented in this thesis, except where otherwise stated, is based on my own research. The work was mainly performed in the Separation and Extraction Technologies Laboratory of the Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa and CICECO – Instituto de Materiais de Aveiro, Universidade de Aveiro, between March 2013 and March 2017, and supervised by Doctor Isabel M. Marrucho (ITQB-UNL) and Doctor Mara G. Freire (CICECO-UA).

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À minha grande família,
juntos alcançamos os nossos sonhos!

"The true sign of intelligence is not knowledge but imagination."
— Albert Einstein

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Evolutions of a birth, a rhyme to tell this crimson tale
Evoke the faithful creed, the sacred secrets of society
Matt Pike

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Contents

Treatment of aqueous effluents contaminated with active pharmaceutical ingredients

Abstract

The need to significantly improve human living conditions led to a large increase in the worldwide consumption of pharmaceutical drugs. Over the past few years, the development of advanced analytical tools and investigations on wastewater samples confirmed the presence of residual amounts of active pharmaceutical ingredients (APIs) in wastewater treatment plants (WWTPs), sewage treatment plants (STPs), groundwater and drinking water. Even at low concentrations ($ng.L^{-1}$ – $\mu g.L^{-1}$), the regular contact and ingeston of APIs can lead to deleterious effects towards living organisms. Numerous studies demonstrated that APIs present in WWTPs are a matter of concern towards wildlife and public health. Given the widespread occurance of these drugs in aquatic ecossystems and their deleterious effects, the development of strategies able to mitigate their introduction in the aquatic environment is of great importance.

This thesis addresses the development and study of two water cleaning processes to be included in WWTPs, namely liquid-liquid extraction (LLE) and solid-phase extraction (SPE), making use of a novel class of compounds – ionic liquids (ILs). Ionic liquids present excellent solvating qualities, which can be additionally tailored to guarantee the success of specific extractions. In particular, aqueous biphasic systems (ABS) composed of ILs and supported ionic liquid phases (SILPs) were investigated for the removal of non-steroidal anti-inflammatory drugs (NSAIDs) and antibiotics (fluoroquinolones – FQs), two classes of APIs frequently found in aquatic environments. The work presented herein starts with the use of IL-based ABS, formed by the addition of a citrate-based salt ($C_6H_5K_3O_7$), for the extraction and concentration of FQs and NSAIDs from aqueous media (Chapter 2). Afterwards, the extraction capacity of ABS composed of ILs and $Al_2(SO_4)_3$ was evaluated for four NSAIDs and six FQs. $Al_2(SO_4)_3$ was used as a salting-out species, as it is already used as a flocculating agent in the purification of drinking water processes (Chapters 3 and 4). In these works, the recyclability of the IL-based ABS was always attempted. In addition to

IL-.based ABS strategies, SILPs were synthetized by crafting 1-methyl-3-propylimidazolium chloride into the surface of silica, ensuing the preparation of six materials with different counter ions. Adsorption kinetics and isotherms were performed with diclofenac solutions (Chapter 5). Further, the most promising materials was evaluated to remove three NSAIDs, by carrying out adsorption kinetics and isotherms. In order to develop a continuous removal method, a packed column with SILP was also prepared. Finally, the recovery of NSAIDs and the SILP reusability were evaluated (Chapter 6).

The obtained results demostrate that it is possible to design chemical structures of ILs so that they can be efficiently used to remove target pollutants from aqueous environments. The processes here developed are envisioned to be included in the final stage of WWTPs and STPs.

Resumo

A necessidade de melhorar significativamente as condições de vida humana conduziu a um grande aumento no consumo de medicamentos. Ao longo dos últimos anos, o desenvolvimento de ferramentas analíticas avançadas e estudos em amostras de águas residuais, confirmou a presença de quantidades vestigiais de ingredientes farmacêuticos ativos (IFAs) em estações de tratamento de águas residuais (ETARs), plantas de tratamento de esgoto (PTE), águas subterrâneas e água potável. Mesmo em baixas concentrações ($ng.L^{-1}$ - $\mu g.L^{-1}$), o contato regular e a ingestão de IFAs podem conduzir a efeitos nocivos para os organismos vivos. Numerosos estudos demonstraram que os IFAs presentes nas ETARs são motivo de preocupação para o ambiente e a saúde pública. Dada a ocorrência generalizada destas drogas em ecossistemas aquáticos e seus efeitos nocivos, o desenvolvimento de estratégias capazes de mitigar sua introdução no meio aquático é de grande importância.

Esta tese aborda o desenvolvimento e estudo de dois processos de limpeza de água a serem incluídos nas ETARs, nomeadamente extração líquido-líquido (ELL) e extração em fase sólida (EFS), utilizando uma nova classe de compostos – líquidos iónicos (LIs). Os LIs apresentam excelentes qualidades de solvatação, que podem ser adicionalmente adaptados para garantir o sucesso de extrações específicas. Em particular, os sistemas bifásicos aquosos (SAB) compostos por LIs e fases líquidas iónicas suportadas (FLIS) foram investigados para a remoção de anti-inflamatórios não esteróides (AINEs) e antibióticos (fluoroquinolonas - FQs), duas classes de IFAs frequentemente encontradas em ambientes aquáticos. O trabalho aqui apresentado começa com o uso de SAB com base em LIs, formado pela adição de um sal à base de citrato ($C_6H_5K_3O_7$), para a extração e concentração de FQs e AINEs, a partir de meios aquosos (Capítulo 2). Posteriormente, avaliou-se a capacidade de extração de SAB composta por LIs e $Al_2(SO_4)_3$ para extração de quatro AINEs e seis FQs. $Al_2(SO_4)_3$ foi utilizado como uma espécie de salting-out, já que já é usado como

agente floculante na purificação dos processos de água potável (capítulos 3 e 4). Nesses trabalhos, foi sempre considerada a reciclabilidade do SAB baseado em LIs. Além das estratégias de SAB baseadas em LIs, as FLIS foram sintetizadas pela elaboração de cloreto de 1-metil-3-propilimidazólio na superfície de sílica, resultando na preparação de seis materiais com contra-iões diferentes. A cinética de adsorção e as isotérmicas foram realizadas com soluções de diclofenaco (Capítulo 5). Além disso, os materiais mais promissores foram avaliados para remover três AINEs, realizando a cinética de adsorção e isotérmicas. Para desenvolver um método de remoção contínua, uma coluna compactada com FLIS também foi preparada. Finalmente, avaliou-se a recuperação de AINEs e a reutilização FLIS (Capítulo 6).

Os resultados obtidos demonstram que é possível projetar estruturas químicas de LIs para que possam ser usadas eficientemente para remover contaminantes alvo de ambientes aquosos. Os processos aqui desenvolvidos devem ser incluídos nos estágios finais das ETARs e PTEs.

Publications

Thesis publications (5)

Hugo F. D. Almeida, Sara Azevedo, Isabel M. Marrucho and Mara G. Freire, *Adsorption of Non-Steroidal Anti-Inflammatory Drugs from Aqueous Samples using Ionic-Liquid-Silica-based Materials*, manuscript in preparation, 2017.

Hugo F. D. Almeida, Márcia C. Neves, Isabel M. Marrucho and Mara G. Freire, *Supported Ionic Liquids as Efficient Materials to Remove Non-Steroidal Anti-Inflammatory Drugs from Aqueous Media*, manuscript in preparation, 2017.

Hugo F. D. Almeida, Mara G. Freire and Isabel M. Marrucho*, Improved Monitoring of Aqueous Samples by the Concentration of Active Pharmaceutical Ingredients using Ionic-Liquid-based Systems*, Green Chem., 2017, **19**, 4651-4659.

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Other publications (9)

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Thesis Layout

Chapter 1 | Introduction

A brief overview of the state-of-art and general concepts are presented, along with the motivation and objectives of this thesis.

Chapter 2 | Improved Monitoring of Aqueous Samples by the Concentration of Active Pharmaceutical Ingredients using Ionic-Liquid-based Systems

Development of a pre-treatment technique for the extraction and concentration of fluoroquinolones and non-steroidal anti-inflammatory drugs using aqueous biphasic systems composed of ionic liquids and a citrate-based salt.

Chapter 3 | Removal of Non-Steroidal Anti-Inflammatory Drugs from Aqueous Environments with Reusable Ionic-Liquid-based Systems

Application of ionic-liquid-based aqueous biphasic systems formed with aluminium-based salts as extraction techniques of non-steroidal anti-inflammatory drugs from aqueous samples. The recovery of non-steroidal anti-inflammatory drugs and reuse of ionic liquids is also shown.

Chapter 4 | Improved Extraction of Fluoroquinolones with Recyclable Ionic-Liquid-based Aqueous Biphasic Systems

Elaboration of an efficient extraction technique for fluoroquinolones from aqueous samples using ionic-liquid-based aqueous biphasic systems. The recycle and reuse of ionic liquids is shown.

Chapter 5 | Supported Ionic Liquids as Efficient Materials to Remove Non-Steroidal Anti-Inflammatory Drugs from Aqueous Media

Preparation and characterization of novel materials based on silica modified with 1-methyl-3-propylimidazolium. Their performance for the removal of diclofenac from aqueous media is provided.

Chapter 6 | Adsorption of Non-Steroidal Anti-Inflammatory Drugs from Aqueous Samples using Ionic-Liquid-Silica-based Materials

Application of supported ionic liquid materials to remove non-steroidal anti-inflammatory drugs. Development of a continuous removal process. The regeneration and reuse of the material are shown.

Chapter 7 | Concluding Remarks and Outlook

The main achievements and conclusions are highlighted herein. Possible challenges and perspectives for future research are also presented.

Chapter 1

Introduction

1. General Context

The contamination of the water cycle with persistent contaminants remains one of the primary challenges of the 21st century, since new chemicals are being continuously developed and introduced in disposable consumer products thus ending in the sewage and largely contributing to the contamination of the water streams. New formulations of toothpastes, soaps, perfumes, artificial sweeteners, insect repellents, deodorants, prescribed drugs, plastic bottles and containers, among others, are examples of these products.[1] The presence of persistent contaminants in the aqueous environment is a key concern in environmental chemistry, since these compounds are only partially removed by conventional wastewater treatment plants (WWTP).[2-6] Besides, they are ubiquitous, persistent and bioaccumulative in wildlife and humans, raising a number of health concerns,[2, 4, 7-9] and are thus called chemicals of emerging concern or emerging micro-pollutants. Major international organizations, like the World Health Organization (WHO), United States Geological Survey (USGS), United States Environmental Protection Agency (EPA) and the European Commission (EU) expressed their concerns about the increasing concentrations, diversity and toxicity of micropollutants.[1] Although emerging micro-pollutants still have no clear definition, different authors have proposed different descriptions. According to Kümmerer,[10] emerging micro-pollutants are defined as unregulated or limited regulated compounds, which are present in the environment at concentrations below $\mu g.L^{-1}$, independently of their chemical structure. Marcoux et al.[11] summarized emerging micro-pollutants as new risky detected or already risky identified substances in the environment, or substances already known but whose recent use in products can cause problems during their future treatment as wastes or that due to difficulties in their analytical quantification are only being studied as suggested by Kurwadkar et al.[12]. These emerging contaminants are not of recent origin, but their analytical quantification, exposure routes and pathways and consequent human health and ecological effects are now becoming known. For

these reasons their occurance, environmental fate and toxicity are still largely unknown.[12, 13] Pharmaceuticals, personal care products, endocrine disrupting compounds, nanomaterials and perfluorinated compounds are some of the emerging micro-pollutants in water streams.

Active pharmaceutical ingredients (APIs) are a group of micropollutants that have been gaining huge attention not only from the scientific community but also from the general population, due their occurrence and persistence in the aquatic environment,[4, 5, 14, 15] in very low concentrations ($\mu g.L^{-1}$ or $ng.L^{-1}$).[6, 16] More than 5000 pharmaceuticals were synthesized and are available in the market for human and animal consumption.[17] Presently, the annual global drug consumption is in the range of 100,000 – 200,000 tons, being North America, Central Europe, Brazil, Russia, India, China, Australia and South Africa the major consumers.[6, 18] Several classes of pharmaceutical compounds, including prescription drugs, over-the-counter medications, drugs used in hospitals and veterinary drugs, have been found throughout the water cycle.[2-5, 19, 20] Although most of these drugs can be administered orally or by injection, part is excreted in urine or faeces, due to the incomplete assimilation in humans and animals, and eventually end up in WWTPs.[1, 3, 18, 21] Therefore, the consumption of water or/and aquatic organisms which might have pharmaceutical residues accumulated can expose humans to further unknown risks.[2, 4, 8]

WWTPs are responsible for the quality of waterstreams and drinking water and thus play a crucial and relevant role in the prevention of the aquatic environment contamination. However, as highlighted by several authors,[18, 22-27] WWTPs are not designed for the removal/degradation of these contaminants and thus exhibit low efficiency in the removal of APIs. This is in agreement with the detection of APIs in urban aquatic environment and drinking water, leading to their continuous bioaccumulation into the environment and consequent human exposure.[6, 9, 16, 28, 29]

1.1. Active Pharmaceutical Ingredients in Water Effluents

A large number of studies in Europe and United States have shown the contamination of the water cycle with active pharmaceutical ingredients (APIs).[6, 30] For instance, APIs have been detected in wastewater treatments plants (WWTP) as well as in surface water, in concentrations between 1000 – 10000 ng.L^{-1} and 100 – 1000 ng.L^{-1}, respectively.[30] In hospital wastewater, these concentrations can reach much higher values.[4, 5, 20, 30, 31] This is mainly due to the fact that the currently used processes in WWTPs were not designed to remove APIs and thus are not able to do it. On the other hand, according to the IMS Institute for Healthcare Informatics reports,[32] most countries will continue to increase pharmaceutical spending per capita by 2018, especially in North America. This statement is confirmed by the data shown in Figure 1, where the pharmaceutical spending per capita in 2013 and 2018 in several countries is compared.

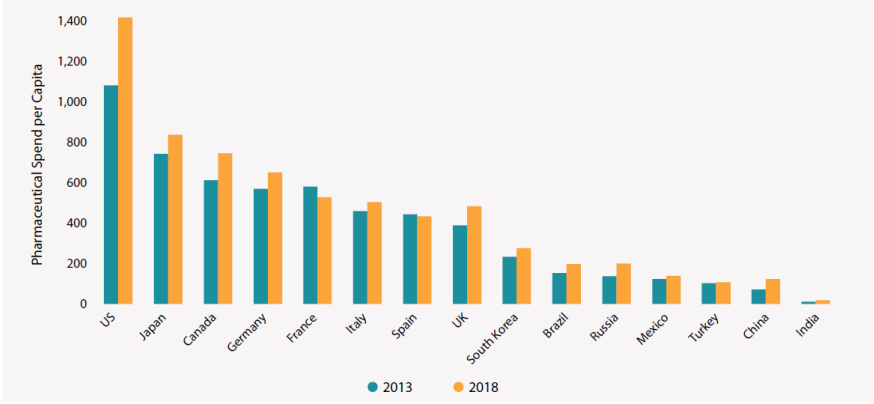

Figure 1. Comparison of pharmaceutical spending per capita (U.S. Dollar) in several countries in 2013 versus 2018, adapted from the IMS Institute for Healthcare Informatics.[32]

It is foreseen that the huge progress in APIs manufacture all over the world will remain strong in the next decades, mainly due to the expiration of patents, allowing a larger number of products to be further explored.[3, 32, 33]

Consequently, the correct and controlled use and disposal of APIs as well as the monitoring of their presence, either in metabolized or unmetabolized forms in water systems, will continue to be a relevant topic of research and regulation. The Global Water Research Coalition (GWRC) started investigations in order to come up with a reliable selection of APIs that are present in water cycle and represent a risk for human health.[30] The identification of the most important APIs and definition of evaluation criteria were major topics in those prioritization studies, which mainly occurred in North America, Europe, Australia and East Asia. The establishement of representative priority APIs list furthered studies on analytical methods of detection and occurrence, treatability, and potential risks associated with exposure. One hundred and fifty-three APIs were listed in the final data-base, where twenty four of these compounds are presented in two priority lists, sixteen show up in three to five lists, and nineteen in more than five lists. These lists were made taking into account different criteria for selection and/or prioritization of pharmaceuticals.[30, 34] According to de Voogt et al.,[34] APIs can be classified in three classes, representing pharmaceutical with high, medium and low priority. In Figure 2, the chemical structures of APIs in Class 1 – high priority, are presented. All of these compounds are listed as toxic, persistent and resistant to treatment. This list certainly needs to be updated over the time, depending on the outcomes of future studies, where especial attention should be given to the increasing occurrence data set. Recently, the European Union proposed the first watch list for emerging water pollutants in wich APIs are included,[35] and it is expected that by 2017 the EC proposes measures to address the environmental effects of pharmaceutical substances.

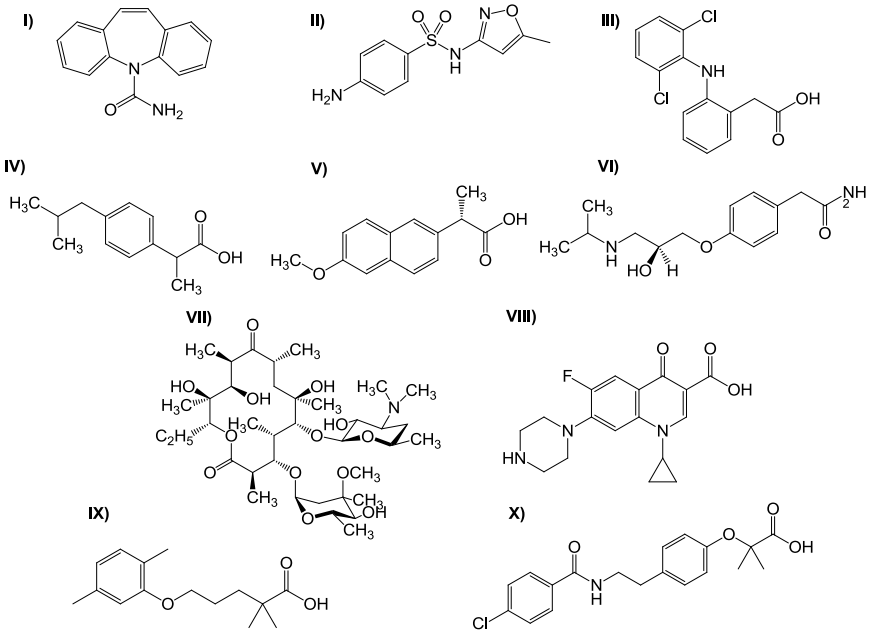

Figure 2. Structure of priority pharmaceutical active compounds – Class 1: I) carbamazepine, II) sulfamethoxazole, III) diclofenac, IV) ibuprofen, V) naproxen, VI) atenolol, VII) erythromycin, VIII) ciprofloxacin, IX) gemfibrozil, and X) bezafibrate.

1.2. Classification of Active Pharmaceutical Ingredients

Active pharmaceutical ingredients (APIs) are defined as relatively low molecular weight compounds, presenting a variety of physico-chemical and biological properties and functionalities.[2, 5, 16] Although APIs can be classified by their chemical structures within medicinal chemistry subgroups,[2] they are usually classified according to their purpose and biological activity.[5] Thus, the main classes of APIs include non-steroidal anti-inflammatory drugs, antibiotics, steroid hormones, blood lipids regulators, antihypertensive and neuroactive drugs.

Non-steroidal anti-inflammatory drugs (NSAIDs) are used as pain killers in human and veterinary medicine. NSAIDs are one of the primary classes of pharmaceutical compounds prescribed in human medical care, sold with and

without prescription. Diclofenac, acetylsalicylic acid, ibuprofen, acetaminophen, naproxen, codeine, propyphenazone and antipyrine are the most frequently prioritized NSAIDs.[4, 36, 37] For instance, 75 tons of prescribed diclofenac are annually sold in Germany alone.[4, 15] High NSAIDs concentrations have been detected in aquatic environment as a result of their high consumption, potentially leading to a high bioaccumulation, according to their octanol-water partition coefficient values (K_{ow}).[38]

Antibiotic is, by definition, a natural or synthetic drug that presents a selective toxic action on bacteria or other unicellular microorganism,[5, 36, 39] exhibiting a considerable benefit in public health.[40] It is estimated that the worldwide consumption of antibiotics reaches the hundredths of thousands of tons annually, where tetracyclines, penicillins, sulphonamides, macrolides, aminoglycosides, and fluoroquinolones are the most frequently found.[36, 39] In human medicine, antibiotics represent a third of the most commonly prescribed drugs, while in veterinary medicine that fraction is larger than 70 %.[39, 41-43] Thus, due to their extensive use, the entrance of antibiotics into aquatic environment is unavoidable, being the main pathways excretion, flushing of old prescriptions and hospital waste.[4, 5, 43] Regarding antibiotics consumed by animals, only 30 % up to 90 % are metabolized, while the remaining stay unchanged as biologically active metabolites. Subsequently, these metabolites are discharged directly to the soil through the use of manure as fertilizer or by pasture animals excreting faeces and urine.[44] Nevertheless, the largest parcel of antibiotics in the environment comes from agricultural related activities since they are used worldwide for feed additive purposes. Recent reviews by Puckowski et al.[36] and Martinez et al.[45] give a perspective on the dissemination of antibiotics, for both human and veterinary use, in the environment.

Steroidal hormones are a diverse class of synthetic and natural compounds that can modify the hormonal systems functions and might cause unfavorable health effects, affecting a large part of the body reactions responsible for the homeostasis, reproduction, development and behavior. Generally, steroidal

hormones constitute a coherent group of organic compounds that contain a characteristic arrangement of four cycloalkane rings.[36] Hormones are mainly used in estrogen replacement therapy,[46] as oral contraceptives,[47] for growth enhancement,[48] and in athletic performance enhancement.[49] as well as in veterinary.[50] The most frequently priority groups of steroid hormones are estrone, 17α-estradiol, 17β-estradiol and estriol, while the most popular synthetic hormone is 17α-ethynylestradiol, widely used in contraceptive pills.[36] Hormones were the first APIs to be found and reported in sewage effluents, reservoirs, rivers and potable water, being the first pharmaceutical compounds to draw the attention of environmental scientists, regulatory agencies and organizations.[5, 51] They are potent endocrine disruptors, being for example responsible for the feminization of male fish.[52, 53]

Lipid regulators are used to lower the levels of triglycerides and to raise the levels of high density cholesterol in the blood. There are two main classes of lipid regulators: statins and fibrates. Some lipid regulators (eg. clofibric acid, gemfibrozil and bezafibrate) have an acidic group in their molecular structure and thus exhibit similar properties to NSAIDs. The first blood lipids regulator drug found in aquatic environment was clofibric acid.[4, 54-56] This drug metabolite was even detected in drinking water from Berlin as a consequence of artificial groundwater enrichement.[57] Ternes[27] reported bezafibrate, gemfibrozil and clofibric acids in sewage treatment plants (STPs) and rivers, indicating a high stability of these pharmaceutical drugs under environment conditions. According to Coimbra et al.,[58] zebrafish exposed during their whole lifetime to clofibric acid display growth effects, lowered triglyceride muscle content and decreased fecundity. Bezafibrate, clofibric acid, gemfibrozil, and fenofibric acid are also toxic towards primary consumers such as the cyanobacteria *Anabaema*.[57]

Cardiovascular diseases are classified as the chronic diseases of the 21st century. Thus, a large consumption of antihypertensive drugs is expected in the next generations. The most prescribed antihypertensive drugs include calcium channel blockers, beta-blockers, enzyme inhibitors.[59] According to Santos et al.,[60]

beta-blockers have been increasingly detected in aquatic environment. Several beta-blockers, namely metoprolol, propanolol, betaxolol, bisoprolol, and nadolol were found in STPs, WWTPs and groundwater.[27, 61-63] Although some reports disclose no or low toxicity of these APIs,[64] some authors suggest that beta-blokers are endocrine disruptors[65] and affect normal behavior and stress responses in fish.[66]

Antiepileptics and antidepressants are classified as neuroactive drugs and are a group of pharmaceutical drugs used in treatment of depression, eating disorders and personality disorders.[36] The most frequently found compounds in aquatic environment are paroxetine, carbamazepine, fluoxetine and sertraline, and it is believed that these drugs are among the most toxic ones.[2, 3] According to Zenker et al.[67] and Hazelton et al.,[68] low levels of these compounds induce a relevant biological effect in aquatic organisms, where reproduction and physiological development are inhibited, causing stress responses and deficient locomotion in fish and invertebrates. Also, Brooks[69] showed that prozac influenced the beahaviour on aquatic animals, namely it induced a stress responsive, where fishes acted with a more violent reaction.

1.3. Metabolites and Transformation Products of Active Pharmaceutical Ingredients

The presence of active pharmaceutical ingredients (APIs) in the aquatic environment raises additional concerns: since APIs can undergo a variety of different structural changes by biotic and abiotic processes, in the organism itself after being ingested and also after being released in the environment. Such changes lead to the formation of other unknown and potentially harmful chemical species.[70, 71] On the other hand, technological processes in effluent treatment, such as oxidation, hydrolysis, and photolysis[72-74] also lead to the formation of new chemical compounds.

The term metabolite is somehow unclear since it is usually used for compounds which suffered structural transformations within the human body, without differentiating biochemical processes performed by human enzymes or bacterial activity in the digestive system. Moreover, metabolites are also referred as compounds derived from structural changes by fungi and bacteria and non-biotic processes, such as oxidation, hydrolysis and photolysis in aquatic environment, such as surface water, soil or sewage treatment.[2, 72] According to Längin et al.,[75] the term metabolite should only be used for compounds in which their chemical structures was modified within the body or on the skin of humans and animals. In Figure 3, a scheme of the APIs structural transformations, where metabolites and transformation products are separated according to the Längin et al.[75] metabolite definition, is presented. According to this definition, compounds that are formed after APIs excretion should be referred to as transformation products, covering both biotic and non-biotic processes.

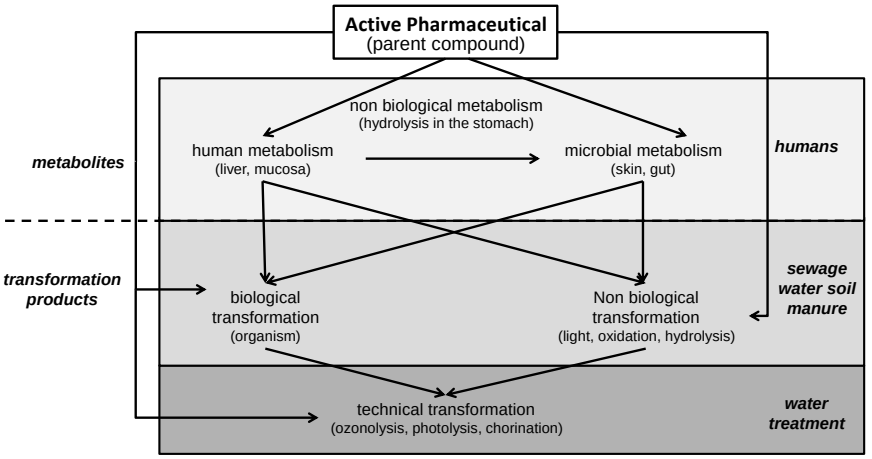

Figure 3. Scheme of structural transformations of APIs – metabolites and transformation products.[2]

Since the degradation and transformation mechanisms of most APIs are not still well studied and understood, it is very difficult to associate the presence of metabolites and transformation products to the consumption of a specific API.

Recently Li et al.[76] developed rapid process-oriented screening approach to prioritize and identify transformation products of several APIs. Despite recent improvements,[76, 77] the detection of metabolites and transformation products in water streams and the development of adequate methodos for their removal is still an emerging research field.

2. Occurrence of Active Pharmaceutical Ingredients in water streams

The entrance of large quantities of diverse pharmaceutical drugs into the environment after its use is, nowadays, unavoidable. After the use of active pharmaceutical ingredients (APIs), they are excreted in their native form or as metabolites to aquatic systems through different pathways. If APIs, their metabolites and transformation products are not treated and eliminated in the sewage treatment plants (STPs) or wastewater treatment plants (WWTPs), they eventually reach drinking water.[2, 4-6, 78, 79] Several studies around the world have investigated the possible sources and pathways of occurrence of APIs in aquatic environment. According to Heberer,[4] pharmaceutical residues have a large number of possible sources and pathways, being the medical products for human and animals use the main one. According to Figure 4, sewage is found to be the most common source of APIs, leading to their transfer into surface and drinking waters.

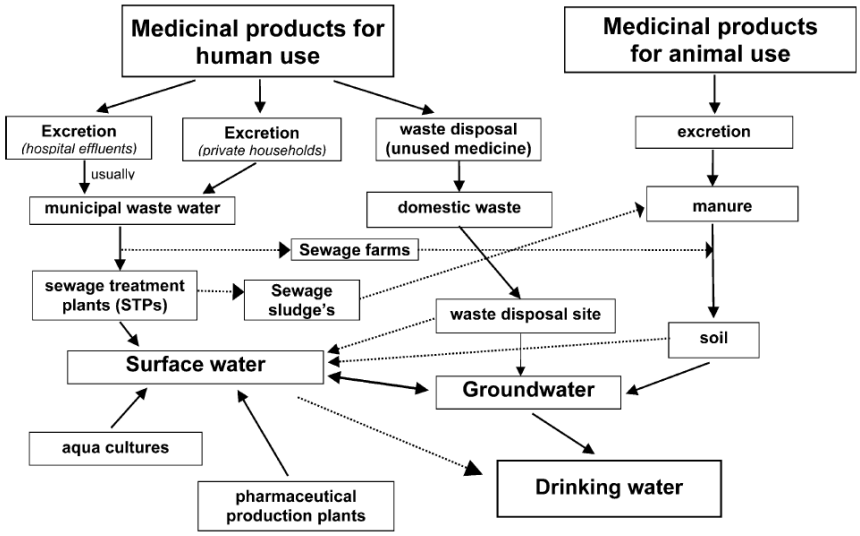

Figure 4. Representative pathways scheme for the occurrence of pharmaceutical residues in the aquatic environment.[4]

Also, the improper disposal of unused and expired pharmaceutical drugs is a common practice.[80] Several regulatory agencies have proposed mechanisms to minimize the release of pharmaceutical waste. The U.S. Food and Drug Administration,[80] proposes specific disposal instructions on the prescription drugs, advertising not to eliminate pharmaceutical drugs down the sink or toilet. The World Health Organization proposes[81] several measures to mimimize pharmaceutical waste including encapsulation and burial in landfills, incineration at high temperatures and dilution and sewer discharge. Furthermore, several reccomendations on waste minimization are also proposed. Nevertheless, developing countries are yet to come to terms with the need to standardize their environmental regulatory framework on emerging micro-pollutants.[82]

In developed countries, municipal wastewater is the principal route of aquatic environment contamination.[4, 6, 83] In addition, hospitals and drugs manufacturers wastewaters are a significant source of contamination.[3, 5, 84, 85]

In general, large quantities of various APIs classes have been detected up to $\mu g.L^{-1}$ in sewage surface and groundwater globally.[2, 4, 5] However, detailed

data of the total worldwide use of pharmaceutical drugs is still not available due to their large consumption and use.[2, 4] Even so, it is possible to analyze global occurrences and fate of the main pharmaceutical compounds in the environment.[6]

2.1. Concentration of Active Pharmaceutical Ingredients in Aqueous Effluents

Generally, anti-inflammatory and antibiotic drugs are the most abundant active pharmaceutical ingredients (APIs) in the aquatic environment, presenting the highest concentrations (up to and above $\mu g.L^{-1}$). Antibiotics are used in animal husbandry for veterinary purposes and as growth promoters, particularly in animal farming and intensive livestock treatment. Therefore, farm soil and groundwater are the primary reservoirs for antibiotic residuals, where the direct application of animal manure is the principal source of environmental contamination.[39, 42, 43] According to Tasho and Cho,[39] it is necessary to find a similar or more efficient alternative to veterinary antibiotics and to impose stricter regulations on their use and proper disposal. Other groups of pharmaceutical compounds are also present in lower concentrations in the aquatic environment (up to $ng.L^{-1}$), in accordance to their usage.[6, 34, 78]

Recently, aus der Beek et al.[6] reviewed the global occurrence of pharmaceuticals in surface water, groundwater, tap/drinking water and also in manure and soil. The number of pharmaceutical and related substances detected worldwide in sewage treatment plants (STPs), wastewater treatment plants (WWTPs) inflow/effluent/sludge, and also in surface waters, groundwater, or tap/drinking water by country is represented in Figure 5a and 5b, respectively.[6] It can be observed that a high number of pharmaceutical drugs have been detected in North America, Central Europe, China and Australia in either STPs, WWTPs inflow/effluent/sludge or in surface waters, groundwater, or tap/drinking water. It should be remarked that no data is available for the vast majority of Africa, several countries in South America and several countries in Middle East. Furthermore,

Brazil, Russia, India, Turkey, Nigeria, Pakistan and Alaska present higher amounts of pharmaceuticals and related compounds in surface waters, groundwater, or tap/drinking water than in STPs, WWTPs inflow/effluent/sludge. This is due to the large use of pharmaceutical compounds in veterinary[43, 86] and animal waste.[39, 44] In several countries, animal waste is applied as a supplement to fertilizers. Because antibiotics are poorly adsorbed by animals, they are excreted in large quantities into manure that will be applied as fertilizer supplement and therefore they will enter surface and groundwater reservoirs which will lead to higher detection of pharmaceuticals and related compounds in surface waters, groundwater, or tap/drinking water.[39, 43]

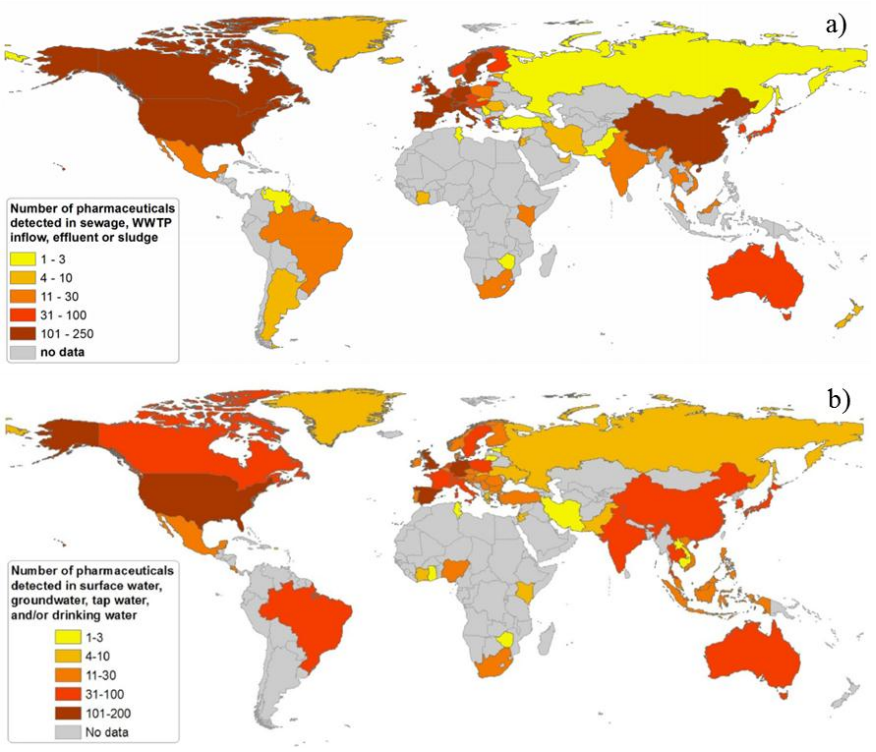

Figure 5. Number of pharmaceuticals and related compounds detected worldwide in a) sewage, wastewater treatment plants inflow/effluent/sludge and b) surface waters, groundwater, or tap/drinking water.[6]

From the 713 different pharmaceuticals and related compounds tested, aus der Beek et al.[6] also reported that 631 were present above the detection limits of the analytical methods employed. Also, the same substances were found in different countries around the world, and anti-inflammatory and antibiotic drugs residues were detected in drinking water from 50 countries. These findings corroborate the results previously compiled by Fent at al.,[87] that compared the concentrations of specific APIs in several countries wastewater and surface water, as shown in Figure 6. In this scheme it can be observed that in STPs effluents pharmaceutical concentrations range from ng.L^{-1} to μg.L^{-1}, while in rivers, lakes and seawater, they are present in concentrations around ng.L^{-1}.

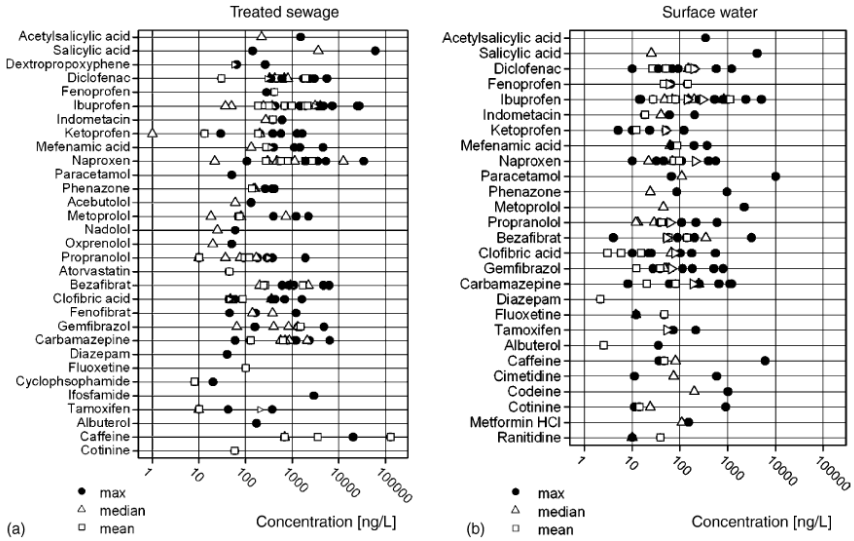

Figure 6. Concentration of pharmaceuticals in treated wastewater (a) and surface water (b).[87]

Data on the pharmaceutical drugs present in aquatic environment grouped by therapeutic classes, and their relative percentage is represented in Figure 7.[60] According to Figure 7, two major classes of APIs can be found in aquatic environments, namely non-steroidal anti-inflammatory drugs (NSAIDs) (16 %) and antibiotics (15 %), followed by blood lipid lowering agents (12 %), sex

hormones (9 %), antiepileptics (8 %) and beta-blockers (8 %). This pattern clearly follows the developments on the pharmaceutical industry according to the incidence of human diseases.

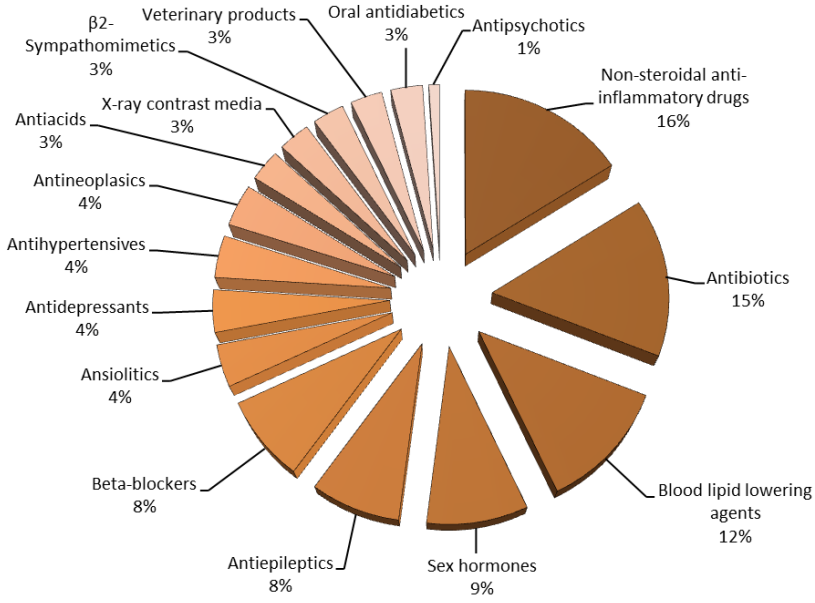

Figure 7. Therapeutic classes of pharmaceutical drugs detected in the aquatic environment, expressed in relative percentage. Data collected from 134 articles published between 1997 and 2009 (Adapted from Santos et al.[60]).

In aquatic environments, the concentration of NSAIDs range from 0.7 to 6100 ng.L^{-1}, where diclofenac, ibuprofen, naproxen and acetylsalicylic acid are the most frequently detected compounds.[24, 36, 88] For example, Heberer et al.[89] identified diclofenac as one of the most important APIs present in the water cycle, which was detected in concentrations of 3.02 and 2.51 μg.L^{-1} in influents and effluents of STPs municipal, respectively. Years later, de Voogt et al.,[34] included diclofenac in a priority list of relevant pharmaceutical drugs in the water cycle. Besides diclofenac, several other analgesics, namely 4-aminoantiyrine, aminophenazone, codeine, fenoprofen, ketoprofen, naproxen, and

propyphenazone, have also been detected in non-negligible concentrations in sewage and surface water samples.[4, 88]

Antibiotics detected in the environment may come from human medicine,[90] or from veterinary use and agriculture.[43, 44, 86] Several studies investigating the occurrence of antibiotics drugs in aquatic environment have been conducted. Erythromycin, sulfamethoxazole, and trimethoprim are the most frequently detected compounds.[88] Also, clarithromycin, dehydroerythromycin (metabolite of erythromycin), roxithromycin, lincomycin, sulfonamides (sulfadimethoxine, sulfamethazine, and sulfathiazole), and fluoroquinolones (ciprofloxacin, norfloxacin, and enrofloxacin) have been found up to μg.L^{-1} levels in the aquatic environment.[4, 91]

As mentioned before, estrone, 17α-estradiol, 17β-estradiol and estriol are the most frequently identified hormones, while 17α-ethynylestradiol is the top priority synthetic hormone.[36] These pharmaceutical drugs have been detected in influents and effluents of STPs in different countries, where concentrations of steroidal hormones ranging from 7.4 to 1267 ng.L^{-1} have been disclosed.[6, 92] They are also detected in river waters and aquifers.[92] Although steroidal hormones present low solubility in water, they may sediment into soils and reach surface water reservoirs in concentrations up to 28 ng.L^{-1}.[28]

In what concerns blood lipid regulators, clofibrate has been detected in water samples from STPs in the U.S., in concentrations up to 2379 ng.L^{-1}.[56] In Germany, concentrations of clofibrate up to 4 μg.L^{-1} were detected in groundwater samples collected from former sewage irrigation fields. Besides clofibrate, bezafibrate and gemfibrozil have also been detected up to the 190 and 340 ng.L^{-1} levels, respectively, in groundwater in Germany.[15] Clofibric acid has been detected with a low frequency in groundwater from a drinking water treatment plant in Berlin, Germany, in concentrations up to 270 ng.L^{-1}.[21]

According to Ternes,[27] the beta-blockers metoprolol and propranolol were detected in STP discharges and river streams in Germany, where metoprolol concentrations of 730 ng.L^{-1} in STP effluents, and 450 ng.L^{-1} in the river and

stream waters, have been found. Also, propranolol was detected in concentrations ranging from 35 to 107 ng.L^{-1} in WWTP and surface waters of the lower river Tyne, UK.[62]

Regarding neuroactive drugs, paroxetine, carbamazepine, fluoxetine and sertraline are the most frequently detected compounds in rivers, wastewater, streams, sediments, marine waters and groundwater at the levels of μg.L^{-1} to ng.L^{-1}.[87]

3. Environmental and Health Hazards of Active Pharmaceutical Ingredients

The continuous consumption of pharmaceutical drugs, even at low therapeutic concentrations, represents nowadays a threat to the environment and also to public health. Pharmaceutical drugs are designed to target specific metabolic and molecular pathways.[2, 60, 87] However, these compounds usually have important side effects, where different organs, tissues, cells or biomolecules, may be affected. Also because the different phylogenetic groups share some metabolic and molecular pathways, pharmaceutical grugs may also affect other species.[93]

Pharmaceutical toxicity experiments should be designed for specific targets on non-mammalian animals, including vertebrates, invertebrates, plants, and other taxonomic groups based on their modes of actions. Furthermore, the active pharmaceutical ingredients (APIs) toxicity effect on non-target organisms needs to be carried out, including the development of specific tests by registration of mortality rates and chronic effects.[5, 87, 94] The risk to aquatic organisms is generally calculated as the ratio between the predicted environmental concentration (PEC), and the predicted no effect concentration (PNEC), where the PEC in water can be estimated according to the EU draft guidance (CEC/III/5504/94, draft 6, version 4). According to Thompson et al.,[95] if the PEC exceeds or is similar to PNEC then further considerations on the physical and

chemical properties of the pharmaceutical, in particular the adsorption to organic matter and elimination to sewage sludge, should be considered.

As observed in Figure 7, the major classes of APIs that can be found in aquatic environments are: non-steroidal anti-inflammatory drugs (NSAIDs) and antibiotics. Despite the chemical diversity of compounds present in these two classes of APIs, it is important to summarize their common health hazards and to discriminate within each class their toxic potential.

3.1. Non-Steroidal Anti-Inflammatory Drugs

Non-steroidal anti-inflammatory drugs (NSAIDs) are generally detected in the environment in low concentrations, however there is a limited knowledge about the long-term effects of low concentrations of these drugs on living organisms.[96]

As an example of site contamination, the severe collapse of the population of a common vulture species in India and Pakistan (95 % decline since 1990) was directly linked with NSAIDs (diclofenac), which was systematically used for veterinary medication and its residues entered into vultures once they fed on dead domestic livestock.[97]

The toxic effects of diclofenac, acetaminophen and ibuprofen towards the cladoceran *Daphnia magna* displays remarkable time-dependent and concentration-dependent effects, with diclofenac presenting the highest toxicities according to Jones et al.[98] Other authors[99] evaluated the toxicity of diclofenac, ibuprofen, naproxen, and acetylsalicylic acid using *Daphnia magna* and also the planktonic chlorococcale green alga *Desmodesmus subspicatus*. Toxicities to these species were relatively low, with EC_{50} values in the range from 68 to 166 $mg.L^{-1}$ and from 72 to 626 $mg.L^{-1}$, for *D. magna* and *D. subspicatus*, respectively.

Several toxicity tests in fish have been carried out for NSAIDs. A 30-day toxicity test with ketoprofen was carried out on embryos and larvae of the common carp (*Cyprinus carpio*). The exposure to ketoprofen showed no effect on mortality,

but revealed significant delays in hatching and development.[100] Further, sub-lethal toxic effects of diclofenac in rainbow trout (*Oncorhynchus mykiss*) were evaluated, by exposing *O. mykiss* to diclofenac concentrations ranging from 1 μg.L^{-1} to 500 μg.L^{-1}, over a period of 28 day. The histopathological examinations of the exposed fish revealed alterations of the kidney and in the gills necrosis of pillar cells leading to the damage of the capillary wall within the secondary lamellae. All these symptoms were detected for a concentration of diclofenac of 5 μg.L^{-1}.[101] Corroborating these results, a set of biotests performed for diclofenac using the cladocera *Daphnia magna* showed toxicological effects at concentrations below 100 mg.L^{-1}, leading to the conclusion that diclofenac displayed the most acute toxic nature among NSAIDs.[102] The Japanese fish, *Oryzias latipes*, was exposed to different concentration of ibuprofen over six weeks, presented a sharp rise in liver weight, together with an enhanced egg production and a reduction in the number of weekly spawning events.[103] Regarding aquatic photosynthetic organisms, a five day exposure to concentrations of ibuprofen of 10 μg.L^{-1} disclosed a stimulation in the of the cyanobacterium *Synechocystis* sp, while for the duckweed plant *Lemna minor* the growth was inhibited.[104]

As a consequence of repeated application of sewage sludge (biosolids) and increased reclaimed wastewater usage for irrigation, human pharmaceuticals compounds are accumulated in soils where they have been detected in concentrations raging from μg.L^{-1} to mg.L^{-1}.[105, 106] Pharmaceuticals in soils can then be absorbed by plants. In this context, the effects of pharmaceutivcals in plants have been studied. Schmidt and Redshaw studied the effects of different NSAIDs on germination, development, growth and physiology of two crop plants, namely radish (*Raphanus sativus*) and lettuce (*Lactuca sativa*). The study revealed that the growth of edible crops can be affected by NSAIDs. In fact, compounds from the fenamic acid class were found to affect *R. sativus* root endpoints (root length and water content), while diclofenac, naproxen and ibuprofen affected early root development of the lettuce.[105]

3.2. Antibiotics

The enormous interest in antibiotics toxicity studies is associated to bacteria resistance mechanisms, which can compromise public health through a decrease in the efficacy of treatment.[60, 94] In fact, antibiotic resistance was estimated in 2014 to account for 700,000 deaths every year.[107] At low therapeutic concentrations, antibiotic may provide the development of bacterial resistance and further potential appearance of cross-resistance between different classes of antibiotics shared with humans.[98] Although the monitoring of the antibiotics presence in the environment has gained a great attention in the media in recent years, there are still no extensive regulations and/or requirements implemented for the environmental assessment of antibiotics.[108]

In general, antibiotics are classified as extremely toxic to microorganism (EC_{50} values above 0.1 $mg.L^{-1}$) and very toxic to algae (EC_{50} between 0.1 and 1 $mg.L^{-1}$).[98] Several antibiotics of diverse families, namely erythromycin, oxytetracyclin, sulfamethoxazole, ofloxacin, lincomycin and clarithromycin, were tested on aquatic organisms belonging to different trophic levels (bacteria, algae, rotifers, crustaceans and fish), and the results showed that the acute toxicity level was in the order of $mg.L^{-1}$, while chronic toxicity appeared at concentrations in the order of $\mu g.L^{-1}$, mainly for algae.[109]

The presence of antibiotics in STP influents can also damage bacteria-based treatment processes, causing toxic effects in the aquatic and/or terrestrial ecosystems at different trophic levels.[14] Bacterial cultures from sewage bioreactors, receiving water from a sewage treatment plant (STP), were tested for resistance against a large pool of antibiotics, such as ciprofloxacin, tetracycline, ampicillin, trimethoprim, erythromycin and trimethoprim/sulphamethoxazole. Results have shown that bacterial cultures are resistant to erythromycin and ampicillin.[110] The biodegradability and inhibition of cefotiam, ciprofloxacin, meropenem, penicillin G and sulfamethoxazole in wastewater bacteria, gram-negative bacteria *Pseudomonas putida*, was also assessed.[111] According to Al-

Ahmad et al.,[111] ciprofloxacin was the most toxic compound, with 50 % of growth inhibition at a concentration of 80 $\mu g.L^{-1}$. The antibacterial activity of the parent compounds and their oxidation mixtures was determined according to electrochemical oxidation mechanisms of ciprofloxacin, norfloxacin and ofloxacin. It was found that the oxidation products of ciprofloxacin and norfloxacin maintained the antibacterial properties of the gram-negative bacteria *Salmonella typhimurium*, although with lower activity when compared to their parent compounds.[112]

Furthermore, antibiotics that are released in waters and in soils may also have impacts on the local microbial community, althought the changes induced in the community composition and activity are still not completely understood.[113]

Toxicity tests were performed on five aquatic organisms, with seven fluoroquinolone antibiotics, namely ciprofloxacin, lomefloxacin, ofloxacin, levofloxacin, clinafloxacin, enrofloxacin, and flumequine, where overall toxicity values ranged from 7.9 to 23000 $\mu g.L^{-1}$.[114] According to Robinson et al.,[114] the cyanobacterium *Microcystis aeruginosa* was the most sensitive organism (EC_{50} values ranging from 7.9 to 1960 $\mu g.L^{-1}$), followed by duckweed *Lemna minor* (EC_{50} values ranging from 53 to 2470 $\mu g.L^{-1}$) and the green alga *Pseudokirchneriella subcapitata* (EC_{50} values ranging from 1100 to 22700 $\mu g.L^{-1}$). Toxicity tests with fish and ciprofloxacin were also performed. The effects of ciprofloxacin in fertilized eggs of the common carp (*Cyprinus carpio*) were evaluated after exposure to concentrations between 1 and 3000 $\mu g.L^{-1}$.[115] Accelerated hatching was found in all groups exposed to ciprofloxacin, but significant growth reduction was only observed at the highest concentration (3000 $\mu g.L^{-1}$). An increased number of macroscopic morphological anomalies were observed on day 6 of the test (after hatching). Also, the effects of subchronic exposure of zebrafish (*Danio rerio*) to norfloxacin were evaluated, where an increase in the activity of glutathione peroxidase, glutathione S-transferase, and catalase was found, having a negative impact on specific biochemical processes connected with the production of reactive oxygen species in fish.[116]

3.3. Mixture Effects

Generally, the risk evaluation is based on single compounds or families. However, contaminants such as pharmaceutical drugs do not occur isolated in the environment, they are present as multi-component mixtures. Although still scarce, toxicological data have shown that mixtures hold different unexpected toxic effects.[2, 60, 117] According to Richards et al.,[118] outdoor aquatic microenvironment exposed to a mixture of ibuprofen, fluoxetine and ciprofloxacin over 35 days showed that sunfish (*Lepomis gibbosus*) and aquatic plants (*Lemna gibba* and *Myriophyllum spp.*) died after 4 days of exposure for higher mixture concentrations (600, 1000, and 1000 $mg.L^{-1}$ for ibuprofen, fluoxetine and ciprofloxacin, respectively). Further, Cleuvers[102] demonstrated that a mixture of diclofenac and ibuprofen presents a stronger toxicity than predicted in *D. magna*. When more than two non-steroidal anti-inflammatory drugs (NSAIDs) (naproxen and acetylsalicylic acid) were used, a considerable toxicity on *Daphnia* was reported, even at concentrations at which the single NSAID do not exhibit any effects. This addictive toxicity was also demonstrated for marine phytoplankton species *Dunaliella tertiolecta* when exposused to mixtures of NSAIDs and pharmaceuticals and personal care products (PPCPs).[117] Also, Stancova et al.,[119] observed that a 35-day-long exposure of tench (*Tinca tinca*) to a mixture of ibuprofen, diclofenac, and carbamazepine at concentration of 60 $\mu g.L^{-1}$, leads to an increase in mortality, as well as to significant increase in growth and elevated incidence of malformations. On the other hand, mixture effects of clofibric acid and diclofenac towards the estuarine shrimp *Palaemon longirostris*, revelead a similar effect of the mixture as compared with clofibric acid in isolation.[120]

It should be stressed however, that all the toxicity responses were observed at concentrations well below the equivalent pharmacologically active concentrations in mammals; and thus, a long term exposure could enhance adverse changes in mammals, leading to irreversible effects.

4. Pharmaceutical Wastewater Treatment Options

The overall demand for quality water has been continuously increasing, whether it is for drinking, sanitation, irrigation, or industrial use. There are different categories of water, such as potable water, process water, feedwater for utilities, water recycling, wastewater, water coming from byproduct treatment, water used for odor treatment, water from desalination, and water for irrigation, that have different compositions and thus need different treatments. Therefore, water treatment and reuse, within the strictest standards, has been a raising concern in recent years.[121] According to Figure 8, there is a marked difference between the countries in the northern hemisphere with those from the south in which the treatment is scarse. On a global scale, more than 90 % of the water used suffers no treatment prior to discharge.[121] Furthermore, it is estimated that around half of the pharmaceutical wastewater produced worldwide is discarded without treatment.[91, 121, 122] In the case of sewage treatment plants (STPs), the studies on the elimination rates are primarily based on measurements of influents and effluents concentrations of pharmaceutical drugs, varying according to the season and performance, hydraulic retention time and construction and technology of the STP. Since pharmaceuticals form a heterogeneous group of compounds, a wide range of elimination efficiencies (0 to 99) % has been observed.[27, 87]

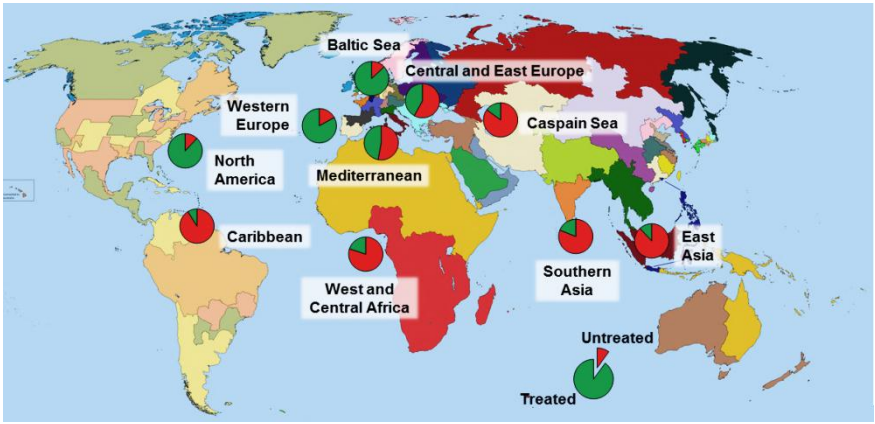

Figure 8. Ratio of treated and untreated wastewater reaching water bodies from 10 regions across the globe (Adapted from Gadipelly et al.[121]).

Fate and behavior of pharmaceutical drugs and their metabolites in the aquatic environment is still not fully known. However, the available data discloses that the distribution of active pharmaceutical ingredients (APIs) in the environment occurs primarily through aqueous transport, due to the low volatility of pharmaceuticals.[55, 87] Generally, conventional wastewater treatment plants (WWTPs) are designed to remove solid wastes, suspended solids, easily biodegradable dissolved organic matter and nutrients (phosphorus and nitrogen) from wastewater (Figure 9).[123] In general, the simple function of wastewater treatment is to speed up the natural processes, where water is purified.

There are two basic stages in the treatment of water. In the primary stage, large floating objects, such as rags and sticks that might clog pipes or damage equipment, are removed from wastewater and the small particles are removed from sewage in a sedimentation tank, followed by a subsequent treatment for use as a fertilizer, or disposed off in a land fill or incinerated. The secondary stage of treatment removes about 85 % of the organic matter in sewage, where biological treatments to improve the purification of wastewater may be used.[124]

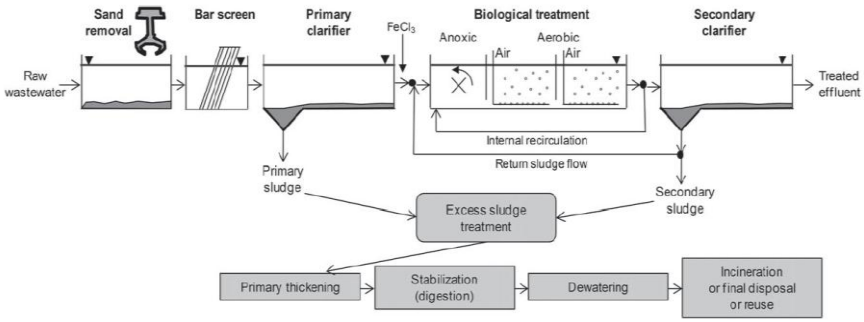

Figure 9. Scheme of a conventional WWTP with three treatment stages: 1st primary clarifier, 2nd biological treatment, and 3th secondary clarifier.[123]

Solid wastes, suspended solids, easily biodegradable dissolved organic matter and nutrients are mainly eliminated in WWTPs through two process, namely adsorption and biodegradation.[123] However, since these plants are not designed to treat pollutants such as pharmaceutical compounds, which are insufficiently affected by the physical, chemical, and biological processes implemented in WWTPs, they are continuously being accumulated in the water cycle.[55, 87, 125] According to Figure 9, the main mechanisms for solid wastes, suspended solids, easily biodegradable dissolved organic matter, nutrients and pharmaceutical drugs are adsorption onto particulate matter (primary and secondary clarifier), biological transformation (biological treatment), and abiotic degradation. Adsorption and volatilization consist on the transference of certain compounds from one compartment (water) to another (solid or gas), whereas biological transformation and abiotic degradation lead to the transformation of the APIs in different compounds.[123]

Regarding adsorption, hydrophobic and electrostatic interactions of pharmaceutical with particulates and microorganisms are crucial.[55, 87, 126] In this sense, experimental variables such as pH play an important role in the adsorption performance. For instance, acidic pharmaceutical compounds, such as non-steroidal anti-inflammatory drugs (NSAIDs), with pK_a values that range from 4.9 to 4.1, as well as blood lipid regulators, such as clofibric acid and bezafibrate (pK_a =

3.6)[36], are usually present in water streams in a negatively charged form. If the adsorption sludge presents a neutral pH, the amount adsorbed of these APIs is not significant.[55, 126] In other words, adsorption is an inefficient process for the elimination of pharmaceuticals from wastewater and surface water.[87]

Biodegradation is suggested to present the highest efficiency among the elimination processes in wastewater treatment. It can occur in aerobic or anaerobic media, in activated sludge treatment, or anaerobically in sewage sludge digestion. The best results are generally obtained when hydraulic retention time and aged activated sludge treatment is applied.[87, 123] Once pharmaceutical related compounds reach surface water, photodegradation can play an important role for their degradation. However, hydrolysis is not effective in the degradation of pharmaceutical drugs at the water surface level,[87] since it depends on the strength of the solar irradiation and on the constituents present in water that can act as photosensitizers.[127]

In general, pharmaceutical water treatment processes can be generally divided into the following three categories and subcategories (Figure 10): (1) Biological Treatment Processes: (a) Aerobic and (b) Anaerobic Treatment; (2) Advanced Processes: (a) Membrane Processes, (b) Adsorption, and (c) Filtration; and (3) Advanced Oxidation Processes: (a) Chlorination; (b) Ozonation, (c) Perozonation, (d) Fenton Reactions, and (e) Photocatalysis.[121]

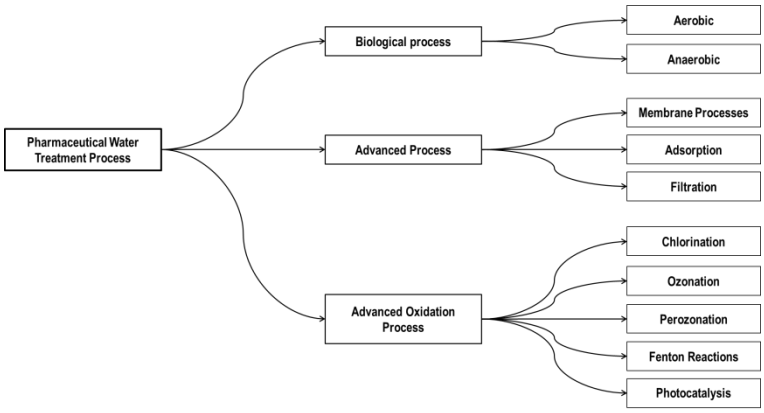

Figure 10. Pharmaceutical water treatment processes schematic.

Biological treatment methods may be subdivided into aerobic and anaerobic processes. Aerobic applications include activated sludge and anaerobic methods include up-flow anaerobic stage reactors.[128, 129] Advanced treatment processes of pharmaceutical wastewater are considered as primary treatment or pretreatment processes to accelerate the removal of contaminants. The efficiency of these methods for the treatment of pharmaceutical wastewater varies significantly, where membrane processes, adsorption and filtration with activated carbon are included.[121]

Advanced oxidation reactions have mainly been used to supplement instead of replacing conventional systems, to enhance the treatment of water containing pharmaceutical drugs.[130] A chemical agent source (hydrogen peroxide, ozone, transition metals and metal oxides) and an energy source (ultraviolet-visible radiation, electric current, gamma-radiation and ultrasound) are required for advanced oxidation process.[131] These reactions produce free radicals, in particular hydroxyl radicals, that enable the conversion of contaminates to less harmful and more biodegradable compounds. However, degradation compounds need to be identified and monitored, since they can be equally or more toxic than the parent compounds.[132] Advanced oxidation process includes chlorination, ozonation, perozonation, fenton reactions, and photocatalysis.[121] Despite improved efficiencies these methods generally require high energy cost and therefore are still not widely used in WWTPs.

4.1. Biological Treatment Processes

4.1.1. Activated Sludge

The characteristics of wastewater play an important role in the selection of biological treatments. Active pharmaceutical ingredients (APIs) and metabolites are biologically recalcitrant substances.[128, 131] Conventional activated sludge (a common aerobic treatment) with a long hydraulic retention time has been the

election method for pharmaceutical industry wastewater treatments.[133] This wastewater treatment method has the advantage of presenting a lower capital cost when compared to more advanced techniques. However, high energy consumption, large amounts of sludge production and operation problems, including foaming and bulking in secondary clarifies, are disadvantages that need to be overcame or minimized.[131, 133] Also, other factors that may interfere in the efficient removal of APIs are the hydraulic retention time, temperature, pH, microbial community, and presence of toxic or recalcitrant substances.[128] It has been showed that the key factor in the efficiency of activated sludge facilities is the operation temperature, since it directly influences individual microbial species selection.[128, 134] Although activated sludge is a relatively efficient method for APIs removal, some of the pharmaceutical drugs in the aquatic environment are not removed by this method.[26] For instance, while *β*-lactam antibiotics are susceptible to aerobic oxidation, presenting a high biodegradability due to hydrolitic cleavage of the β-lactam ring, lincomycin and sulphonamides do not show significant degradation.[135] Furthermore, ibuprofen, naproxen, bezafibrate and estrogens showed a high degree of degradation using activated sludge, while for sulfamethoxazole, carbamezapine and diclofenac this technique showed limited efficiency.[135] Nevertheless, recent improvements namely in the selection of the involved bacteria and the bactch conditions (i.e. temperature) can promote the degradation of some of these NSAIDs, including diclofenac.[136, 137]

4.1.2. Up-flow Anaerobic Batch Reactor

The advantages of anaerobic treatments over aerobic processes are the capability to deal with higher wastewater volume, lower energy inputs, sludge yield, small nutrient requirements, lower operating costs, lower space requirement and improved biogas recovery. However, because an extensive range of pharmaceutical drugs in wastewaters are recalcitrant and non-biodegradable to the microbial mass within the conventional treatment system, anaerobic treatment is not constantly effective in removing these contaminants.[121, 131] So, up-flow

anaerobic stage reactors (UASRs) are used as a pre-treatment to activated sludge in industrial effluent, presenting good efficiency for pharmaceuticals removal, even at high concentrations.[121, 129, 138] According to Chelliapan et al.[138], UASRs fed with wastewater containing antibiotics showed 70-75 % of chemical oxygen demand and an average of 95 % of drug reduction, where a hydraulic retention time of 4 days was applied.

4.2. Advanced Processes

4.2.1. Membrane Process

Membrane processes, such as microfiltration, ultrafiltration, nanofiltration and reverse osmosis, are commonly used for the removal of active pharmaceutical ingredients (APIs).[139] Although microfiltration and ultrafiltration are not fully effective in removing pharmaceutical drugs, mainly due to the pore sizes, nanofiltration and reverse osmosis have been the focus of attention for the treatment of drinking water.[139, 140] According to Snyder et al.[139] and Watkinson et al.,[140] reverse osmosis is efficient in the removal of thirty six active pharmaceutical ingredients, namely, antibiotics, lipid regulators, hormones and oral contraceptives, antiepileptics and analgesics. Furthermore, Secondes et al.[141] investigated the removal efficiency of diclofenac, carbamazepine, and amoxicillin using a novel hybrid process that applies simultaneously membrane ultrafiltration, activated carbon adsorption, and ultrasound irradiation, achieving high extraction efficiencies percentages (> 99 %) for all the pharmaceuticals reported.

4.2.2. Adsorption and Filtration

Adsorption using activated carbon is considered a conventional technology in wastewater treatment. It is applied as a powdered feed or in packet filter granulated form.[142] Snyder et al.[139] demonstrated that powdered activated carbon and granular activated carbon could remove 90 % of estrogens, although

the dissolved organic compounds, surfactants and acids can block the pores in the activated carbons structure. Also, when powdered activated carbon was used at a pilot scale, the removal of 19 of the existing 26 active pharmaceutical ingredients was achieved, with an efficiency rate above 90 %. However, the main drawback in the use of activated carbon treatment lies in the separation of the carbon from water, which is usually done by precipitation or by filtration, which requires additional energy. The use of activated carbon is also only efficient for the treatment of pre-treated wastewaters with a low load of organic compounds.[131]

4.3. Advanced Oxidation Processes

4.3.1. Chlorination

Chlorination is the most popular disinfectant method used either in the form of gaseous chlorine or as sodium hypochlorite (liquid bleach). The action time dependents on the concentration of chlorine applied, type of pathogenic agents present, pH and water temperature. The oxidation results from successive reactions which firstly induce chloramine, where an initial mechanism of electrophilic attack of HOCl on the chloramine nitrogen is observed. So, in NH_3 presence during chlorination, the prediction of chloramine formation and decomposition is important to optimize disinfection and minimize undesirable by-product formation.[143, 144] Quintana et al.[145] reported that diclofenac reacts with chlorine, forming chloro-diclofenac, bromo-diclofenac, decarboxy-diclofenac, and chloro-decarboxy diclofenac as the major products. Also, Simazaki et al.[146] demonstrated that non-steroidal anti-inflammatory drugs (NSAIDs) are decomposed by the addition of sodium hypochlorite with a reaction time of 24 h. These authors also showed that compounds such as naproxen and diclofenac have a percentage of removal over 70 %, while that for ibuprofen and ketoprofen degradation is less than 20 %. Furthermore, chlorination has been shown to be effective in the removal of hormonal pharmaceutical drugs, namely 17α-

ethinylestradiol and 17 β-estradiol.[147] Also, it was found that chlorine dioxide is effective in the removal of sulfamethoxazole, roxithromycin, 17 α-ethinylestradiol and diclofenac.[148]

4.3.2. Ozonation

Due to its strong sterilization and disinfection properties, ozone has been used in the treatment of pharmaceutical wastewaters. The mode of action is through the formation of OH^- radicals, since ozone molecules present large oxidation capacities, where compounds with a C=C bond or aromatic structures seem to be susceptible to ozonation, while compounds with amide structures are resistant to it.[73, 149] Ozonation has been an important principal wastewater treatment, enhancing the biodegradability and efficiency of the overall treatment.[150] Ozonation displays a good performance for the removal of antibiotics (> 90 %).[73, 130] However, removal efficiencies below 50 % were observed for lipid regulators and β-blockers.[73] The removal degree and mineralization of APIs in pure water or synthetic industrial effluent lead to reliable results, however its use "real" pharmaceutical wastewater is still not widespread.[150] This is due to the fact that ozone production is an energy intensive process, making it costly to implement. Ozonation may increase the energy demand of the conventional wastewater treatment plant by 40-50 %. Also, an additional treatment, such as sand filtration, is required after ozonation to break down the reactive oxidation products.[151] Recently, ozonation coupled with activated carbon (AC) demonstrated to be an efficient method to remove metoprolol and fluoxetine at different pHs.[152]

4.3.3. Perozonation

Perozonation is a combination between hydrogen peroxide and ozone, and it has been successfully used in the degradation of the penicillin formulation effluent.[130, 150] It is based on the fact that the conjugate base of H_2O_2, at low concentrations, increases the rate of decomposition of O_3 into hydroxyl radicals.[130]

Cokgor et al.[153] investigated the pretreatment of the synthetic penicillin effluent (procaine penicillin G) with the O_3/H_2O_2 process, and the effects of the resulting chemicals in terms of acute toxicity and biodegradability. The results demonstrated a 70 % of chemical oxygen demand removal and a 50 % decrease in the acute toxicity for *Daphnia magna*. Moreover, the combination of UV, O_3, and H_2O_2 treatments was applied to a municipal wastewater treatment plant in a German Municipal wastewater treatment plant, containing seventeen APIs (antibiotics, β-blockers, antiepileptics, antiphlogistics and lipid lowering agents). The removal of all APIs, followed by 18 min of contact time with an ozone dose of 10-15 $mg.L^{-1}$ was achievedwith the exception of diatrizoate, iopamidol, iopromide and iomeprol which showed removal efficiencies lower than 14 %.[73]

4.3.4. Fenton Reactions

Fenton reactions involve hydrogen peroxide in the presence of iron to generate hydroxyl radicals, where ultraviolet light enhances the reaction by photo reduction of Fe (III) to Fe (II). Since iron is abundant and non-toxic, Fenton reactions are a viable option for wastewater treatment. Photo-Fenton reactions have been used for the degradation of diclofenac.[74, 154] According to Ravina et al.,[74] the complete mineralization of diclofenac and its intermediates via photo-Fenton reactions is observed. Also, a relevant feature of Fenton reactions is the possibility of using sunlight in the mineralization reaction.[154] On the other hand, the strong pH dependence (pH optimum of 2-4 for the production of $OH^{\bullet}$), concentrations of hydrogen peroxide and Fe^{3+}/Fe^{2+} ions, and the disposal of the iron sludge, are limiting factors that need to be taken into consideration.[155]

4.3.5. Photocatalysis

Photocatalysis is the acceleration of a photochemical transformation by the action of a catalyst, like TiO_2 or Fenton's reagent,[131] where the catalyst most commonly employed for pharmaceutical photocatalysis is rutile TiO_2.[121] The

removal of APIs, such as antibiotics, lipid regulators, antiepileptics and antiphlogistics, using TiO_2 has been achieved.[156, 157] Some authors reported divergent removal efficiencies when used in combination with UV, ranging from 98 % for antibiotics[158] to 10 % for carbamazepine.[156] However, higher removal efficiencies for carbamazepine can be obtained if nano composites catalysts are used.[159] The main obstacle to the implementation of photocatalysis processes on a large scale is the number of operating parameters that need to be target-optimized, namely the type and geometry of reactor, the photocatalyst, and optimum energy use and wavelength of radiation.[131]

4.4. New Technologies in Pharmaceutical Wastewater Treatment

The continuous search on novel, efficient and environmentally friendly technological processes for wastewater treatment is mainly due to the fact that the conventional technologies are not completely efficient in the removal of active pharmaceutical ingredients (APIs). Nowadays, new technologies for the complete eradication of APIs from aquatic environment are being proposed and tested. Most of these new technologies combine conventional techniques and smart tailored-made materials, which impart higher performances to the traditionally used processes. For instance, Pan et al.[160] designed a novel photocatalyst, TiO_2-doped low-silica X zeolite (TiO_2-LSX) to study the degradation of the 17-ethinylestradiol , where a higher compound removal efficiency was observed with UV-TiO_2-LSX when compared to UV-TiO_2 or UV alone. According to the authors, this novel TiO2-doped zeolite system provides a promising application for the UV disinfection process in wastewater treatment plants. Shan et al.[161] developed a simple and convenient method to prepare granular carbon nanotubes (CNTs) to enhance the adsorption of pharmaceuticals. The proposed granular adsorbent exhibited high surface area and pore volume, making CNTs more dispersible in the formation of the granular cake. CNTs presented higher surface area available

for adsorption and were more easily separated from solution. Furthermore, this adsorbent exhibited a relatively fast adsorption capacity for carbamazepine, tetracycline, and diclofenac sodium salt, with a maximum adsorption capacity of 369.5, 284.2, and 203.1 μmol.g^{-1}, respectively.

Secondes et al.[141] investigated the efficiency of a hybrid process, combining membrane ultrafiltration, activated carbon adsorption, and ultrasound irradiation to remove diclofenac, carbamazepine, and amoxicillin from water. Alturki et al.[162] highlighted the potential and challenges in the development of a novel osmotic membrane bioreactor (OMBR) process for the treatment of 50 organic compounds, namely pharmaceutically active compounds, pesticides, steroid hormones, and other endocrine disrupting chemicals, from municipal wastewater. According to the authors, the removal of 25 out of 27 trace organic compounds, with molecular weight higher than 266 g.mol^{-1}, was above 80 %, where physical separation of the forward osmosis membrane and biodegradation played the major rule. However, the removal efficiency of the remaining 23 trace organic compounds, with molecular weight lower than 266 g.mol^{-1}, was low. Such results, highlight the importance of coupling traditional technologies with emerging technologies and materials in order to achive higher efficiencies in the removal of APIs at lower costs.

5. Ionic Liquids-Based Extraction Technologies

5.1. Ionic Liquids

Ionic liquids (ILs), known as salts with a melting temperature below a conventional temperature of 100 ºC,[163] have been largely explored for a wide variety of applications and are at last reaching their place in industry.[164] ILs are typically composed of an organic cation and an organic or inorganic anion. The ionic nature and the large array of cation-anion combinations of ILs are the main characteristics responsible for some of their outstanding properties, namely a

negligible vapor pressure, a high ionic conductivity, non-flammability, high thermal and chemical stabilities and enhanced solvation ability for a large number of compounds. However, the characteristic that makes ILs unique is the capacity to tune their properties, through the clever manipulation of their chemical structure, so that a specific objective can be accomplished with high efficiency - task specific fluids.[164-168]

In Figure 11, the chemical structures of common IL cations and anions are shown.

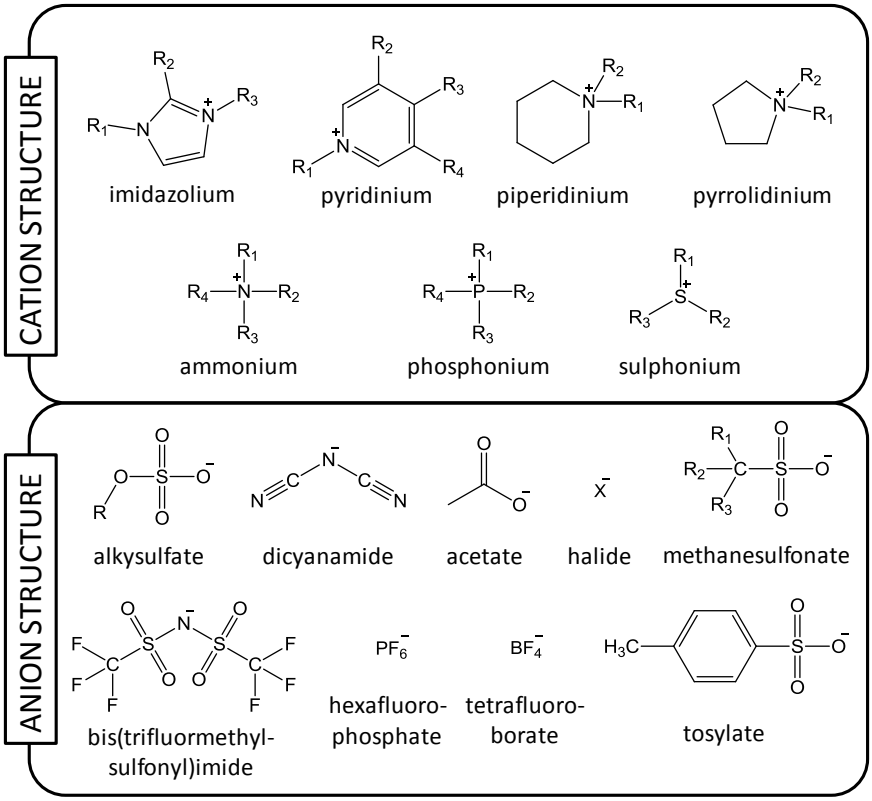

Figure 11. Examples of the chemical structures of cations and anions present in common ionic liquids.

In addition to a large number of applications, ILs have been widely used in the extraction of APIs, through liquid-liquid extraction (LLE), in particular

aqueous biphasic systems (ABS),[169] and by solid-phase extraction (SPE) processes, in particular supported ionic liquid phase (SILP).[170]

5.2. Aqueous Biphasic Systems

Aqueous biphasic systems (ABS) fit within the liquid-liquid extraction techniques, and are formed by the addition of two water-soluble phase-forming components (two polymers, a polymer and a salt or two salts) which undergo phase separation above given concentrations.[171, 172] The partition and/or extraction of target compounds occurs between the coexisting aqueous-rich phases, while the chemical nature and physical properties of both the phase-forming components and solute play a major role. Even so, the limited polarity differences between the two phases and the restricted type of interactions between the solute and the phase forming components, aiming the maximization of the extraction efficiency and selectivity, are the major drawbacks of conventional polymer-salt and polymer-polymer-based ABS. To overcome this constraint, in the past few years, both the functionalization of polymers and the addition of ligands have been investigated.[173]

In 2003, Rogers and co-workers[174] showed that the addition of a "kosmotropic" salt to an aqueous solution of a given IL (hydrophilic IL, water-soluble) leads to phase separation. After this proof of concept, in the following years a new plethora of extraction/separation routes was created through the combined use of ILs and a large number of salts, amino acids, carbohydrates and polymers.[169] The major advantages of IL-based ABS versus conventional polymer-based ABS are the tailoring ability of the phases' polarities and affinities by an adequate choice of the ILs ions.[168, 175-177] As a result, a superior performance of IL-based ABS was perceived.[169, 178-185] Although hydrophobic ILs can be used to extract or separate pharmaceutical drugs from water, since they are immiscible with water at the temperatures of interest, they display some

drawbacks.[186] Usually, hydrophobic ILs display a lower performance to extract pharmaceutical compounds.[169]

Domínguez-Pérez *et al.*[182] used ABS composed of the ionic liquid 1-butyl-3-methylimidazolium triflate ($[C_4C_1im][CF_3SO_3]$) and lysine for the extraction of ciprofloxacin and ciprofloxacin.HCl from aqueous media, with extraction efficiencies of around 85 %. In the same line, Shahriari *et al.*[183] proposed the use of ABS composed of cholinium-based ILs for the extraction of tetracycline and ciprofloxacin from aqueous media. Also, the extraction of macrolide antibiotics was investigated using ABS formed by 1-butyl-3-methylimidazolium tetrafluoroborate ($[C_4C_1im][BF_4]$) and diverse inorganic salts, where extraction efficiencies ranging from 91.8 to 96.2 % and 89.6 to 92.2 % were obtained for azithromycin and mydecamycin, respectively.[184] Further, Han *et al.*[178] used $[C_4C_1im][BF_4]$ and an organic salt aiming an easier identification and quantification of chloroamphenicol in water, milk and honey samples, where the recovery of the antibiotics ranged between 90.4 and 102.7 %. ABS composed of tetrabutylammonium chloride ($[N_{4444}]Cl$) and citrate buffer were further used in a process to valorize pharmaceutical wastes through the extraction and recovery of pharmaceutically active compounds, namely ibuprofen.[181] According to the authors, extraction efficiency up to 98 % was obtained in a single-step. In addition to antibiotics, the extraction of paracetamol using ABS formed by six quaternary ammonium-based IL and three different salts (potassium citrate, potassium carbonate and potassium phosphate) was attempted, where extraction efficiencies ranging from 80 to 100 % have been obtained.[179] Zawadzki et al.[180] developed a sustainable process for the extraction and recovery of amitriptyline hydrochloride from pharmaceutical wastes using ABS formed by ILs, where extraction efficiencies between 93 and 100 % were obtained. The synthetic hormone ethinylestradiol was also extracted and concentrated by using ABS formed by several hydrophilic ILs and $KNaC_4H_4O_6$, with extraction efficiencies of ethinylestradiol for the IL-rich phase ranging between 92 and 100 %.

In general, ABS is a good technique to be employed in the removal of pharmaceutical drugs from aquatic environment due to the high and tailored extraction efficiency, quick phase separation and low viscosity. However, further research is neded for a better understanding and employment of ABS in water treatment plants. According to Daugthon and Ternes,[5] and Kümmerer,[3] pharmaceutical compounds are not totally degraded in STPs and WWTPs, which results in the in the contamination of rivers, lakes, groundwater and drinking water by the discharged treated effluents. Therefore, aqueous biphasic systems as a liquid-liquid extraction technique can be inserted at the final of the secondary stage, the 3rd stage of a wastewater treatment plant (WWTP) (Figure 9) to remove the remaining pharmaceutical compounds after biological treatment, advanced and advanced oxidation processes.

5.3. Supported Ionic Liquid Phases

In the past years, it has been proved that ILs can be immobilized onto silica and polymer supports, although mainly used for the separation of natural compounds from biomass extracts. These supported ionic liquid phases (SILPs) share some of the advantages of neat ILs: non-volatility, non-flammability and high tailoring ability by the modification of materials with the most diverse ILs. In SILs, the cation is usually covalently attached to the support, and thus the same range of interactions are expected to occur between the IL and the adsorbate,[170, 187] therefore allowing the tuning of these materials separation or removal performance.

In 2005, Jiang and co-workers were pioneers in the use of SILPs as an adsorption technique, where a solid-phase microextraction (SPME) with a disposable ionic liquid coating was developed for headspace extraction of benzene, toluene, ethylbenzene, and xylenes in paints.[188] Since then, extraction, separation and pretreatment processes were explored, through the combined use

of ILs and a number of supported materials, being silica and polymer the most used ones.[170, 189-193]

Regarding the SILPs use for APIs removal from aqueous samples, Fontanals et al.[192] reported the use of a crosslinked polymer-supported imidazolium trifluoroacetate salt (IL-CF_3COO^-) for the removal of salicylic acid, 4-nitrophenol, carbamazepine, nalidixic acid, flumequine, naproxen, fenoprofen, diclofenac sodium, ibuprofen and gemfibrozil from different aqueous samples (ultrapure, tap and river). The favorable combination of the properties of ILs and the advantages of a solid support enabled the complete extraction of the studied acidic compounds. Furthermore, two imidazolium supported ionic liquid phases (SILPs) containing different anions, namely trifluoromethanesulphonate ($[CF_3SO_3]^-$), and tetrafluoroborate ($[BF_4]^-$), were evaluated as sewage treatment plant (SPE) sorbents for extracting salicylic acid, carbamazepine, antipyrine, trimethoprim, metoprolol, naproxen, fenoprofen, diclofenac, ibuprofen, and gemfibrozil from aqueous samples under strong anion-exchange conditions, which results in an complete and effective cleanup of the sample.[193] Also, Vidal et al.[189] functionalized silica materials with imidazolium, *N*-methylimidazolium and 1-alkyl-3-(propyl-3-sulfonate) imidazolium and applied as SPE for organic acids, amines and aldehydes, where extraction efficiencies for organic acids ranged from 87 to 100 % and 0 to 37 % for amines and aldehydes.

Although excellent extraction results have been achieved using SILPs, no comprehensive review dedicated to SILPs as adsorption has yet been published,[170] with the exception of the work by Vidal et al.[191] who reviewed SILPs together with other stationary phases for separation purposes. According to Figure 9, SILPs could be placed also at the final of the secondary stage, the 3rd stage of a WWTP, in combination or without the ABS.

6. Objectives

The main objective of this work is to develop new technologies based on a novel class of ubiquitous compounds – ionic liquids (ILs) - in order to efficiently remove non-steroidal anti-inflammatory drugs (NSAIDs) and fluoroquinolones (FQs), which belong to the top ten priority active pharmaceutical ingredients list encountered in water cycles, and that may have significant impact on environmental and human health. The development of a advanced technologies to preserve the environment using new engineered solvents, as well as the formation of qualified human resources in frontier areas of knowledge are the general goals of this proposal.

The work presented in this thesis explores de development of two different technologies, currently used in wastewater treatment plants (WWTPs), namely liquid-liquid extraction (LLE) and solid-phase extraction (SPE). These technologies will be re-formulated aiming at the efficient removal of APIs from aqueous streams through the use of ILs. Specifically, the tailoring ability of ILs, *i.e.* the ILs chemical versatility, will be used to attain specific properties so that the complete extraction of the proposed APIs is attained. To guarantee the sustainability of proposed technologies and that no hazardous impact towards the environment is observed, the recycling of the ILs and related materials was investigated.

7. Thesis outline

The research developed during the time of my PhD project is displayed in a article-based thesis format, constituted by seven chapters. The present chapter will introduce the main topics discussed and the thesis objective. The following chapters (2 to 6) are based on published (or submitted) scientific articles and aim to give the reader a perspective of the evolution of the two fields studied in this thesis, starting with the extraction of active pharmaceutical drugs using aqueous

biphasic systems, and ending with the usage of a silica column for active pharmaceutical drugs removal. It should be however remarked that the chapters are not shown by their chronological order of publication/submission. Additionally, lists of figures, tables and abbreviations or symbols are not presented in this thesis, since the above-mentioned contents are carefully identified in each individual article-based chapter.

In Chapter 2 the development of pre-treatement techniques for aqueous samples containing non-steroidal anti-inflammatory drugs and fluoroquinolones is the main focus. IL-based ABS composed of imidazolium-, piperidinium-, pyrrolidinium- and phosphonium-based IL and a citrate-based salt ($C_6H_5K_3O_7$) were evaluated for the single-step extraction and concentration of three NSAIDs, namely naproxen, diclofenac and ketoprofen, and three FQs, namely ciprofloxacin, enrofloxacin and norfloxacin.

After acquiring some understanding on the extraction of NSAIDs and FQs using IL-based ABS, ABS containing ILs and $Al_2(SO_4)_3$ were evaluated. In the next two chapters, Chapters 3 and 4, the extraction capacity of ABS composed of different ILs based on imidazolium and phosphonium cations was evaluated for four NSAIDs (naproxen, diclofenac, ibuprofen and ketoprofen) and six FQs (ciprofloxacin, enrofloxacin, moxifloxacin, norfloxacin, ofloxacin and sarafloxacin). Taking into account the goal to introduce the developed ABS-based technology in the final stage of a wastewater treatment plant (WWTP), $Al_2(SO_4)_3$ was used as a salting-out agent, since this inorganic salt is already used as a flocculating agent in the purification of drinking water.[194] It is well-known and clearly demonstrated in Chapters 2, 3 and 4 that the use of ILs as phase-forming components of ABS leads to outstanding extraction performances compared to more traditional routes. The IL regeneration, recycling and reuse also were accomplished warranting the development of more sustainable processes.

Chapter 5 focus on the development of silica supported ionic liquids materials and their use for the adsorption/removal of diclofenac. Silica supported ionic liquid phase materials were synthetized by crafting 1-methyl-3-

propylimidazolium into the silica surface. Six new materials were prepared by ion exchange and properly characterized. Adsorption isotherm and kinetic studies were conducted with diclofenac. In Chapter 6, the most efficient SILP was investigated to remove three NSAIDs, namely naproxen, diclofenac and ketoprofen. Kinetic and isotherm studies were performed for the three NSAIDs. Furthermore, a continuous removal method was developed by applying a packed column with the IL-based material. The recovery of NSAIDs and the SILP reusability were finally addressed.

Finally, in Chapter 7, the primary results presented in the previously described chapters are summarized. Possible future work is also highlighted.

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Chapter 2

Improved Monitoring of Aqueous Samples by the Preconcentration of Active Pharmaceutical Ingredients using Ionic-Liquid-based Systems

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The author was involved in all the experiments, as well as on the discussion and interpretation of the data and preparation of the manuscript.

1. Abstract

Fluoroquinolones (FQs) and Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are two classes of Active Pharmaceutical Ingredients (APIs), widespreadly used in human healthcare and as veterinary drugs, and that have been found throughout the water cycle in the past years. These two classes of APIs are commonly present in aqueous streams in concentrations ranging from ng.L^{-1} to μg.L^{-1}. Despite such low concentrations, these contaminants tend to bioaccumulate, leading to serious environmental and health issues after chronic exposure. The low concentrations of FQs and NSAIDs in aqueous media also render difficult identification and quantification, wich may result in an unefficient evaluation of their environmental impact and persistence. Therefore, the development of alternative pre-treatment techniques for their extraction and preconcentration from aqueous samples is a crucial requirement. In this work, liquid-liquid systems, namely ionic-liquid-based aqueous biphasic systems (IL-based ABS), were tested as simultaneous extraction and preconcentration platforms of FQs and NSAIDs. ABS composed of imidazolium-, ammonium- and phosphonium-based ILs and a citrate-based salt ($C_6H_5K_3O_7$) were evaluated for the single-step extraction and preconcentration of three FQs (ciprofloxacin, enrofloxacin and norfloxacin) and three NSAIDs (diclofenac, naproxen and ketoprofen) from aqueous samples. Outstanding one-step extraction efficiencies of APIs close to 100 % were obtained. Furthermore, preconcentration factors of both FQs and NSAIDs were optimized by an appropriate manipulation of the phase-forming components compositions to tailor the volumes of the coexisting phases. Preconcentration factors of 1000-fold of both FQS and NSAIDs were obtained in a single-step, without reaching the saturation of the IL-rich phase. The preconcentration of APIs up to the mg.L^{-1} allowed their easy and straightforward identification and quantification by High-Performance Liquid Chromatography

(HPLC) coupled to an UV detector, as shown for both model systems (distilled water) and real effluent samples from a wastewater treatment plant.

2. Introduction

The presence of active pharmaceutical ingredients (APIs) in non-negligible levels in sewage treatment plants (STPs), wastewater treatment plants (WWTPs), surface water effluents, river waters and seawater has been a topic of growing concern.[1-4] The increasing consumption of large number of different pharmaceuticals along time has had a significant impact in the public health and wildlife. Within APIs, antibiotics, and in particular fluoroquinolones (FQs), and non-steroidal anti-inflammatory drugs (NSAIDs) are of particular concern since they are consumed in relatively high amounts,[5, 6] which results in their inherent excretion into the waste water cycle (either as metabolized or unchanged species) or by the simple direct discharge of expired or non-consumed drugs.[7-9] APIs are known as mutagenic, carcinogenic and endocrine disruptors and have been detected in worldwide effluents in concentrations up to $\mu g.L^{-1}$ and in rivers and oceans in concentrations generally up to $ng.L^{-1}$.[1-3]

FQs (ciprofloxacin, norfloxacin and enrofloxacin) are synthetic antibiotics broadly used in the treatment of infectious diseases, such as respiratory and urinary tract infections, since they act against a wide range of aerobic gram-positive and gram-negative organisms.[10] Due to their high effectiveness, FQs have been largely used by humans, food producing animals (cattle and aquaculture fish, e.g.), and companion animals. However, these pharmaceutical drugs have negative impacts towards humans and wildlife, namely in the development and reproductive functions of fish, invertebrates, plants and algae, particularly after prolonged exposure periods. Nowadays, ciprofloxacin and norfloxacin are the second-generation FQs most prescribed in the world. In Europe, for example, in 2012 ciprofloxacin accounted for 71 % of the consumption of second generation quinolones in all countries.[11-14] Due to this large

consumption, ciprofloxacin was already found not only in effluents from WWTP/STP but also in river waters. In effluents, the levels tend to be higher ranging from 40 to 3353 $ng.L^{-1}$ in Europe; from 110 to 1100 $ng.L^{-1}$ in North America; and from 42 to 720 $ng.L^{-1}$ in Asia and Australia.[15] In WWTP influents and hospital wastewaters, the levels are, as expected, much higher (in the $\mu g.L^{-1}$ range).[4, 10] In Switzerland, e.g., ciprofloxacin levels in hospital wastewater ranged between 3 and 87 $\mu g.L^{-1}$.[16] In river waters, the levels are generally in the low $ng.L^{-1}$ range. Nevertheless, exceptionally high levels have also been reported: concentrations of ciprofloxacin as high as 2745 $ng.L^{-1}$ were found in one location of a Polish river.[17]

NSAIDs (diclofenac, naproxen and ketoprofen) are a class of pain killers used in human and veterinary medicine, being one of the primary classes of pharmaceutical compounds prescribed in human medical care, with many compounds sold without prescription.[2] Due to their widespread use, NSAIDs are continuously discharged in the aquatic environment where they are pseudo-persistent. They have the potential to bioaccumulate and they can be reactive to non-target organisms. They are known to be toxic towards a wide variety of organisms including invertebrates and fish.[2] Diclofenac was already found in effluents from WWTP/STP in levels ranging from 460 to 3300 $ng.L^{-1}$ in Europe; from < 0.5 to 177 $ng.L^{-1}$ in North America; and from 8.8 to 127 $ng.L^{-1}$ in Asia and Australia.[15] It was also detected in freshwater rivers in levels varying between 2 and 41 $ng.L^{-1}$ in Europe, 11-82 $ng.L^{-1}$ in North America and 1.1 and 6.8 $ng.L^{-1}$ in Asia and Australia.[15]

Since diclofenac, naproxen and ciprofloxacin are the most frequently found APIs in water cycles, the Global Water Research Coalition (GWRC) pointed them out as high priority pharmaceutical drugs.[5, 18, 19] Furthermore, diclofenac is considered as priority hazardous substance in the European Union.[20] In Figure 12, the chemical structures of the FQs and NSAIDs studied in this work are depicted.

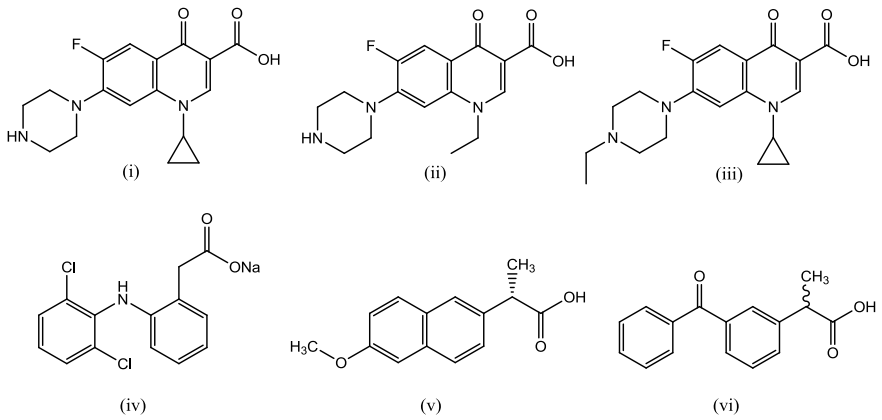

Figure 12. Chemical structures of fluoroquinolones: I) ciprofloxacin, II) enrofloxacin, and III) norfloxacin; and non-steroidal anti-inflammatory drugs: IV) diclofenac, V) naproxen, and VI) ketoprofen.

Although STPs and WWTPs use advanced technologies for the removal and elimination of pollutants/contaminants, the complete elimination of APIs is extremely difficult and thus these contaminants were detected even in drinking water.[1] According to Deblonde et al.[21], the removal efficiency of ciprofloxacin, norfloxacin, diclofenac, naproxen and ketoprofen in WWTPs is 62.3, 54.3, 34.6, 81.6 and 31.1 %, respectively. Therefore, the entry of these contaminants into the environment is a continuous process which will result in significant environmental and human hazards in the near future. For an adequate monitoring of their concentration, environmental risks, persistence and occurrence, there is the need to develop improved analytical methods for their detection and quantification. The accurate identification and quantification of APIs often requires pre-treatment strategies of aqueous samples, both to increase their concentrations up to values that can be quantified by analytical equipment or to remove major interferences. The commonly used technique for the pre-treatment of aqueous samples is solid-phase extraction (SPE). For instance, Vieno et al.[22] applied SPE as an isolation and preconcentration procedure, aiming at an improved detection of three FQS, in which preconcentration factors of 2000, 1000, 500 and 200 for ground water,

surface water, STP effluent and STP influent samples, respectively, were obtained. Prat et al.[23] used SPE to preconcentrate 10 quinolones (preconcentration factor up to a 250-fold) followed by reversed-phase high-performance liquid chromatography coupled to a fluorescence detector analysis. Lopes et al.[24] developed a modified SPE method for identification of emerging contaminants from large sample volumes, where the preconcentration of bisphenol-A, acetaminophen, salicylic acid and diclofenac was determined by high-pressure liquid chromatography combined with time-of-flight mass spectrometry (HPLC-MS-TOF). Although SPE is the most commonly used technique for the pre-treatment and preconcentration of aqueous samples containing APIs, it requires an additional desorption step of the analyte, usually carried out with hazardous volatile organic solvents. In this context, it is of high relevance to develop alternative and more efficient pre-treatment techniques for aqueous samples containing APIs envisaging their accurate monitoring in the aquatic environment.

In this work, ionic-liquid-based aqueous biphasic systems (IL-based ABS) will be evaluated as an alternative pre-treatment strategy for two major families of APIS, FQs and NSAIDS. ABS are liquid-liquid systems formed by at least two (ideally non-volatile) compounds dissolved in a water-rich medium. In general, two polymers, a salt and a polymer or two salts above given concentrations lead to the creation of two-phase aqueous systems.[25, 26] In 2003, Rogers and co-workers[27] demonstrated that the addition of a “kosmotropic” salt to aqueous solutions of ILs results in two-phase separation. Since then, IL-based ABS, formed by the combination of ILs with a large number of salts, amino acids, carbohydrates or polymers, have been the focus of intensive research regarding their use in extraction, separation and purification approaches.[28-32] This boom in research derives from the ILs exceptional properties, namely a negligible vapor pressure, non-flammability, high thermal and chemical stabilities, and the ability to tailor the phases’ polarities and affinities by an adequate choice of the chemical structures

of the ILs ions.[33-36] While most studies reported in the literature are devoted to the use of IL-based ABS for purification purposes,[29] their use in the extraction and preconcentration of target compounds has also been investigated. Passos et al.,[37] envisaging an adequate monitoring of endocrine disruptors in human fluids, demonstrated the complete extraction of bisphenol A from human urine and its preconcentration up to a 100-fold using IL-based ABS. Later, Dinis et al.[38] studied the simultaneous extraction and preconcentration of ethinylestradiol with IL-based ABS, achieving a preconcentration factor up to 1000-fold in a single-step. In conclusion, IL-based ABS are promising candidates to pre-treatment strategies of aqueous samples allowing for a better monitoring of APIs in aqueous environmental samples. Therefore, in this work, ABS composed of a wide range of ILs and a citrate-based biodegradable salt (potassium citrate, $C_6H_5K_3O_7$) were investigated for the extraction and preconcentration of FQS, namely ciprofloxacin, norfloxacin and enrofloxacin, and of NSAIDs, namely diclofenac, naproxen and ketoprofen. The liquid-liquid ternary phase diagrams corresponding to the ABS used for the extraction and preconcentration purposes were previously reported by Passos et al.[39] however, new ternary mixtures and their respective compositions at the coexisting phases have been determined in this work. An initial screening of the ability of these systems to extract FQS and NSAIDs was carried out, followed by the use of the most promising systems for the simultaneous extraction and preconcentration the two classes of APIs. Model systems, using distilled water and also real effluent samples from a WWTP were used to evaluate the matrix effect on the extraction performance of the proposed technology.

3. Experimental Section

3.1. Materials

Three FQs, namely ciprofloxacin hydrochloride (CAS# 86393-32-0), enrofloxacin (CAS# 93107-08-5), and norfloxacin (CAS# 70458-96-7), and three NSAIDs, namely diclofenac sodium salt (CAS# 15307-79-6), naproxen (CAS# 22204-53-1) and ketoprofen (CAS# 22071-15-4), were used in this work. Ciprofloxacin hydrochloride, norfloxacin, diclofenac sodium salt, naproxen and ketoprofen were acquired from Sigma-Aldrich, whereas enrofloxacin was purchased from BioChemika. The chemical structures of the studied FQs and NSAIDs are depicted in Figure 12.

The ILs investigated to form ABS were tetrabutylphosphonium chloride, $[P_{4444}]Cl$, (97 wt%); tetrabutylammonium chloride, $[N_{4444}]Cl$, (≥ 97 wt%); 1-butyl-1-methylpiperidinium chloride, $[C_4C_1pip]Cl$, (≥ 99 wt%); 1-butyl-1-methylpyrrolidinium chloride, $[C_4C_1pyr]Cl$, (≥ 99 wt%); 1-butyl-3-methylimidazolium chloride, $[C_4C_1im]Cl$, (99 wt%); 1-methyl-3-octylimidazolium chloride, $[C_8C_1im]Cl$, (99 wt%); 1-butyl-3-methylimidazolium bromide, $[C_4C_1im]Br$, (99 wt %); 1-butyl-3-methylimidazolium thiocyanate, $[C_4C_1im][SCN]$, (98 wt%); 1-butyl-3-methylimidazolium dicyanamide, $[C_4C_1im][N(CN)_2]$, (98 wt%); 1-butyl-3-methylimidazolium trifluoromethanesulfonate, $[C_4C_1im][CF_3SO_3]$, (99 wt%). Phosphonium-based ILs were gently supplied by Cytec Industries Inc., while the imidazolium-, piperidinium-, and pyrrolidinium-based fluids were purchased from Iolitec. Tetrabutylammonium chloride was purchased from Sigma-Aldrich. To decrease the volatile impurities and water contents, individual samples of ILs were purified at room temperature under constant stirring and vacuum for a minimum of 24 h. In particular for $[P_{4444}]Cl$, the temperature was raised up to 373 K and this sample was kept under vacuum for a minimum of 72 h, due to its higher amounts of water. The purity of each IL was confirmed by ^{1}H and ^{13}C NMR and found to be

in accordance with the purities given by the suppliers. The chemical structures of the studied ILs are depicted in Figure 13.

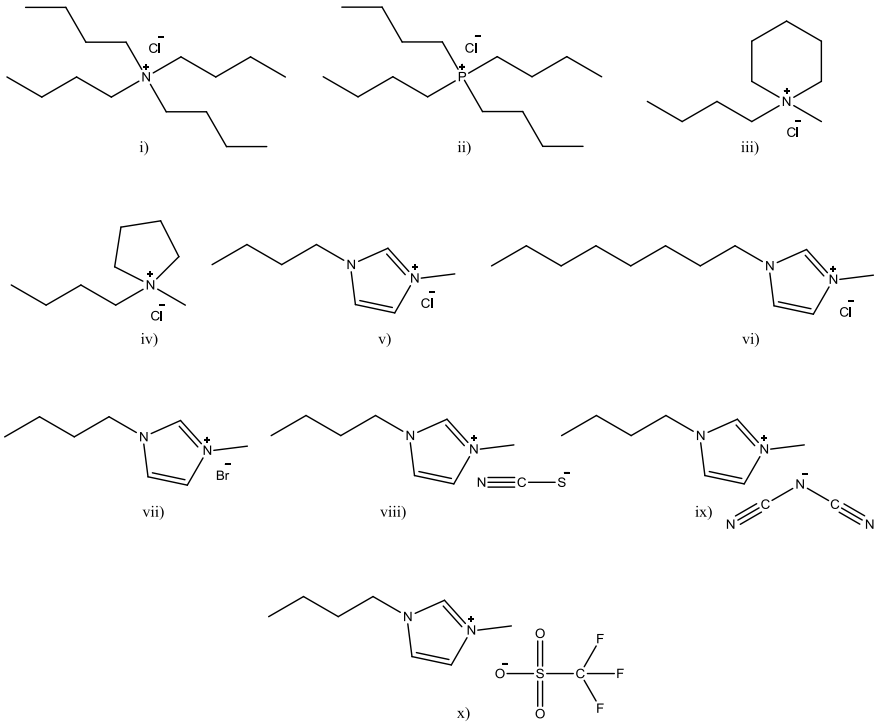

Figure 13. Chemical structures of the ionic liquids used: i) $[N_{4444}]Cl$, ii) $[P_{4444}]Cl$, iii) $[C_4C_1pip]Cl$, iv) $[C_4C_1pyr]Cl$, v) $[C_4C_1im]Cl$,vi) $[C_8C_1im]Cl$, vii) $[C_4C_1im]Br$, viii) $[C_4C_1im][SCN]$, ix) $[C_4C_1im][N(CN)_2]$, and x) $[C_4C_1im][CF_3SO_3]$.

The potassium citrate tribasic monohydrate salt, $C_6H_5K_3O_7.H_2O$ (≥ 99 wt%) used in the ABS was acquired from Sigma–Aldrich. The water used in the extractions experiments was double distilled, passed across a reverse osmosis system and further treated with a Milli-Q plus 185 water purification equipment. Effluent samples from a waste water treatment plant serving a population of about 20 000 inhabitants located in central Portugal were used to test the validity of the developed concentration technique. Buffers solutions of pH of 4.00 and 7.00, acquired from Panreac, were used for the calibration of the pH meter.

3.2. Screening of IL-based ABS for the complete extraction of APIs

The liquid-liquid ternary phase diagrams corresponding to the ABS used for the extraction and preconcentration purposes carried out in this work were previously reported by Passos et al.[39] Each tie-line (TL), which gives the mixture compositions to be used in the extraction experiments, was determined in this work by an established gravimetric method proposed by Merchuk et al.[40] Further details are provided in the Supporting information.

ABS composed of IL + $C_6H_5K_3O_7$ + water for the extraction of FQs and NSAIDs, corresponding to ternary mixtures in the biphasic region, were prepared gravimetrically using a Sartorius CPA225D Analytical Balance, within ± 2×10^{-5} g. Glass ampoules (15 cm^3) were used for the ABS preparation, by adding appropriate amounts of IL, inorganic salt and water solutions containing each of the FQs and NSAIDs. The concentration of ciprofloxacin, norfloxacin, enrofloxacin, diclofenac, naproxen and ketoprofen used in the initial aqueous solutions was $5x10^{-2}$ $g.L^{-1}$, while a ternary mixture composed of 40 wt% of IL, 19 wt% of $C_6H_5K_3O_7$ and 41 wt% of aqueous solution of APIs was used for extraction/preconcentration purposes. These mixtures were vigorously stirred and left to equilibrate for 24 h at (25 ± 1) ºC, to allow the equilibrium and complete separation of both phases. Subsequently, both the IL and salt-rich phases were carefully separated and weighted. The amount of each FQ and NSAID in each phase was quantified through UV-spectroscopy, using a Shimadzu UV-1800, Pharma-Spec UV-Vis Spectrophotometer, at a wavelength of 276, 275, 275, 276, 230 and 256 nm for ciprofloxacin, norfloxacin, and enrofloxacin, diclofenac, naproxen and ketoprofen, respectively, using calibration curves previously established. Ternary mixtures at the same weight fraction composition were prepared, using pure water instead of the aqueous solutions containing the FQs or NSAIDs, for blank control purposes. The pH values (± 0.02) of the IL-rich phase

were measured at (25 ± 1) ºC, using a Mettler Toledo S47 SevenMulti™ dual meter pH/conductivity equipment.

The extraction efficiencies of FQs ($\%EE_{FQs}$) and NSAIDs ($\%EE_{NSAIDs}$) are defined as the ratio between the total mass of each FQ or NSAID present in the IL-rich phase to that in the total mixture (both phases). Three replicates were prepared for each extraction assay, allowing for the determination of the average extraction efficiency and respective standard deviation.

3.3. Preconcentration of APIs using IL-based ABS

After the ILs screening for the extraction of both FQs and NSAIDs, the system composed of $[N_{4444}]Cl$ + $C_6H_5K_3O_7$ + water with a TLL of 88 was selected to develop APIs´s preconcentration platforms. The preconcentration factors of FQs and NSAIDs were determined using ternary systems with different initial compositions along the same TL. In the same TL, the composition of both phases in equilibrium remains constant, only the ratio of the volume or mass of the two phases changes. This step was initially carried out using the same initial concentration ($5x10^{-2}$ $g.L^{-1}$) of ciprofloxacin and diclofenac in the aqueous solutions used in the screening step. Furthermore, and in order to simulate representative preconcentrations of APIs in aqueous environments, aqueous solutions of ciprofloxacin and diclofenac, at concentrations of *circa* 7×10^{-6} and 5×10^{-6} $g.L^{-1}$, respectively, were used.

ABS with a total weight of 50 g were prepared. After equilibration and careful separation of the phases, the amount of ciprofloxacin and diclofenac in each phase was quantified through UV-spectroscopy, using a Shimadzu UV-1800, Pharma-Spec UV-Vis Spectrophotometer, at a wavelength of 276 nm. For the studies of real water samples, high-performance liquid chromatography (HPLC) was used to quantify ciprofloxacin and diclofenac, using a HPLC-UV from Shimadzu, Prominence Modular HPLC, using calibration curves previously determined. The chromatographic separation was achieved using a Reprosil C-18

analytical column, with porous spherical silica of 5 μm and pore diameter of 100 Å, from GmbH. The column size was of 250 × 4.6 mm. The operation temperature of the column was set at 25 ºC. The mobile phase used was a mixture of methanol (A), and water adjusted to pH 2.5 with concentrated formic acid (B). The volume ratio of solvent A to solvent B was 70:30. The elution was performed at the flow-rate of 0.8 $mL.min^{-1}$ and the injection volume was 10 μL. The wavelength of the UV detector was set at 278 and 275 nm for the quantification of ciprofloxacin and diclofenac, respectively.

The preconcentration factor was determined as the ratio between the concentrations of each API in the IL-rich phase and in the initial aqueous solution/sample. The preconcentration factors of FQs and NSAIDs were determined with model systems (distilled water) and real wastewater samples from a municipal waste water treatment plant. Since in the real sample, no detectable levels of the target APIs were found, the sample was spiked with 2 $mg.L^{-1}$ of ciprofloxacin and diclofenac.

An important parameter when developing IL-based ABS as preconcentration techniques, is the solubility of ciprofloxacin and diclofenac in the IL-rich phase. Thus, the solubility of both APIs at (25 ± 1) ºC in the $[N_{4444}]Cl$-rich phase, namely in an aqueous solution composed of 62.4 wt% $[N_{4444}]Cl$ + 2.1 wt% $C_6H_5K_3O_7$ + 35.5 wt% H_2O, was determined. A total weight of 1 g of the IL-rich phase was used, in which individual amounts of FQs and NSAIDs were continuously added - (0.002 – 0.005) g - and kept under controlled stirring and temperature (25 ± 1) ºC. The solubility was determined by the visual detection of the cloud point, i.e. the appearance of solid that does not dissolve in 24 h. Three replicates were prepared for each solubility assay, allowing the determination of the average solubility value and respective standard deviation.

4. Results and Discussion

4.1. Screening of IL-based ABS for the complete extraction of APIs

A fixed ternary mixture composition (IL ≈ 40 wt%, salt ≈ 19 wt%) was initially used to evaluate the ability of ILs of different chemical structures to extract FQs and NSAIDs from aqueous media. Although the liquid-liquid ternary phase diagrams used in the current work were previously reported by Passos et al.,[39] the compositions of the two phases in equilibrium (TLs) for each extraction experiment were determined in this work and are reported in the Supporting Information. Figure 14 and Figure 15 depict the single-step extraction efficiencies of the investigated ABS at 25 ºC for FQs ($\%EE_{FQs}$) and NSAIDs ($\%EE_{NSAIDs}$) (*cf.* the Supporting Information with detailed data). In all systems investigated, the studied FQs and NSAIDs preferentially partition to the IL-rich phase (top phase of the studied systems, with the exception of the system formed by $[C_4C_1im][CF_3SO_3]$ in which an inversion on the phases densities occurs).

The $\%EE_{FQs}$ and $\%EE_{NSAIDs}$ of the studied ABS for the IL-rich phase range between 59 % and 100 %, and between 83 % and 100 %, respectively. Overall, the extraction efficiencies of IL-based ABS for FQs and NSAIDs follow the rank: $[N_{4444}]Cl \approx [C_4C_1pip]Cl \approx [C_4C_1pyr]Cl \approx [C_4C_1im]Cl \approx [C_8C_1im]Cl \approx [P_{4444}]Cl > [C_4C_1im]Br > [C_4C_1im][N(CN)_2] > [C_4C_1im][SCN] \approx [C_4C_1im][CF_3SO_3]$. No significant differences are found between the extraction efficiencies of the investigated IL-based ABS for the different FQs and NSAIDs when using ILs with the chloride (Cl^-) anion (combined with the $[P_{4444}]^+$, $[N_{4444}]^+$, $[C_4C_1pip]^+$, $[C_4C_1pyr]^+$ and $[C_4C_1im]^+$ cations), suggesting that the IL anion plays a dominant role in the systems extraction performance. In the same line, an increase in the IL cation alkyl side chain length (from $[C_4C_1im]Cl$ to $[C_8C_1im]Cl$) has no significant impact on the $\%EE_{FQs}$ and $\%EE_{NSAIDs}$.

When analyzing ILs with the same cation core ($[C_4C_1im]^+$) combined with different anions, namely Cl^-, Br^-, $[SCN]^-$, $[N(CN)_2]^-$, and $[CF_3SO_3]^-$, more significant differences in the extraction efficiencies can be found. Among these, ABS containing ILs bearing the chloride-anion or anions of higher hydrogen-bond basicity[41] are more efficient extraction platforms for both NSAIDs and FQs.

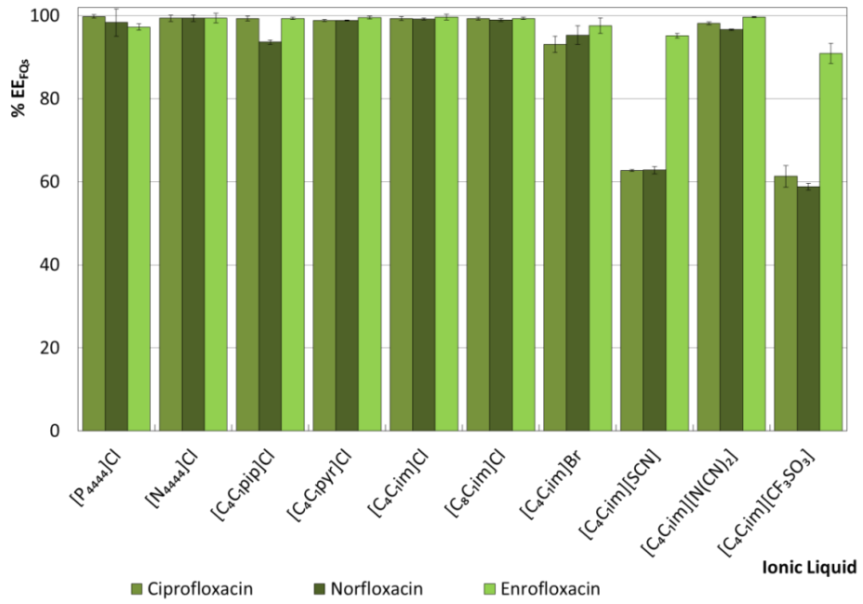

Figure 14. Extraction efficiencies of ABS composed of IL + $C_6H_5K_3O_7$ + H_2O at 25 ºC for FQs ($\%EE_{FQs}$).

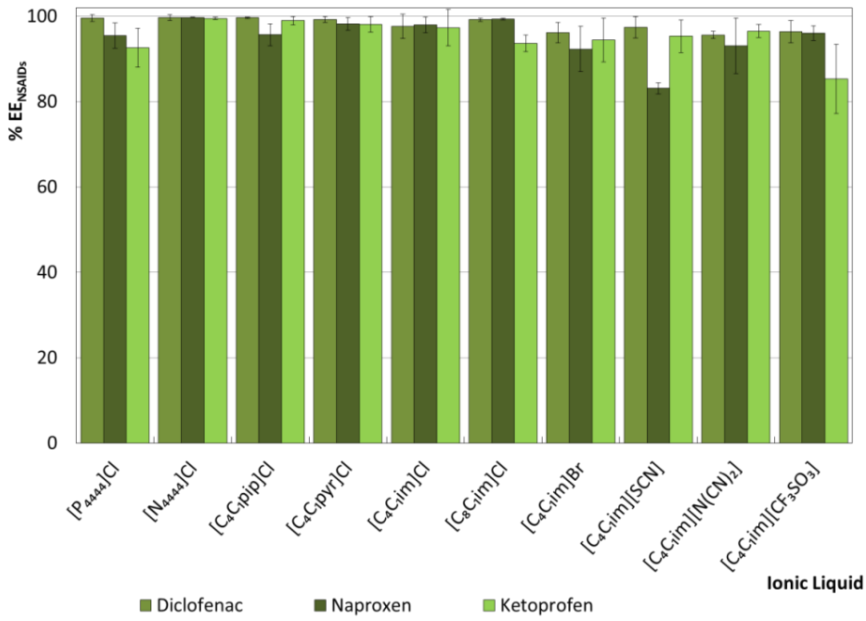

Figure 15. Extraction efficiencies of ABS composed of IL + $C_6H_5K_3O_7$ + H_2O at 25 ºC for NSAIDs (%EE_{NSAIDs}).

The pH values of the IL-rich phase range between 7 and 10, as a result of the alkaline character of the $C_6H_5K_3O_7$ aqueous solutions. According to the FQs and NSAIDs pK_a values, all compounds are mainly present in their zwiterionic and negatively charged forms. The detailed pH data and speciation curves of each API are shown in the Supporting Information. In previous works,[42, 43] IL-based ABS composed of IL + $Al_2(SO_4)_3$ + H_2O were proposed as removal techniques of APIs from aqueous streams. Since these systems were highly acidic, both FQs and NSAIDs were mainly present in their protonated form. Very high %EE_{FQs} and %EE_{NSAIDs}, up to 98 %[42] and 100 %,[43] were obtained in a single-step extraction, respectively. Taking into account this information and the maximum extraction efficiencies found in this work, it can be concluded that electrostatic interactions between the charged APIs and IL or salt ions do not play a major role in the solutes partitioning/extraction.

Considering the extraction efficiencies of the studied APIs by the different IL-based ABS, it can be concluded that $[N_{4444}]Cl$ is one of the most promising candidates for extracting FQs and NSAIDs from aqueous media. Although rarely explored as phase-forming component of ABS,[39, 44] quaternary ammonium-based ILs present a higher aptitude to form ABS, due to their higher hydrophobicity, afforded by the four alkyl side chains. As a result, there is also a small loss of this type of ILs to the salt-rich phase (or cross-contamination). For the investigated mixtures, the amount of $[N_{4444}]Cl$ in the salt-rich phase is *circa* 2 wt% (TL data shown in the Supporting Information). In addition, $[N_{4444}]Cl$ also has a low cost[45] and low toxicity.[46] The toxicity of ILs mainly depends on the IL cation and particularly on the size of the alkyl chains in the pyridinium, imidazolium and quaternary ammonium salts.[47] The IL anion exhibits thus a less significant effect than the IL cation. In general, the studies of environmental fate and toxicity of ILs demonstrate that their effects vary considerably across organisms and trophic levels, and no general conclusions can be established. Even so, and among the several possibilities of ILs, Cl- and Br-based ILs are amongst the less harmful choices to the environment. Due to these advantages, the ABS constituted by $[N_{4444}]Cl$ and $C_6H_5K_3O_7$ was used in the preconcentration studies.

4.2. Preconcentration of APIs using IL-based ABS

A primary requisite to use ABS as preconcentration platforms is the existence of long tie-lines. The TLL is an indicator of the differences in the compositions between the two phases and it is usually used to correlate the partitioning trend of solutes between both phases.[29] A long TLL not only decreases the cross-contamination of each phase by the component enriched in the opposite layer, but also affords higher preconcentration factors.[29] The manipulation of the mixture compositions along the same TL enables the tailoring of the volumes of the coexisting phases, without changing their composition. As it

was mentioned before, the ABS formed by $[N_{4444}]Cl$ + $C_6H_5K_3O_7$ exhibits a large biphasic region, leading to long TLs and thus the possibility of obtaining high preconcentration factors. For the study of the preconcentration factors, ciprofloxacin and diclofenac were chosen as representatives of the FQs and NSAIDs classes, mainly because these two APIs are classified as high priority pharmaceuticals.[5]

Figure 16 presents the extraction efficiencies of the $[N_{4444}]Cl$-based system as a function of the TLL for ciprofloxacin and diclofenac. Detailed data are provided in the Supporting Information. In general, the extraction efficiencies of the studied ABS for FQs and NSAIDs are maintained at 100 % in a single-step in the different TLs evaluated, meaning that no saturation of APIs in the IL-rich phases occurs. Therefore, the longest TLL (*circa* 88) was further studied for preconcentration purposes, since it fulfills three criteria: (i) it allows a high preconcentration factor to be obtained; (ii) the total extraction of FQs and NSAIDs is achieved in a single-step; and (iii) there is a low amount of IL in the salt-rich phase or cross-contamination (*circa* 1 wt%).

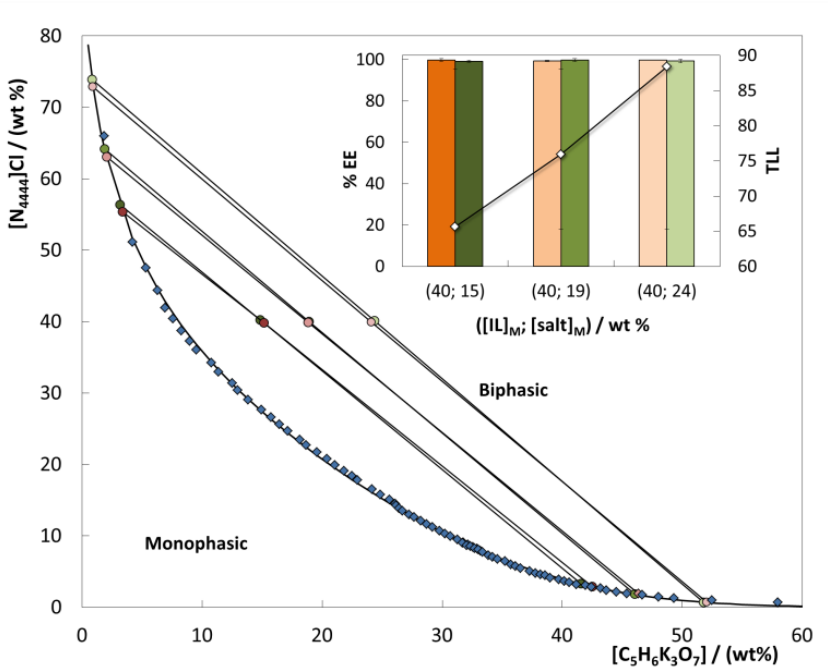

Figure 16. Extraction efficiencies (%EE) of the $[N_{4444}]Cl$-based ABS for ciprofloxacin (orange) and diclofenac (green) at (25 ± 1) ºC using different TL: binodal curve data (◆);[39] TL data (●); and TLL values (◇).

Figure 17 shows the composition of the initial mixtures (*cf.* the Supporting Information with detailed data) along the TL with a TLL *circa* 88 for the $[N_{4444}]Cl$ + $C_6H_5K_3O_7$ + H_2O ABS,[39] (*cf.* the Supporting Information with detailed data). The extraction efficiencies values and preconcentration factors afforded by these mixtures for ciprofloxacin and diclofenac are also shown. As discussed before, ciprofloxacin and diclofenac are enriched in the IL-rich phase, due to their preferential migration to this phase. Preconcentration factors ranging from 0.6 to 9.0-fold for ciprofloxacin and from 0.7 to 8.0-fold for diclofenac were attained, showing that it is possible to preconcentrate FQs and NSAIDs in the IL-rich phase up to a 9-fold without losing the extraction efficiency performance or saturating the IL-rich phase. These preliminary results carried out with model systems allow to

conclude about the possibility to use IL-based systems to preconcentrate FQs and NSAIDs, and thus overcome their difficult detection and quantification resulting from their low concentrations in aquatic real samples. It should be highlighted that in these experiments, initial concentrations of ciprofloxacin and diclofenac of *circa* $5x10^{-2}$ $g.L^{-1}$ were used and thus the final concentrations of both APIs in the two phases in equilibrium were here determined by UV-spectroscopy. Therefore, the maximum obtained preconcentration factors of 9.0-fold and 8.0-fold represent concentrations of ciprofloxacin and diclofenac in the IL-rich phase of 0.45 and 0.40 $g.L^{-1}$, respectively.

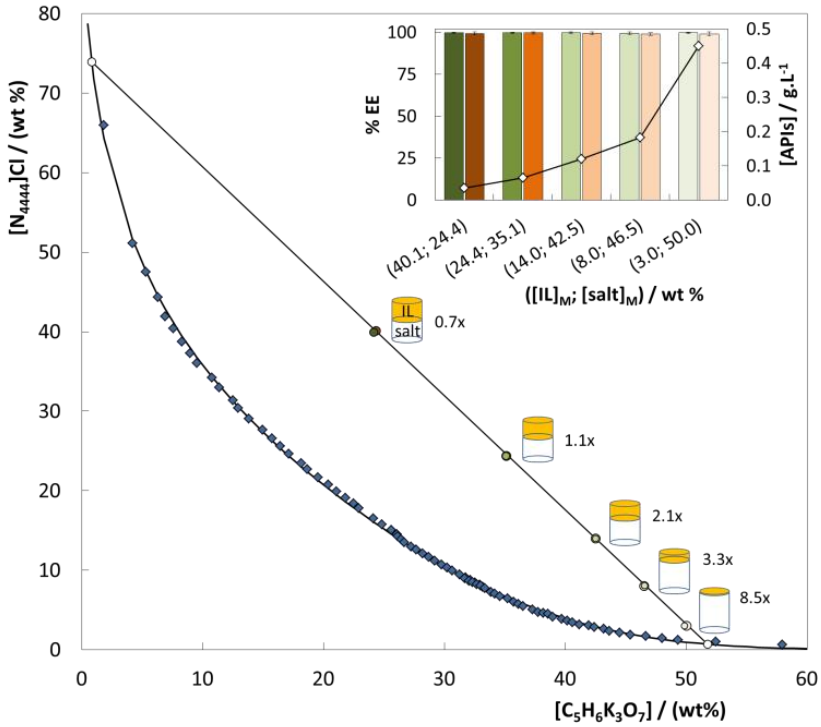

Figure 17. Extraction efficiencies (%EE) of the $[N_{4444}]Cl$-based ABS for ciprofloxacin (orange) and diclofenac (green) for different initial compositions of ABS phase forming components, along the same TL at 25 ºC: (◆), binodal curve data;[39] (○), TL data, (●), initial composition ($[IL]_M$; $[salt]_M$); and (◇), final concentration of ciprofloxacin and diclofenac in the IL-rich phase ([APIs]/$g.L^{-1}$).

In order to explore higher preconcentration factors, still along the same TL, lower initial concentrations of IL and APIs had to be used in the ABS formation and concomitantly the quantification of APIs in the IL-rich phase was carried out by HPLC-UV. The initial mixture compositions of ciprofloxacin and diclofenac used here are presented in Supporting Information (Table S5). Since the determined limit of detection (LOD) of ciprofloxacin and diclofenac in the HPLC is of 0.5 $mg.L^{-1}$ and 0.2 $mg.L^{-1}$, respectively, it is possible to quantify samples contaminated with APIs in the order of $\mu g.L^{-1}$. HPLC chromatograms of aqueous solutions of ciprofloxacin and diclofenac, with concentrations ranging between 1×10^{-4} and 5×10^{-2} $g.L^{-1}$ are presented in the Supporting Information. Aqueous solutions of ciprofloxacin and diclofenac with a concentration *circa* 7×10^{-6} and 5×10^{-6} $g.L^{-1}$, respectively, representative of the APIs levels that may be found in aqueous environments, were used in the study of higher preconcentration factors. According to Figure 17, the complete extraction of both APIs and experimental preconcentration factors of 1000-fold were achieved in a single-step (*cf.* the Supporting Information, 1085 and 1164 for ciprofloxacin and diclofenac, respectively). Furthermore, and since the retention time of ciprofloxacin and diclofenac are different (2.9 min and 13.2 min, respectively), aqueous solutions containing both ciprofloxacin and diclofenac were further preconcentrated, and again preconcentration factors of 1000-fold were obtained (*cf.* the Supporting Information, 1010 and 998 for ciprofloxacin and diclofenac, respectively). This fact allows the conclusion that IL-based ABS are powerful tools to simultaneously extract and preconcentrate different classes of APIs.

In order to confirm that the saturation the two studied APIs in the IL-rich phase was not reached, their solubility was determined. Solubility values of 2.0 ± 0.2 $g.L^{-1}$ and 5.4 ± 0.2 $g.L^{-1}$ in the IL-rich phase were obtained at (25 ± 1) ºC for ciprofloxacin and diclofenac, respectively. These values are well above the equipment LOD and final concentrations determined for the highest preconcentration factors obtained. It should also be remarked that FQs and

NSAIDs can be easily recovered from the IL-rich phase by the addition of water as anti-solvent and changes in pH, which lead to the drugs precipitation and allow the IL reuse with minimal losses, as previously demonstrated.[42, 43]

In order to further evaluate the feasibility of using the $[N_{4444}]Cl$-based ABS as extraction and preconcentration platforms for ciprofloxacin and diclofenac, real effluent samples from a wastewater treatment plant spiked with ciprofloxacin and diclofenac were used. Although a preliminary preconcentration step was carried out with non-spiked samples, diclofenac and ciprofloxacin were not identified, meaning that, they are present in concentrations below $\mu g.L^{-1}$. Such results are not a surprise, since many surveys conducted in Europe disclosed concentrations in the order of $ng.L^{-1}$. Also, our effluent samples come from a WWTP that serves a population of only 20 000 inhabitants. Figure 18 shows the HPLC-UV chromatograms at 278 nm and 275 nm, in which the peaks corresponding to ciprofloxacin and diclofenac are clearly identified and can be quantified, after reaching preconcentration factors of 1000-fold in a single-step (experimental preconcentration factors of 1006 and 1014 for ciprofloxacin and diclofenac, respectively). The chromatograms of the non-spiked and spiked WWTP effluent samples with no ABS pre-treatment are also shown. In summary, analysis of the chromatograms shows that there is no interference of the ABS phase-forming components and any other compounds present in the real sample and thus that it is possible to individually quantify ciprofloxacin and diclofenac.

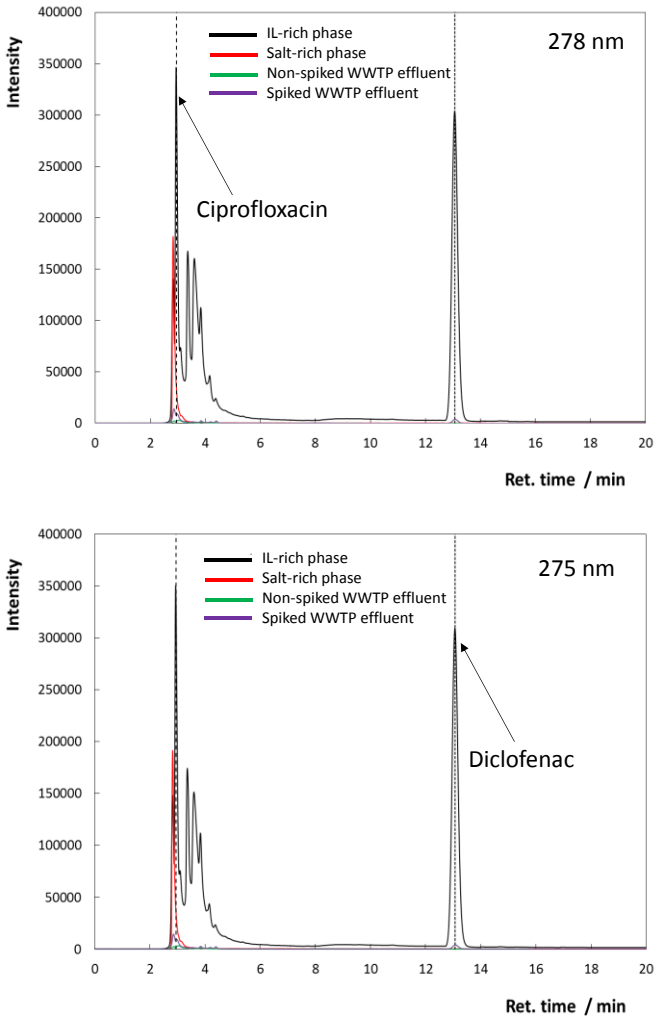

Figure 18. HPLC-UV chromatograms corresponding to the identification/quantification of ciprofloxacin and diclofenac simultaneously extracted from WWTP effluent and 1000-fold preconcentrated. Peaks corresponding to ciprofloxacin and diclofenac are identified at both wavelengths. The remaining peaks correspond to the phase-forming components of the ABS and other compounds present in the real sample.

The highest detected concentration values of ciprofloxacin and diclofenac in WWTP effluents in Europe was 3.3 μg.L^{-1}.[15] Therefore, the here proposed technique allows to identify and quantify APIs, with concentration in the μg.L^{-1} range, in real effluents by HPLC-UV, with LOD of 0.5 mg.L^{-1} and 0.2 mg.L^{-1} for ciprofloxacin and diclofenac, respectively. This is due to the high preconcentration factors, around 1000, achieved. Although some studies have been found in the literature,[23,24] regarding the pre-treatment of water samples for identification and quantification of FQs and NSAIDs, most of them reached lower enrichment factors and use of a SPE approach, which requires an additional desorption step, usually carried out with hazardous volatile organic solvents, for the target contaminants analysis. The use of IL-based ABS overcome some of these drawbacks, namely the need of an additional desorption step. From the obtained results it is expected that the proposed extraction/preconcentration procedure using IL-based ABS for the monitoring of APIs could be also applied to influents of a WWTP and in environmental routine analysis of other contaminants present in aqueous samples, namely in river water and seawater were APIs have already been detected.[1, 2, 48]

5. Conclusion

We propose the use of IL-based ABS as effective extraction/preconcentration techniques for FQs and NSAIDs in order to improve the monitoring of aqueous environmental samples, while overcoming some limitations of SPE pre-treatment techniques. ABS composed of $C_6H_5K_3O_7$ and imidazolium-, phosphonium- and ammonium-based ILs were firstly screened to identify the most promising systems able to completely extract the two classes of APIs. Due to the high extraction efficiencies, ability to allow high preconcentration factors, and lower environmental hazards, [N_{4444}]Cl-based ABS were further investigated as preconcentration platforms for ciprofloxacin and diclofenac as model FQs and NSAIDs. By playing around with the initial mixture compositions

along the same TL, it was possible to decrease the IL-rich phase volume and reach preconcentration factors of ciprofloxacin and diclofenac of 1000-fold in a single-step, shown for both model system, using distilled water, and real WWTP effluent samples. The proposed technology allows the simultaneous extraction/preconcentration of the two important classes of APIs up to values of $mg.L^{-1}$ and their simple identification and quantification by HPLC-UV. IL-based ABS are thus potential candidates to pre-treat environmental aqueous samples aiming at improving the monitoring of APIs.

6. Acknowledgment

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7. Supporting Information

7.1. Determination of Tie-Lines (TLs) and Tie-Line Lengths (TLLs)

The specific composition of each phase in equilibrium was determined based on the initial mixture composition and on the weight of each phase, using the following equations (eqs. (1) – (4)) with four unknown values ($[IL]_{IL}$, $[IL]_{salt}$, $[salt]_{IL}$, $[salt]_{salt}$): [40]

$$[\mathrm{IL}]_{\mathrm{IL}} = \mathrm{A}\exp[(\mathrm{B} \times [\mathrm{salt}]_{\mathrm{IL}}^{0.5}) - (\mathrm{C} \times [\mathrm{salt}]_{\mathrm{IL}}^{3})] \quad (1)$$

$$[\mathrm{IL}]_{\mathrm{salt}} = \mathrm{A}\exp[(\mathrm{B} \times [\mathrm{salt}]_{\mathrm{salt}}^{0.5}) - (\mathrm{C} \times [\mathrm{salt}]_{\mathrm{salt}}^{3})] \quad (2)$$

$$[\mathrm{IL}]_{\mathrm{IL}} = \frac{[\mathrm{IL}]_{\mathrm{M}}}{\alpha} - \frac{1-\alpha}{\alpha} \times [\mathrm{IL}]_{\mathrm{salt}} \quad (3)$$

$$[\mathrm{salt}]_{\mathrm{IL}} = \frac{[\mathrm{salt}]_{\mathrm{M}}}{\alpha} - \frac{1-\alpha}{\alpha} \times [\mathrm{salt}]_{\mathrm{salt}} \quad (4)$$

where IL, salt and M are the ionic-liquid-rich phase, the salt-rich phase and the mixture, respectively. [IL] and [salt] correspond to the weight fraction percentage of ionic liquid and salt, respectively, and α is the ratio between the weight of the ionic-liquid-rich phase and the total mass of the mixture.

For the calculation of the tie-line length (TLL), the following equation was used:

$$\mathrm{TLL} = \sqrt{([\mathrm{salt}]_{\mathrm{IL}} - [\mathrm{salt}]_{\mathrm{salt}})^2 + ([\mathrm{IL}]_{\mathrm{IL}} - [\mathrm{IL}]_{\mathrm{salt}})^2} \quad (5)$$

where IL and salt represents the ionic-liquid-rich phase and salt-rich phase, respectively. [IL] and [salt] correspond to the weight fraction percentage of ionic liquid and salt, as described before.

7.2. Supporting Tables

Table S1. Weight fraction percentage (wt%) of each compound at the coexisting phases of the ABS investigated.

	Fluorquinolones					
	Weight fraction percentage / wt (%)					
Ionic Liquid	**$[IL]_{salt}$**	**$[salt]_{salt}$**	**$[IL]_{M}$**	**$[salt]_{M}$**	**$[IL]_{IL}$**	**$[salt]_{IL}$**
$[P_{4444}]Cl$	2.7	40.3	40.2	18.8	66.5	3.8
$[N_{4444}]Cl$	1.9	46.4	39.8	18.8	62.8	2.1
$[C_4C_1pip][Cl$	3.1	58.9	40.1	18.9	51.9	6.1
$[C_4C_1pyr]Cl$	5.6	54.1	40.0	18.9	53.1	5.5
$[C_4C_1im]Cl$	9.1	48.9	39.9	18.8	49.9	8.9
$[C_8C_1im]Cl$	12.7	44.4	39.9	18.7	46.9	12.2
$[C_4C_1im]Br$	4.6	43.1	39.9	18.9	63.7	2.7
$[C_4C_1im][SCN]$	0.0	34.7	40.1	19.0	87.4	0.5
$[C_4C_1im][N(CN)_2]$	0.1	40.6	39.8	18.9	71.9	1.4
$[C_4C_1im][CF_3SO_3]$	1.1	34.2	40.1	19.0	85.7	1.2
	Non-steroidal anti-inflammatory drugs					
	Weight fraction percentage / wt (%)					
Ionic Liquid	**$[IL]_{salt}$**	**$[salt]_{salt}$**	**$[IL]_{M}$**	**$[salt]_{M}$**	**$[IL]_{IL}$**	**$[salt]_{IL}$**
$[P_{4444}]Cl$	2.8	40.1	40.0	18.9	66.7	3.7
$[N_{4444}]Cl$	2.1	45.9	40.0	18.9	64.0	1.9
$[C_4C_1pip][Cl$	3.1	58.8	39.8	18.9	51.6	6.2
$[C_4C_1pyr]Cl$	2.9	61.4	40.1	18.9	51.0	6.4
$[C_4C_1im]Cl$	9.6	48.2	40.0	18.9	51.1	8.2
$[C_8C_1im]Cl$	11.9	45.6	40.0	18.8	47.2	12.0
$[C_4C_1im]Br$	4.4	43.6	40.0	18.9	63.4	2.7
$[C_4C_1im][SCN]$	0.1	34.6	40.1	19.0	87.6	0.4
$[C_4C_1im][N(CN)_2]$	0.0	43.3	39.8	18.8	67.3	1.5
$[C_4C_1im][CF_3SO_3]$	1.0	34.3	40.0	19.0	85.4	1.0

Table S2. Tie-line length (TLL), pH of the IL-rich phase (pH_{IL}), and extraction efficiencies of the studied fluoroquinolones (% EE_{FQs}), and corresponding standard deviations (σ).

Ciprofloxacin			
Ionic Liquid	**TLL ± σ**	**pH_{IL} ± σ**	**% EE_{FQs} ± σ**
$[P_{4444}]Cl$	73.3 ± 0.3	8.52 ± 0.04	99.80 ± 0.39
$[N_{4444}]Cl$	75.5 ± 0.3	9.91 ± 0.06	99.37 ± 0.73
$[C_4C_1pip][Cl$	72.7 ± 1.1	9.51 ± 0.03	99.29 ± 0.59
$[C_4C_1pyr]Cl$	68.9 ± 1.3	9.31 ± 0.01	98.82 ± 0.23
$[C_4C_1im]Cl$	58.1 ± 1.3	9.08 ± 0.02	99.28 ± 0.43
$[C_8C_1im]Cl$	47.4 ± 0.6	7.37 ± 0.01	99.29 ± 0.37
$[C_4C_1im]Br$	71.5 ± 0.2	8.23 ± 0.02	93.06 ± 1.96
$[C_4C_1im][SCN]$	93.8 ± 0.5	7.59 ± 0.03	62.72 ± 0.22
$[C_4C_1im][N(CN)_2]$	81.8 ± 0.0	9.14 ± 0.01	98.13 ± 0.32
$[C_4C_1im][CF_3SO_3]$	90.8 ± 1.1	8.04 ± 0.04	61.33 ± 2.58
Norfloxacin			
Ionic Liquid	**TLL ± σ**	**pH_{IL} ± σ**	**% EE_{FQs} ± σ**
$[P_{4444}]Cl$	73.3 ± 0.3	8.61 ± 0.04	98.35 ± 3.29
$[N_{4444}]Cl$	76.2 ± 0.5	9.95 ± 0.09	99.36 ± 0.72
$[C_4C_1pip][Cl$	70.7 ± 1.1	9.52 ± 0.01	93.62 ± 0.52
$[C_4C_1pyr]Cl$	67.5 ± 0.1	9.28 ± 0.02	98.84 ± 0.16
$[C_4C_1im]Cl$	58.7 ± 0.4	9.08 ± 0.02	99.16 ± 0.25
$[C_8C_1im]Cl$	45.9 ± 1.0	7.37 ± 0.01	98.96 ± 0.40
$[C_4C_1im]Br$	71.9 ± 0.0	8.14 ± 0.02	95.32 ± 2.24
$[C_4C_1im][SCN]$	93.5 ± 0.6	7.50 ± 0.05	62.83 ± 0.87
$[C_4C_1im][N(CN)_2]$	80.8 ± 0.0	9.12 ± 0.04	96.62 ± 0.20
$[C_4C_1im][CF_3SO_3]$	90.4 ± 0.9	7.50 ± 0.01	58.83 ± 0.83
Enrofloxacin			
Ionic Liquid	**TLL ± σ**	**pH_{IL} ± σ**	**% EE_{FQs} ± σ**
$[P_{4444}]Cl$	72.7 ± 0.4	8.22 ± 0.03	97.28 ± 0.78
$[N_{4444}]Cl$	75.9 ± 0.1	9.78 ± 0.00	99.44 ± 1.13
$[C_4C_1pip][Cl$	71.0 ± 0.6	9.72 ± 0.04	99.35 ± 0.31
$[C_4C_1pyr]Cl$	58.2 ± 0.0	9.15 ± 0.02	99.55 ± 0.35
$[C_4C_1im]Cl$	60.1 ± 0.3	9.52 ± 0.04	99.66 ± 0.68
$[C_8C_1im]Cl$	46.0 ± 0.6	7.39 ± 0.01	99.35 ± 0.31
$[C_4C_1im]Br$	72.2 ± 0.1	8.57 ± 0.01	97.57 ± 1.87
$[C_4C_1im][SCN]$	93.9 ± 1.0	7.59 ± 0.03	95.16 ± 0.63
$[C_4C_1im][N(CN)_2]$	82.6 ± 0.0	9.15 ± 0.03	99.60 ± 0.11
$[C_4C_1im][CF_3SO_3]$	90.1 ± 0.8	7.98 ± 0.13	90.89 ± 2.42

Table S3. Tie-line length (TLL), pH of the IL-rich phase (pH_{IL}), and extraction efficiencies of the studied non-steroidal anti-inflammatory drugs (% EE_{NSAIDs}), and corresponding standard deviations (σ).

Diclofenac			
Ionic Liquid	**TLL ± σ**	**pH_{IL} ± σ**	**% EE_{FQs} ± σ**
$[P_{4444}]Cl$	73.5 ± 0.3	9.55 ± 0.06	99.55 ± 0.81
$[N_{4444}]Cl$	76.0 ± 0.1	9.90 ± 0.04	99.71 ± 0.70
$[C_4C_1pip][Cl$	72.4 ± 1.3	9.51 ± 0.03	99.65 ± 0.16
$[C_4C_1pyr]Cl$	70.6 ± 3.6	9.56 ± 0.01	99.21 ± 0.69
$[C_4C_1im]Cl$	57.7 ± 1.7	8.72 ± 0.02	97.65 ± 2.82
$[C_8C_1im]Cl$	48.8 ± 0.8	7.38 ± 0.01	99.22 ± 0.38
$[C_4C_1im]Br$	71.8 ± 0.6	8.35 ± 0.02	96.16 ± 2.44
$[C_4C_1im][SCN]$	93.7 ± 0.4	7.68 ± 0.03	97.42 ± 2.46
$[C_4C_1im][N(CN)_2]$	80.0 ± 1.3	9.33 ± 0.01	95.61 ± 0.85
$[C_4C_1im][CF_3SO_3]$	90.1 ± 0.8	7.90 ± 0.04	96.41 ± 2.66
Naproxen			
Ionic Liquid	**TLL ± σ**	**pH_{IL} ± σ**	**% EE_{FQs} ± σ**
$[P_{4444}]Cl$	76.1 ± 0.1	9.19 ± 0.04	95.44 ± 2.96
$[N_{4444}]Cl$	78.9 ± 0.1	9.20 ± 0.10	99.75 ± 0.01
$[C_4C_1pip][Cl$	71.2 ± 1.2	9.52 ± 0.01	95.65 ± 2.57
$[C_4C_1pyr]Cl$	70.3 ± 1.7	9.58 ± 0.02	98.21 ± 1.50
$[C_4C_1im]Cl$	67.4 ± 0.5	8.74 ± 0.02	97.98 ± 1.86
$[C_8C_1im]Cl$	47.4 ± 0.7	7.36 ± 0.01	99.34 ± 0.24
$[C_4C_1im]Br$	75.7 ± 0.1	8.45 ± 0.03	92.32 ± 5.33
$[C_4C_1im][SCN]$	93.6 ± 0.7	7.62 ± 0.05	83.14 ± 1.31
$[C_4C_1im][N(CN)_2]$	82.4 ± 0.1	9.29 ± 0.04	93.10 ± 6.50
$[C_4C_1im][CF_3SO_3]$	90.3 ± 0.8	7.84 ± 0.01	96.06 ± 1.72
Ketoprofen			
Ionic Liquid	**TLL ± σ**	**pH_{IL} ± σ**	**% EE_{FQs} ± σ**
$[P_{4444}]Cl$	72.9 ± 0.5	9.76 ± 0.03	92.63 ± 4.53
$[N_{4444}]Cl$	77.5 ± 2.0	9.59 ± 0.00	99.52 ± 0.25
$[C_4C_1pip][Cl$	71.0 ± 0.6	9.72 ± 0.04	98.96 ± 0.94
$[C_4C_1pyr]Cl$	71.4 ± 0.9	9.58 ± 0.02	98.09 ± 1.82
$[C_4C_1im]Cl$	60.5 ± 1.0	8.79 ± 0.04	97.33 ± 4.31
$[C_8C_1im]Cl$	45.9 ± 0.6	7.39 ± 0.01	93.65 ± 1.91
$[C_4C_1im]Br$	71.6 ± 0.1	8.37 ± 0.01	94.46 ± 5.12
$[C_4C_1im][SCN]$	93.8 ± 1.1	7.70 ± 0.33	95.32 ± 3.84
$[C_4C_1im][N(CN)_2]$	80.4 ± 0.7	9.34 ± 0.03	96.53 ± 1.54
$[C_4C_1im][CF_3SO_3]$	90.0 ± 0.7	7.86 ± 0.13	85.33 ± 8.14

Table S4. Initial mixture composition (wt%), percentage extraction efficiencies of ciprofloxacin and diclofenac (% EE_{FQs} and % EE_{NSAIDs}, respectively) and tie-line length (TLL), with corresponding standard deviations (σ).

	Ciprofloxacin							
IL	**Weight fraction percentage / wt %**						**TLL**	**% EE_{FQs} ± σ**
	$[IL]_{salt}$	**$[salt]_{salt}$**	**$[IL]_{M}$**	**$[salt]_{M}$**	**$[IL]_{IL}$**	**$[salt]_{IL}$**		
	2.8	43.5	39.8	15.1	55.2	3.4	65.82 ± 0.17	99.75 ± 0.29
$[N_{4444}]Cl$	1.9	46.4	39.9	18.8	63.3	2.0	77.51 ± 0.28	99.37 ± 0.73
	0.5	53.7	39.9	24.1	71.5	1.0	87.64 ± 0.35	99.77 ± 0.27
	Diclofenac							
IL	**Weight fraction percentage / wt %**						**TLL**	**% EE_{NSAIDs} ± σ**
	$[IL]_{salt}$	**$[salt]_{salt}$**	**$[IL]_{M}$**	**$[salt]_{M}$**	**$[IL]_{IL}$**	**$[salt]_{IL}$**		
	3.3	41.5	40.2	14.8	56.4	3.1	65.51 ± 0.66	99.26 ± 0.51
$[N_{4444}]Cl$	1.8	46.0	40.1	18.9	64.2	1.9	76.40 ± 0.17	99.71 ± 0.70
	0.7	51.8	40.3	24.3	73.9	0.8	89.18 ± 0.77	99.32 ± 0.82

Table S5. Percentage extraction efficiencies of ciprofloxacin and diclofenac (% EE_{FQs} and % EE_{NSAIDs}, respectively) with corresponding standard deviations (σ), initial mixture composition (wt%) and concentration factor (CF).

	Ciprofloxacin			
IL	**Weight fraction percentage / wt%**		**% EE_{FQs} ± σ**	**CF**
	$[IL]_M$	**$[salt]_M$**		
$[N_{4444}]Cl$	39.92	24.14	99.8 ± 0.3	0.6
	24.35	35.07	99.7 ± 0.4	1.1
	13.98	42.52	99.7 ± 0.5	2.0
	8.07	46.54	99.5 ± 0.8	3.3
	2.99	49.85	99.7 ± 0.4	9.0
	0.53	51.74	99.9 ± 0.2	1084.5
	Diclofenac			
IL	**Weight fraction percentage / wt%**		**% EE_{NSAIDs} ± σ**	**CF**
	$[IL]_M$	**$[salt]_M$**		
$[N_{4444}]Cl$	40.11	24.35	99.3 ± 0.8	0.7
	24.38	35.12	99.6 ± 0.7	1.1
	14.01	42.50	99.4 ± 0.8	2.1
	8.02	46.53	99.0 ± 0.8	3.3
	3.02	50.03	99.1 ± 1.2	8.0
	0.53	51.74	99.9 ± 0.2	1164.4

7.3. Supporting Figures

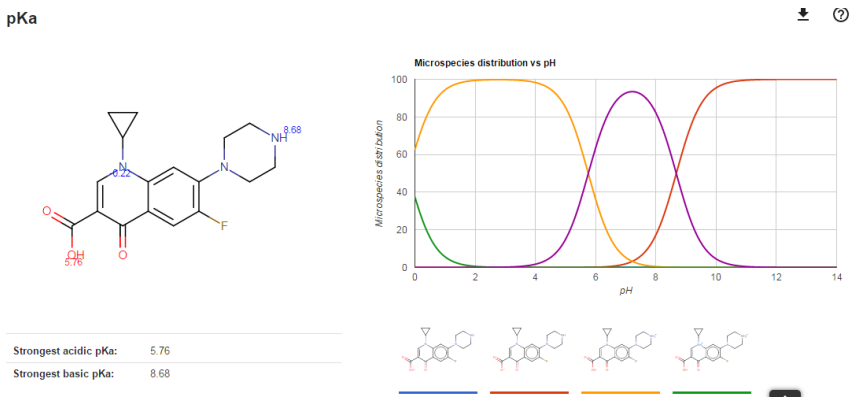

Figure S1. Speciation profile of ciprofloxacin as a function of the pH.[49]

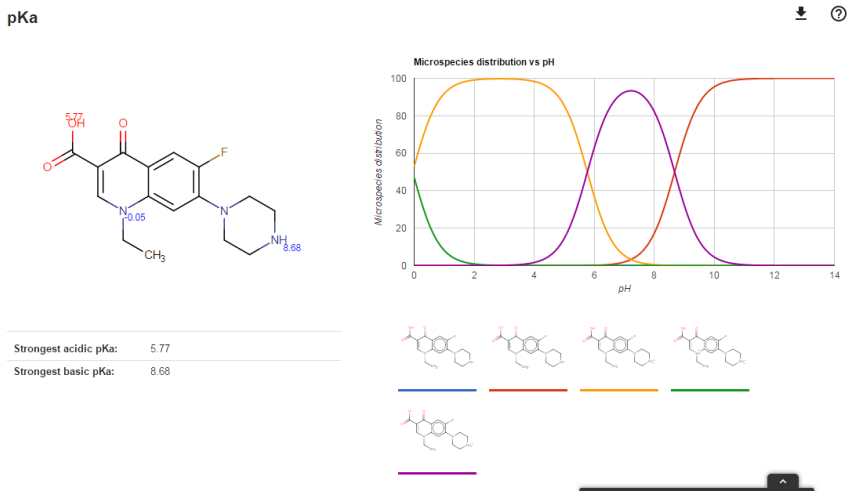

Figure S2. Speciation profile of norfloxacin as a function of the pH.[49]

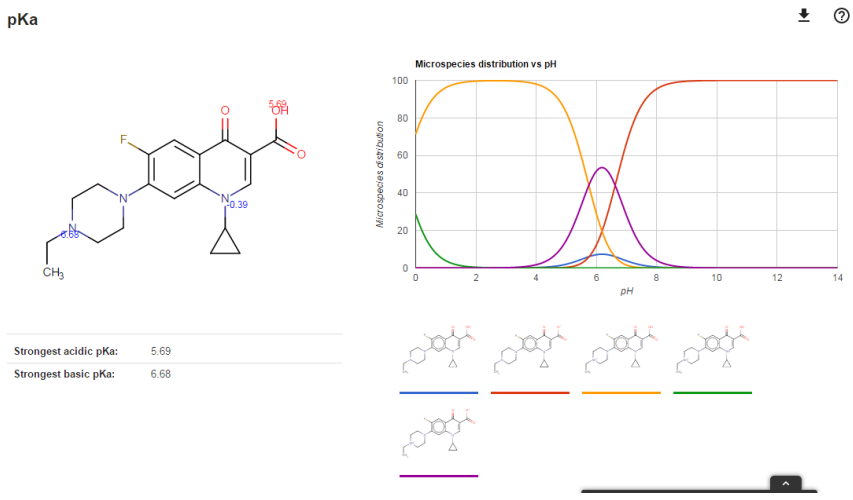

Figure S3. Speciation profile of enrofloxacin as a function of the pH.[49]

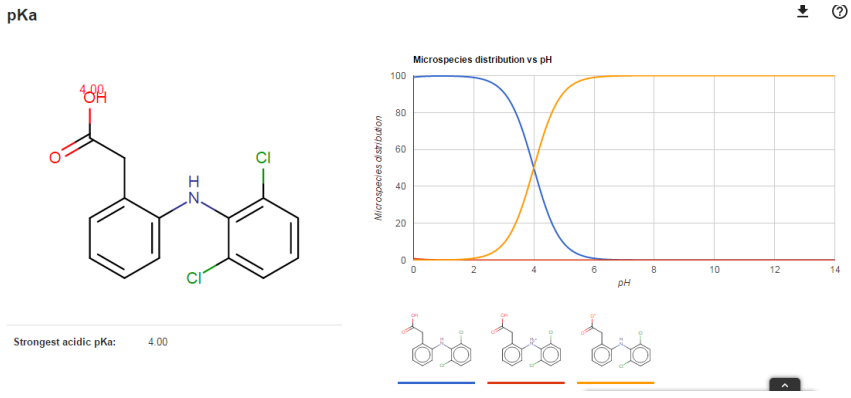

Figure S4. Speciation profile of diclofenac as a function of the pH.[49]

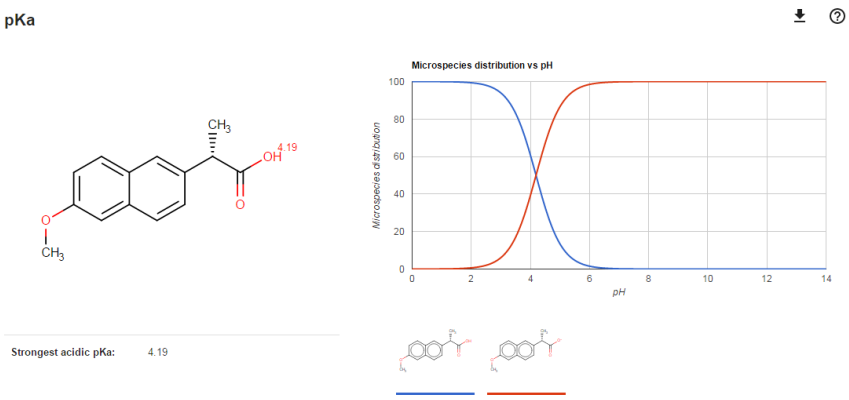

Figure S5. Speciation profile of naproxen as a function of the pH.[49]

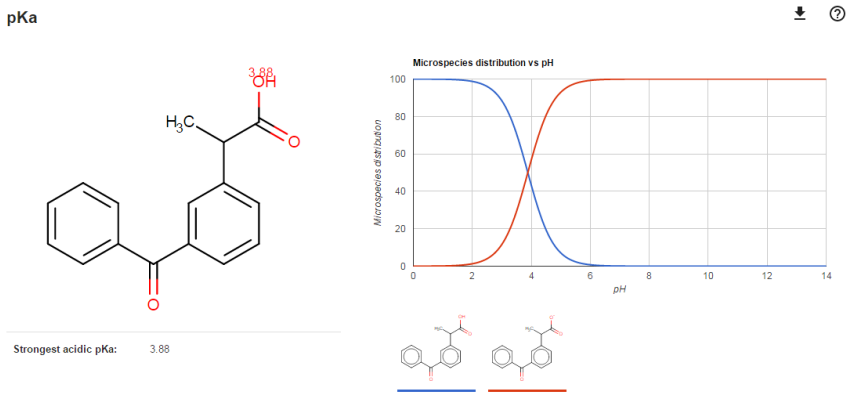

Figure S6. Speciation profile of ketoprofen as a function of the pH.[49]

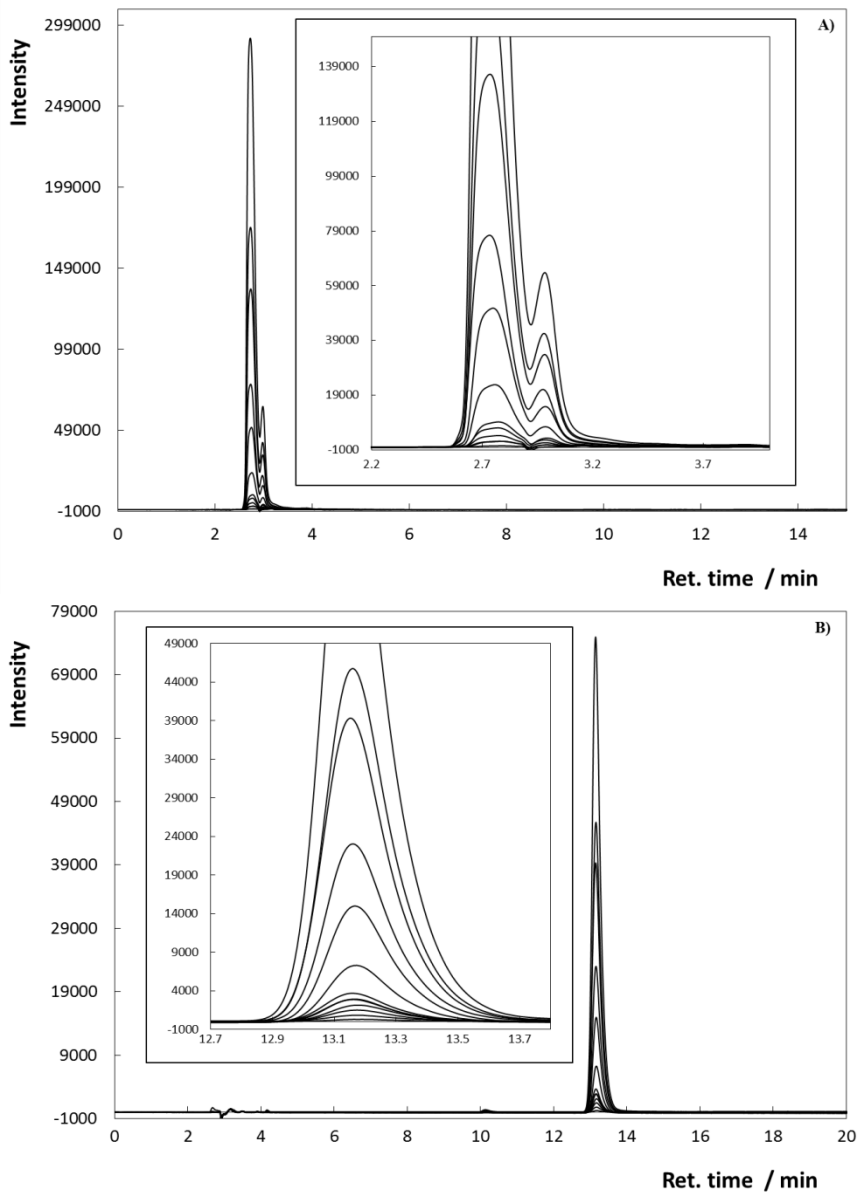

Figure S7. HPLC-UV chromatograms of (A) ciprofloxacin and (B) diclofenac at different concentrations at 278 nm and 275 nm, respectively. The concentrations used were in the range of 0.0002 to 0.0500 $g.L^{-1}$.

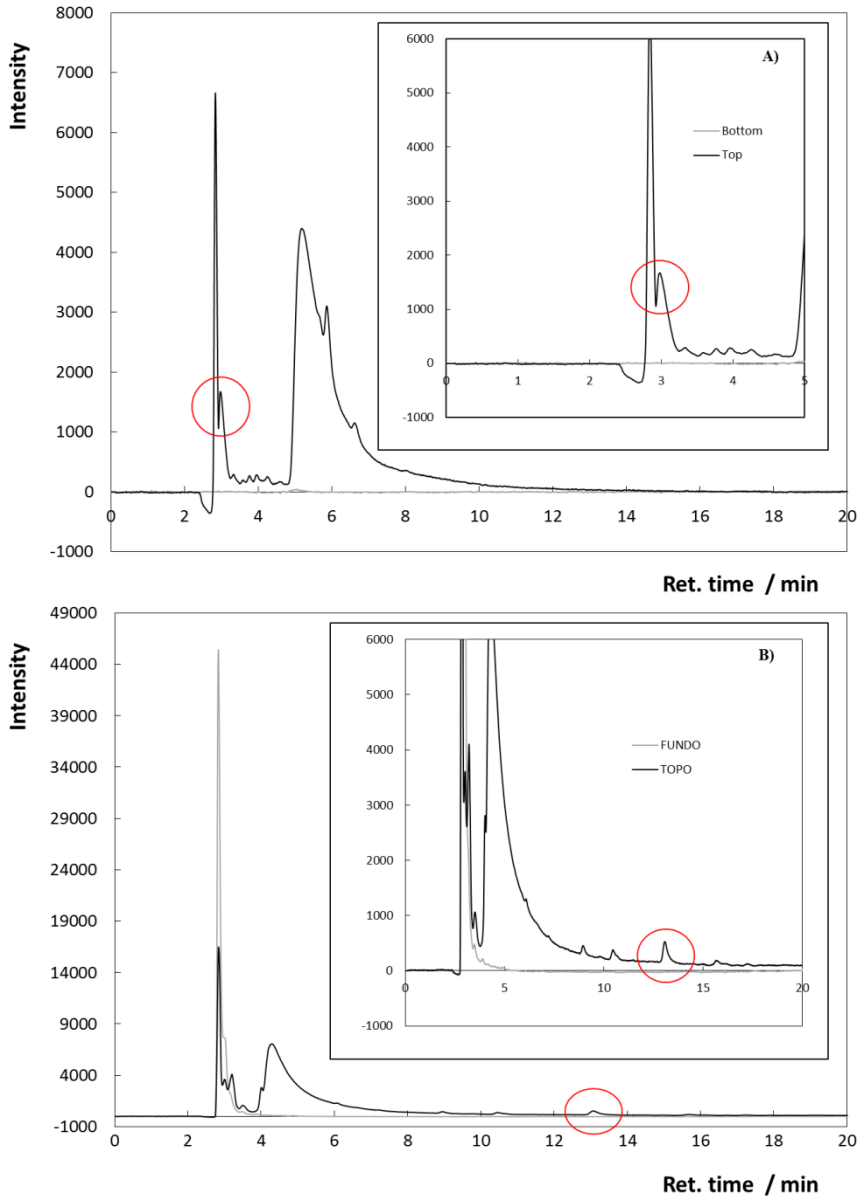

Figure S8. HPLC-UV chromatograms after the ABS concentration step with model systems (distilled water) containing (A) ciprofloxacin and (B) diclofenac, at 278 nm and 275 nm, respectively. Circles are representative of ciprofloxacin and diclofenac.

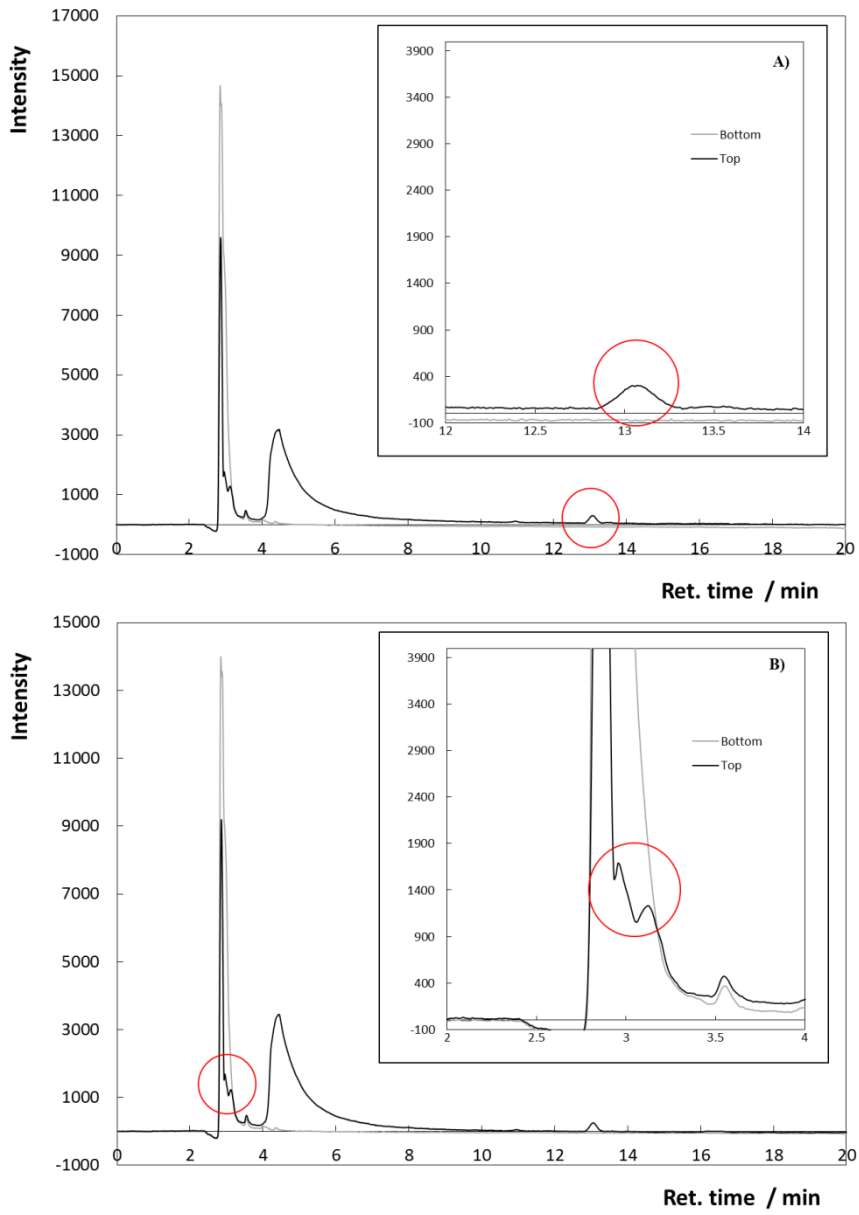

Figure S9. HPLC-UV chromatograms after the ABS concentration step with model systems/distilled water containing a mixture of (A) ciprofloxacin and (B) diclofenac, at 278 nm and 275 nm, respectively. Circles are representative of ciprofloxacin and diclofenac.

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Chapter 3

Removal of Non-Steroidal Anti-Inflammatory Drugs from Aqueous Environments with Reusable Ionic-Liquid-based Systems

Adapted from: **Hugo F. D. Almeida**, Isabel M. Marrucho, and Mara G. Freire; Removal of Non-Steroidal Anti-Inflammatory Drugs from Aqueous Environments with Reusable Ionic-Liquid-based Systems; *ACS Sustain. Chem. Eng.*, **2017**, 5, 2428-2436.

The author was also involved in all the experiments, as well as on the discussion and interpretation of the data and preparation of the manuscript.

1. Abstract

In the current era of human life, we have been facing an increased consumption of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs). Nevertheless, NSAIDs are not entirely metabolized by humans, and are thus excreted into domestical effluents, whereas expired medications are recurrently straightly disposed into wastewaters. Several studies already demonstrated that an extensive diversity of pharmaceuticals is present in aqueous effluents and are therefore a matter of serious concern to wildlife and public health. In this perspective, this work is focused on the use of a liquid-liquid extraction approach for the removal of NSAIDs from aqueous media. In particular, aqueous biphasic systems (ABS) composed of ionic liquids (ILs) and aluminium-based salts were used for the removal of diclofenac, ibuprofen, naproxen and ketoprofen. With these systems, extraction efficiencies of NSAIDs up to 100 % into the IL-rich phase were obtained in a single-step. Further, the recovery of NSAIDs from the IL medium and the recyclability of the IL-rich phase were ascertained aiming at developing a more sustainable and cost-effective strategy. Based on the remarkable increase of NSAIDs solubility in the IL-rich phase (from a 300- to a 4100-fold when compared with pure water), water was used as an effective anti-solvent, where recovery percentages of NSAIDs from the IL-rich phase up to 91 % were obtained. After the "cleaning" of the IL-rich phase by the induced precipitation of NSAIDs, the phase-forming components were recovered and reused in four consecutive cycles, with no detected losses on both the extraction efficiency and recovery of NSAIDs.

2. Introduction

In the past years, the detection of emerging pollutants in diverse environmental matrices has been the focus of large concerns and debate. The

classification of pharmaceuticals and personal care products (PPCPs) as relevant pollutants was firstly recommended by Daughton and Ternes,[1] being currently classified as emerging contaminants according to the United Nations Environmental Program (UNEP).[2] Advances on analytical techniques have allowed their identification in an increasing number of environmental matrices.[3-6] Active pharmaceutical ingredients (APIs) belong to the PPCPs class, and have particularly raised severe concerns in more recent years after their non-negligible levels identification in aqueous environments.[7-14] APIs, known as mutagenic, carcinogenic, and endocrine disruptors, have been found in concentrations up to $\mu g.L^{-1}$ in worldwide effluents of sewage treatment plants (STPs), wastewater treatment plants (WWTPs), freshwaters (rivers and lakes) and estuarine/marine waters.[7, 10, 13, 15-18] A global occurrence and perspective of pharmaceuticals in the environment has been summarized by aus de Beek et al.[18] APIs found in the environment include prescription drugs, drugs used in hospital by humans and veterinary drugs.[10, 19-21] Variable quantities of the taken doses are metabolized by organisms whereas the rest is excreted (in either metabolized or unchanged forms).[9, 10, 22-28] According to Heberer[29] and Daughton and Ternes,[1] the consumed PPCPs are mainly excreted through urine or feaces as a mixture of their original and metabolized forms. For instance, Vieno and Sillanpää[30] investigated the metabolic path of diclofenac in humans, showing that between 65 % and 75 % and between 20 % and 30 % of the orally administered dose is excreted through urine and feaces, respectively, as the parent drug or in the form of metabolites. Furthermore, according to Dias-Ferreira et al.[31] each household keeps an average of 1097 g of pharmaceutical products, with 20 % in current use, 72 % not in use, and 8 % as expired products ready to be discarded. As a result, most of the unnecessary or expired medications are recurrently straightly disposed into wastewaters.[10, 20, 21] Even at low concentrations, the continuous contact with APIs leads to deleterious effects in living organisms.[10] These compounds have important side effects, where different organs, tissues, cells or biomolecules, may be affected.[10]

Based on extensive criteria, the Global Water Research Coalition (GWRC) selected ten priority APIs.[32] This list comprises antibiotics, anti-epileptics, anti-inflammatory drugs, β-blockers and lipid regulators.[10, 20, 21] Although WWTPs use advanced processes for water purification, such as membrane filtration, ozonation, chlorination, flocculation/sedimentation and adsorption, none of these processes was specifically designed to remove APIs,[9, 10, 12-14, 28] and some of these emerging pollutants were already identified in drinking water.[29,33, 34]

Within APIs, the non-steroidal anti-inflammatory drugs (NSAIDs) diclofenac, ibuprofen and naproxen are included in the list of the top 10 persistent pollutants.[19] These compounds display a high-octanol partition coefficient (K_{ow}), and thus a high ability to passively diffuse across biological membranes, low pK_a values and high persistence in aquatic environments.[35] Some classic methods have already been tested for the removal of NSAIDs; in particular, the addition of several salts to promote the coagulation of ibuprofen, naproxen, diclofenac, carbamazepine and diazepam was investigated, whereas the best results were obtained for diclofenac with 50 % of removal efficiency.[36] Ozonation[37] and chloride oxidation[38] have also been studied for NSAIDs degradation, where ozone was found to be the most effective oxidizer. Kahn et al.[39] compared several techniques, such as lime clarification, dissolved air flotation, dual media filtration, activated carbon, combined reverse-osmosis/nanofiltration, ozonation, and UV disinfection units for the removal or degradation of NSAIDs. The authors[39] concluded that reverse osmosis is an effective process for removing a wide range of pharmaceuticals, yet it is highly energy-intensive. Therefore, the development of a cost-efficient removal technique for NSAIDs from aqueous media is an urgent requirement of modern society.

Aqueous biphasic systems (ABS), as liquid-liquid extraction techniques, are formed by two aqueous-rich phases, which result from the dissolution in water of two water-soluble phase-forming components above certain concentrations. Generally, two non-volatile compounds, such as two polymers, a salt and a polymer or two salts, allow the creation of ABS.[40, 41] In addition to the two phase-

forming components, ABS are mainly composed of water and are thus considered as more environmentally friendly liquid-liquid extraction approaches. The partition/extraction of purpose given compound occurs between the two coexisting phases, in which the chemical nature and physical properties of both the phase-forming components and solute are crucial. Nevertheless, more conventional polymer-based ABS display a limited polarity difference between the two phases, resulting in restricted extraction performance and selectivity. In the past few years, the polymers functionalization and addition of ligands have been investigated to overcome this constraint.[42, 43]

In 2003, Rogers and co-workers[44] demonstrated that by adding an inorganic salt to an aqueous solution of a given ionic liquid (IL) there is the formation of ABS. After this pioneering work, it was latter demonstrated that these systems can be created with a large number of salts, amino acids, carbohydrates and polymers, offering a new plethora of extraction/separation systems.[45] Even though many ILs display some exceptional properties, namely a negligible vapor pressure, non-flammability, high thermal and chemical stabilities, and a large liquid temperature range,[46-49] the most important feature conveys on their tailoring ability (by a suitable choice of their ions), which is transferrable to IL-based ABS.[50] In fact, IL-based ABS already proved a superior performance on extraction efficiencies and selectivity for a wide range compounds, comprising proteins, alkaloids, phenolic compounds, dyes, among others.[45] In particular, IL-based ABS have also been investigated for the extraction of pharmaceuticals,[51-56] mainly to evaluate their performance as purification and concentration techniques,[51-54] as well as to recover value-added compounds from pharmaceutical wastes.[55, 56]

From a different perspective to the previously published works regarding the use of IL-based ABS for the concentration and purification of pharmaceuticals,[51-56] herein, we propose an integrated and highly efficient ABS-based strategy to remove and recover NSAIDs (diclofenac, ibuprofen, naproxen, and ketoprofen), as current persistent pollutants, from aqueous environments. Since STPs and WWTPs currently use $Al_2(SO_4)_3$ for the purification of drinking

water, mainly as a flocculating agent, this salt was chosen to create the IL-based ABS under study. Three different stages (mechanical, biological and disinfection treatments) are combined in a simplified version of a WTTP,[57] whereas the ABS strategy designed here for the NSAIDs removal should be introduced in the final stage. Finally, and aiming at developing a more sustainable technique for the removal of persistent pollutants from aqueous environments, the recovery of the investigated NSAIDs from the IL-rich phase and the IL recycling were also ascertained, allowing us to propose an integrated and highly efficient process which comprises the removal and recovery of NSAIDs and the phase-forming components recovery and reuse.

3. Experimental Section

3.1. Materials

The non-steroidal anti-inflammatory drugs investigated were diclofenac sodium salt (2-[(2,6-Dichlorophenyl)amino]benzene acetic acid sodium salt, CAS# 15307-79-6), ibuprofen ((±)-2-(4-Isobutylphenyl)propanoic acid, CAS# 15687-27-1), naproxen ((S)-(+)-2-(6-Methoxy-2-naphthyl)propionic acid, CAS# 22204-53-1) and ketoprofen ((RS)-2-(3-Benzoylphenyl)propionic acid, CAS# 22071-15-4), with a purity level ≥ 99 % for diclofenac, and ≥ 98 % for ibuprofen, naproxen and ketoprofen. All NSAIDs were acquired from Sigma-Aldrich, and used as received. The chemical structures of the NSAIDs investigated are depicted in Figure 19.

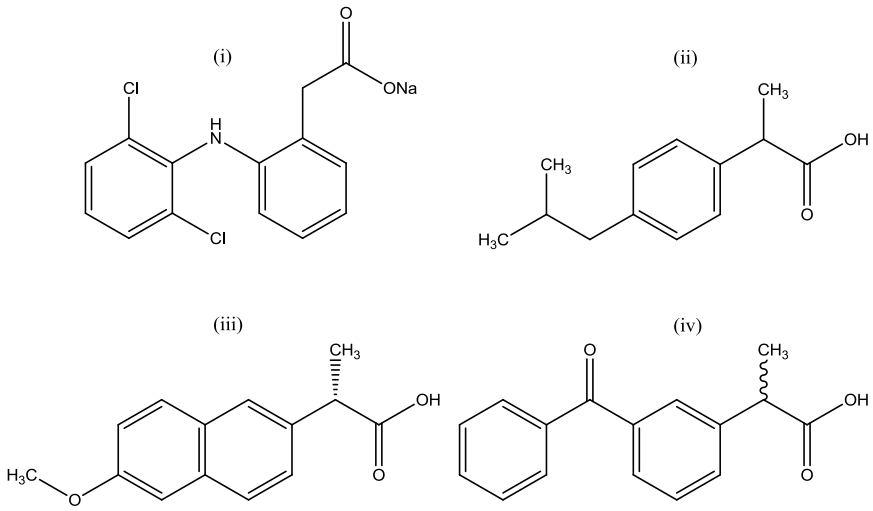

Figure 19. Chemical structures of the NSAIDs investigated: diclofenac sodium salt (i), ibuprofen (ii), naproxen (iii), and ketoprofen (iv).

The ILs used were 1-ethyl-3-methylimidazolium trifluoromethanesulfonate (triflate) ($[C_2C_1im][CF_3SO_3]$, purity 99 wt%, CAS# 145022-44-2); 1-butyl-3-methylimidazolium trifluoromethanesulfonate (triflate) ($[C_4C_1im][CF_3SO_3]$, purity 99 wt%, CAS# 174899-66-2); 1-butyl-3-methylimidazolium tosylate ($[C_4C_1im][Tos]$, purity 99 wt%, CAS# 410522-18-8); tri(isobutyl)methylphosphonium tosylate ($[P_{i(444)1}][Tos]$, purity 98 wt%, CAS# 374683-35-9); tributylmethylphosphonium methylsulfate ($[P_{4441}][CH_3SO_4]$, purity 96-98 wt%, CAS# 69056-62-8); tetrabutylphosphonium bromide ($[P_{4444}]Br$, purity 95 wt%, CAS# 3115-68-2); and tetrabutylphosphonium chloride ($[P_{4444}]Cl$, purity 97 wt%, CAS# 2304-30-5). All imidazolium-based ILs were purchased from Iolitec, while the phosphonium-based fluids were gently supplied by Cytec Industries Inc. In order to reduce the volatile impurities and water content in the IL samples, these were placed under constant stirring, at vacuum and 50 ºC, for a minimum of 24h. Only $[P_{4444}]Br$ and $[P_{4444}]Cl$ were purified at a higher temperature (100 ºC), under vacuum, and for a minimum of 72h, since both samples are commercially provided with higher amounts of

water. The purity of each IL was further checked by ^{1}H and ^{13}C NMR spectra. The chemical structures of the ILs investigated are shown in Figure 20.

The inorganic salt $Al_2(SO_4)_3$ (CAS# 17927-65-0) was acquired from José Manuel Gomes dos Santos, Lda. (purity ≥ 98.0 wt%). The water applied was doubled distilled, passed across a reverse osmosis system and further treated with Milli-Q plus 185 water purification equipment. Buffers solutions with pH of 4.00 and 7.00, acquired from Panreac, were used for the pH meter equipment calibration.

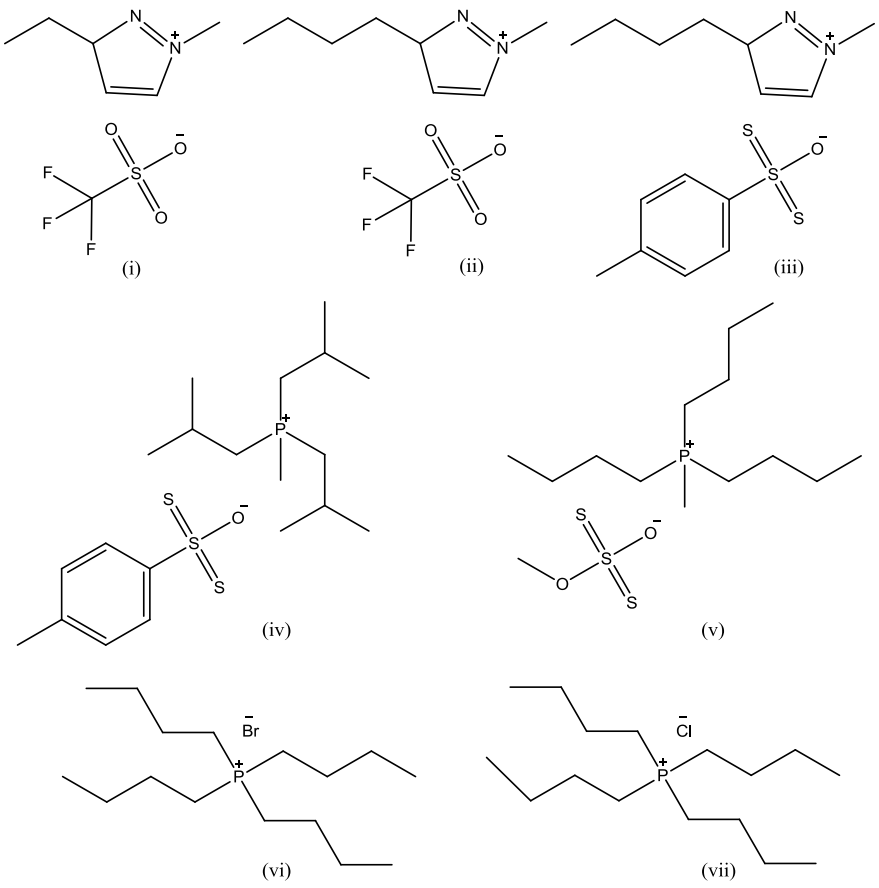

Figure 20. Chemical structures of the ILs used to form ABS: $[C_2C_1im][CF_3SO_3]$ (i), $[C_4C_1im][CF_3SO_3]$ (ii), $[C_4C_1im][Tos]$ (iii), $[P_{i(444)1}][Tos]$ (iv), $[P_{4441}][CH_3SO_4]$ (v), $[P_{4444}]Br$ (vi), and $[P_{4444}]Cl$ (vii).

3.2. Phase diagrams and tie-lines

The ABS ternary phase diagrams used in the current work were taken from the literature.[58] However, additional tie-lines (TLs), which describe the compositions of the phases in equilibrium for given mixture compositions, were determined in this work. Each TL was determined according to the lever-arm rule originally proposed by Merchuk et al.[59] Additional details on the TLs determination and respective length (tie-line length, TLL) are provided in the Supporting Information.

3.3. Removal of NSAIDs using IL-based ABS

IL-based ABS investigated for the removal of NSAIDs from aqueous media require the use of ternary mixtures (ionic liquid + salt + aqueous solutions containing the target NSAID) within the biphasic region of each system. The concentration of NSAIDs in the aqueous solutions was of 0.060 $g.L^{-1}$, 0.049 $g.L^{-1}$ and 0.046 $g.L^{-1}$ for diclofenac sodium salt, naproxen and ketoprofen, respectively. These concentrations are significantly higher than those found in STPs and WWTPs, thus guaranteeing that there is no saturation of each NSAID in the coexisting phases when envisaging the use of the proposed technology in real water samples. The ternary mixtures were prepared gravimetrically within $\pm 10^{-4}$ g, using a Mettler Toledo Excellence XS205 DualRange analytical balance, according to given weight fraction composition percentages (shown thereinafter as wt%). All mixtures were stirred and left in equilibration for 24 h at (25 ± 1) ºC, to allow the complete separation of both liquid phases and consequent NSAIDs partitioning. The two phases were then separated, and both IL- and salt-rich phases were weighted and each NSAID quantified through UV-spectroscopy, using a Shimadzu UV-1700, Pharma-Spec UV-Vis Spectrophotometer, at a wavelength of 276, 221, 230 and 258 nm for diclofenac sodium salt, ibuprofen, naproxen and ketoprofen, respectively, using calibrations curves formerly

determined. To avoid interferences of the IL and salt in the quantification of each NSAID, ternary mixtures with the same weight fraction compositions were prepared using pure water. However, in the extractions of ibuprofen and ketoprofen using the $[C_4C_1im][CF_3SO_3]$- and $[C_4C_1im][Tos]$-based ABS, a large interference of the ILs on the UV-spectroscopy quantification method was observed. Since the extraction efficiencies could not be accurately determined for these two particular systems, they are not presented.

The percentage extraction efficiencies (%*EE*) of each system for NSAIDs are defined according to:

$$\%EE = \frac{[\mathrm{NSAID}]_{\mathrm{IL}} \times w_{\mathrm{IL}}}{([\mathrm{NSAID}]_{\mathrm{IL}} \times w_{\mathrm{IL}}) + ([\mathrm{NSAID}]_{\mathrm{salt}} \times w_{\mathrm{salt}})} \times 100 \qquad (1)$$

where w_{IL} and w_{salt} are the total weight of the IL-rich phase and salt-rich phase, respectively, and $[NSAID]_{IL}$ and $[NSAID]_{salt}$ are the concentration of each NSAID in the IL-rich phase and salt-rich phase, respectively.

At least three individual systems were prepared for each ABS and each NSAID, allowing to determine the average %*EE* value and respective standard deviation. The possible loss of each NSAID (e.g. by precipitation and/or saturation of the phases) was evaluated by comparing the amount of each NSAID added and that quantified in each phase, showing that no losses of NSAIDs occurred in the systems investigated.

3.4. pH determination

The pH values (± 0.02) of the ABS coexisting phases were measured at (25 ± 1) ºC, using a Mettler Toledo S47 SevenMulti™ dual meter pH/conductivity. The calibration of the pH meter was beforehand performed with two buffers solutions with pH values of 4.00 and 7.00.

3.5. Solubility of NSAIDs in the IL-rich phase

To infer on the possible saturation of the systems investigated with NSAIDs, the solubility of each pharmaceutical in the IL-rich phase of the system composed of 58.5 wt% of $[P_{4441}][CH_3SO_4]$ + 2.2 wt% of $Al_2(SO_4)_3$ + 39.5 wt% of H_2O was determined at (25 ± 1) ºC. At least three individual systems were prepared for each NSAID, allowing to determine the average solubility value and standard deviation. To a total weight of 1 g of the IL-rich phase, small amounts of each NSAID were added, (from 0.002 up to 0.005) g, and stirred under controlled temperature (25 ± 1) ºC using an Eppendorf Thermomixer® comfort equipment. The samples were left to equilibrate and NSAIDs were continuously added until the detection of a cloud point (visual identification of the first solid in solution). After the identification of the cloud point, the samples were left under stirring for at least 24 h at (25 ± 1) ºC to guarantee that no further NSAID is dissolved and no saturation of the IL-rich phase was achieved.

3.6. Recovery of NSAIDs and IL Recycling

To ascertain on the recycling ability of the studied ABS, the recovery of the NSAIDs from the IL-rich phase was first addressed followed by the IL reuse in a new cycle of NSAIDs removal. After the extraction step and NSAIDs enrichment in the IL-rich phase, water was added to this phase as an anti-solvent, in different amounts, and the mixture was vigorously stirred. Since NSAIDs have a low water solubility,[60] and considering the recently demonstrated ILs hydrotropic effect,[61] the precipitation of NSAIDs is easily achieved by the simple addition of water. All these steps were carried out at (25 ± 1) ºC. The precipitated NSAIDs were recovered by filtration under vacuum, using a Sartorius Stedim Biotech Cellulose Nitrate filter, with a pore size of 0.45 μm. The acquired precipitate was further washed with 10 mL of deionized water, and dried at 70 ºC until constant weight.

The percentage of recovered NSAIDs (%Recovery) was determined according to:

$$\%\text{Recovery} = \frac{(w_{\text{NSAID}})_{\text{recovered}}}{(w_{\text{NSAID}})_{\text{IL-rich phase}}} \times 100 \qquad (2)$$

where $(w_{NSAID})_{recovered}$ and $(w_{NSAID})_{IL\text{-rich phase}}$ is the total weight of each NSAID after the filtration and drying step and the total mass of each NSAID at the IL-rich phase, respectively.

In order to explore the viability of the ABS reuse, it is necessary to know the composition of the IL-rich phase, so that the necessary weight of $Al_2(SO_4)_3$ and aqueous solutions containing NSAIDs for the formation of a new ABS can be directly added. This information was obtained from the phase's compositions and TLs data given in detail in the Supporting Information (Table S1 to S4). After the recovery step of NSAIDs, the IL aqueous solution was placed in a rotary evaporator at 70 ºC for the removal of excess water. The water content of the IL-rich phase was further determined by Karl-Fischer titration, using a Metrohm 831 Karl Fischer coulometer, with the Hydranal - Coulomat AG from Riedel-de Haën reagent. Then, the concentrated IL aqueous solution was recovered and different amounts of $Al_2(SO_4)_3$ and aqueous solutions of each NSAID were added to continue to a new extraction cycle. The removal of NSAIDs and recycling of the IL-rich was repeated for four consecutive cycles.

4. Results and Discussion

4.1. Removal of NSAIDs using IL-based ABS

The compositions of each ABS used in the removal of NSAIDs from aqueous media ranged between (29.97 and 42.03) wt% for the IL, whereas a fixed composition of 15 wt% was maintained for $Al_2(SO_4)_3$. These compositions were chosen in order to carry out the extraction studies at a fixed TLL (≈70), i.e. to to

maintain the difference between the compositions of the two phases, permitting therefore a better evaluation of the IL chemical structure influence. Furthermore, the use of a long TLL usually leads to an increase in the extraction efficiency[55] and to a lower cross-contamination by the constituent enriched in the opposite phase.[58] As described before, the liquid–liquid ternary phase diagrams used in this work were taken from the literature.[58] However, as stated in the experimental section, additional TLs (composition of each phase for a given mixture) were determined in this work for the mixtures compositions used in the extraction/removal studies of NSAIDs. The detailed initial mixture compositions and respective TLs used in the extraction studies of each NSAID are presented in Tables S1 to S4, in the Supporting Information. The values of the extraction efficiencies and pH of the IL-rich phase, as well as the respective standard deviations, are also provided in the Supporting Information (Tables S1 to S4).

The pH values of the IL-rich phases of the ABS prepared ranged between 2.33 and 2.99 - a consequence of the $Al_2(SO_4)_3$ acidic nature in aqueous media. Therefore, in the studied ABS, the NSAIDs investigated are preferentially in a non-charged form (pKa values > 3.88),[62] meaning that electrostatic interactions do not play a major role in the investigated ABS extraction performance. The only exception occurs for diclofenac that is a sodium salt. However, no major differences in the diclofenac partition behavior are observed, as discussed below, confirming the negligible effect of electrostatic interactions. The respective dissociation curves and pKa values of each NSAID are shown in the Supporting Information (Figures S1 to S4).

Figure 21 depicts the extraction efficiencies (%*EE*) of the investigated ABS for NSAIDs (*cf.* Figures S5 to S8 in the Supporting Information for more details). In general, all studied ABS display a remarkable one-step performance to extract NSAIDs to the IL-rich phase from aqueous media, with %*EE* varying from 91 % to 100 %. NSAIDs are highly hydrophobic molecules (logK_{ow} values ranging between 3.12[63] and 4.51[64] – Supporting Information, Table S5) and thus preferentially partition to the less hydrophilic and of lower ionic strength IL-rich

phase. Also, the preferential partition of NSAIDs to the IL-phase is also a consequence of the strong salting-out effect of the salt used.[65]

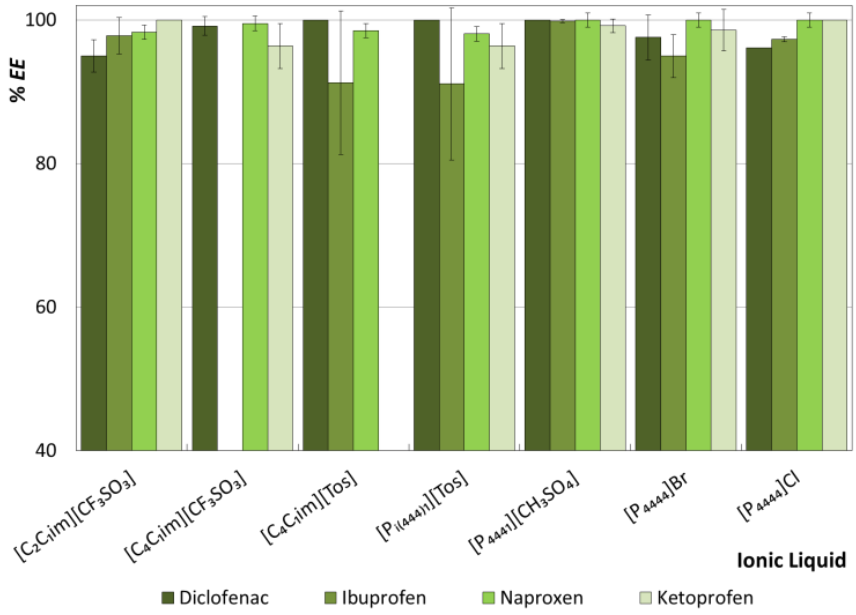

Figure 21. Extraction efficiencies (%*EE*) of ABS composed of IL + $Al_2(SO_4)_3$ + H_2O (at 25 ºC) for non-steroidal anti-inflammatory drugs.

In general, the differences on the %*EE* are dependent on both the IL employed and NSAID used. An increase in the cation alkyl side chain length (from $[C_2C_1im][CF_3SO_3]$ to $[C_4C_1im][CF_3SO_3]$), leads to an increase in the %*EE* for diclofenac and naproxen, and to an opposite behavior for ketoprofen. Regarding the IL anion effect, the $[C_4C_1im][CF_3SO_3]$-based ABS leads to higher %*EE* for diclofenac than $[C_4C_1im][Tos]$-based ones, while the opposite trend is observed for naproxen. On the other hand, small differences are observed in the %*EE* of all NSAIDs with the $[P_{4444}]Br$- and $[P_{4444}]Cl$-based systems, with the exception of ketoprofen where the last ABS seems to be more promising.

Although imidazolium-based ILs are amongst the most investigated ILs for ABS creation and further use in extraction/purification processes,[45] it is here

shown that phosphonium-based ILs display a higher ability to extract NSAIDs from aqueous media. It was already demonstrated that phosphonium-based ILs are more efficient to form ABS,[58, 66, 67] *i.e.*, require lower amounts of IL and salt to undergo phase separation, in agreement with their higher hydrophobic nature. This phenomenon is independent of the salt used and aqueous media pH.[58, 66, 67] This higher hydrophobic nature of phosphonium-based salts mainly derives from the butyl chains at the quaternary cation, which seem to be favorable for the extraction of highly hydrophobic compounds, such as NSAIDs. Moreover, lower losses of IL for the salt-rich phase (cross-contamination) are observed when phosphonium-based ILs are used. For instance, for the mixtures under study, the amount of all phosphonium-based ILs in the $Al_2(SO_4)_3$-rich phase is *ca.* or below 1 wt% – Supporting Information with detailed TL data, Tables S1 to S4. Phosphonium-based ILs also are less toxic, thermally more stable, commercially produced in larger scales, and less expensive than imidazolium-based fluids,[68, 69] which can be seen as further advantages in large-scale operations.

The NSAIDs diclofenac, ibuprofen and naproxen are included in the top 10 persistent pollutants.[19] As mentioned before, several methods have already been tested for APIs removal, such as the addition of salts[36] and reverse osmosis,[39] and APIs degradation, such as ozonation[37] and chloride oxidation.[38] However, the low extraction efficiencies provided by these techniques as well as their high energy requirements clearly indicate that the development of a cost-efficient removal technique for NAIDs from aqueous media is a crucial requirement. In this work, and amongst all the ABS investigated, the $[P_{4441}][CH_3SO_4]$-based one led to %*EE* of 100 % of all NSAIDs to the IL-rich phase at 25 ºC, achieved in a single-step, thus representing a promising alternative strategy for the treatment of aqueous environments. Taking into account these results and the advantages associated to phosphonium-based ILs discussed above, this IL was chosen for the next steps of NSAIDs recovery and IL regeneration and reuse.

4.2. Recovery of NSAIDs and IL Recycling

The solubility of all NSAIDs in the $[P_{4441}][CH_3SO_4]$-rich phase of the respective ABS was determined at 25 ºC for better understanding the high extraction ability of IL-based ABS and to design more sustainable NSAIDs removal techniques.

Table **1** presents the solubility (saturation point) of each NSAID in the $[P_{4441}][CH_3SO_4]$-rich phase and in pure water for comparison purposes.

NSAIDs are highly hydrophobic compounds, and thus present a low solubility in pure water.[60] However, from the data shown in Table 1, it is clearly shown that the solubility of NSAIDs in the $[P_{4441}][CH_3SO_4]$-rich phase is significantly higher. The solubility of NSAIDs in the IL-rich phase increases from a 300- to a 4100-fold (≈4100-fold for diclofenac, ≈1100-fold for ibuprofen, ≈1400-fold for naproxen and ≈300-fold for ketoprofen) when compared with pure water. This increase in solubility closely follows the logK_{ow} values of the investigated NSAIDs, meaning that the higher the hydrophobic nature of the drug (logK_{ow} values shown in Table S5 in the Supporting Information), the higher is the increase in the solubility observed in the IL-rich phase. This remarkable increase in the solubility of NSAIDs in aqueous media is a consequence of the ILs hydrotropic ability recently proposed.[61] Cláudio et al.[61] reported a maximum in the solubility of antioxidants in aqueous solutions of imidazolium-based ILs of 40-fold. In this work, a significantly higher increase in the solubility of NSAIDs was observed further suggesting that phosphonium-based ILs are a skilled class of hydrotropes, and that ILs can act as excellent hydrotropes of highly hydrophobic substances.

Table 1. Solubility of NSAIDs in water[60] and in the $[P_{4441}][CH_3SO_4]$-rich phase at 25 ºC.

	Solubility of NSAIDs / mg.L^{-1}	
	Water[60]	$[P_{4441}][CH_3SO_4]$-rich phase
Diclofenac	2.37	9720 ± 142
Ibuprofen	21.0	23024 ± 257
Naproxen	15.9	22594 ± 210
Ketoprofen	51.0	16780 ± 130

The boosted solvation ability of ILs for drugs (*e.g.* analgesic, non-steroidal anti-inflammatory drugs and antibiotics) has been studied by other authors,[70-72] where a significant dependence on both the IL and drug hydrophobicity-hydrophilicity character was observed. Nevertheless, in all of these studies, pure and non-water miscible ILs were investigated. Although out of the scope of this work, the remarkable ability shown here of phosphonium-based ILs to perform as hydrotopes leading to an exceptional increase on the solubility of highly hydrophobic drugs in aqueous media should be stressed. Aqueous solutions of water-soluble ILs can thus be seen as promising alternatives to increase the bioavailability of relevant pharmaceuticals.

The significantly high solubility values of NSAIDs in the IL-rich phase support the possibility of using the same system to recover large amounts of NSAIDs from aqueous media or to be used in continuous processes before reaching the system saturation. For instance, and amongst the studied NSAIDs, diclofenac presents the lowest solubility in the $[P_{4441}][CH_3SO_4]$-rich phase (9720 mg.L^{-1}). According to Pal et al.,[15] diclofenac is found in WWTP/STP effluents at a concentration *ca*. 0.0033 mg.L^{-1}. Thus, working at the composition studied in this work for the $[P_{4441}][CH_3SO_4]$-based ABS, ideally, it would be possible to treat 3319 L of efluent with 1 g of $[P_{4441}][CH_3SO_4]$, *i.e.*, up to the saturation of diclofenac in the IL-rich phase.

After the IL-rich phase saturation with each NSAID, the drugs recovery was carried out followed by the reuse of the IL, aiming at developing cost-efficient and more sustainable removal technologies. As clearly demonstrated in this work as well as in the literature,[45] the application of ILs as constituents of ABS leads to exceptional extraction performances compared to other traditional routes. Nevertheless, the IL recovery and/or recycling lagged behind and still remain a challenging assignment. Due to the negligible volatility of ILs, the recovery of the compounds extracted and the ILs reutilization are still major obstacles towards the development of more sustainable IL-based techniques. Taking into account the ILs hydrotropic nature and the low solubility of NSAIDs in pure water, the recovery of NSAIDs was herein addressed by induced precipitation from the IL-rich phase through the addition of water (the greenest solvent overall) as an anti-solvent. Several volume ratios of the IL-rich-phase:water were investigated. Table 2 presents de percentage recovery of each NSAID (%Recovery) from the IL-rich phase by the addition of different amounts of water.

Table 2. Recovery of NSAIDs from the IL-rich phase (%Recovery) and respective standard deviation (σ) by adding different volumes of water as anti-solvent.

	Volume ratio of the IL-rich-phase:water		
	1:1	1:3	1:5
	%(Recovery ± σ)		
Diclofenac	53 ± 3	68 ± 6	69 ± 3
Ibuprofen	76 ± 2	80 ± 3	83 ± 3
Naproxen	79 ± 4	86 ± 5	91 ± 2
Ketoprofen	40 ± 3	46 ± 4	48 ± 3

As expected, an increase in the volume of water added (as anti-solvent) leads to an increase of the NSAIDs precipitation, although non-significant

differences are seen between the 1:3 and 1:5 volume ratios. The NSAIDs recovery from the IL-rich phase by induced precipitation ranges between (40 and 91) %, obtained in a single-step. The NSAIDs recovery efficiency follows the order: naproxen > ibuprofen > diclofenac > ketoprofen. With the exception of the diclofenac sodium salt, the recovery of NSAIDs closely follows their hydrophobic nature, *i.e.,* the higher the logK_{ow} value the higher the recovery of each NSAID by the addition of water (*cf.* Table S5 in the Supporting Information). It seems thus that the induced precipitation of a NSAID in a salt form is more difficult to achieve by the addition of water as anti-solvent – an expected trend since salts display a higher solubility in water than their non-charged forms.

Based on the possibility of saturating the IL-rich phase and its further "cleaning", the IL-rich phase was recovered and reused in the formation of new ABS to explore their viability as continuous removal platforms for NSAIDs. At least in four sequential cycles, a decrease on the ABS ability to extract NSAIDs from aqueous media was not observed nor a decrease on the NSAIDs recovery by induced precipitation from the IL-rich phase – Figure 22 (detailed data in Table S6 in the Supporting Information).

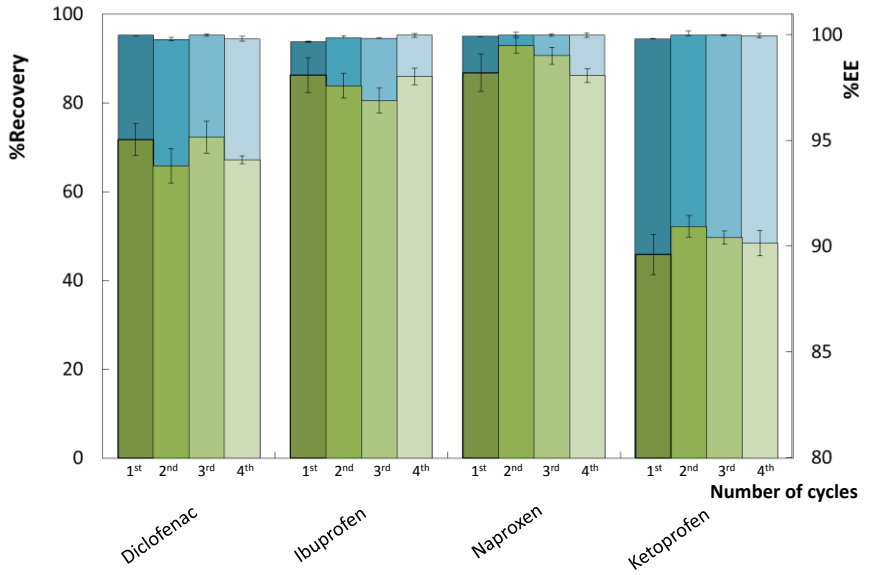

Figure 22. Recovery of non-steroidal anti-inflammatory drugs (%Recovery) from the IL-rich phase (green bars) and extraction efficiencies of non-steroidal anti-inflammatory drugs (%*EE*) (blue bars), in four consecutive cycles.

The %*EE* of the ABS is maintained at 100 %, in a single-step, along the four cycles. Thus, the $[P_{4441}][CH_3SO_4]$-based system does not lose its ability to completely remove NSAIDs from aqueous media after recovery and reuse. In the 4 cycles, more than 94 wt% of the IL was recovered and reused. This remarkable recovery of the IL is a main result of the strong salting-out ability of the salt used, $Al_2(SO_4)_3$, as previously discussed, with the additional advantage of being currently used in the treatment of drinking water as a flocculant agent.[73] Furthermore, the NSAIDs recovery efficiencies in the four cycles are similar to those previously presented (Table 2). Table S6 in the Supporting Information presents the detailed results in the four sequential cycles.

In summary, the use of ABS composed of $[P_{4441}][CH_3SO_4]$ + $Al_2(SO_4)_3$ allows the complete removal of NSAIDs from aqueous media in a single-step, the further cleaning of the IL-rich phase and NSAIDs recovery by the addition of water

as anti-solvent, and further IL reuse in the creation of new ABS. Figure 23 depicts the developed integrated process for NSAIDs removal from aqueous media, followed by the combined steps of NSAIDs removal and IL-rich phase recycling, thus ensuring the sustainability of the proposed process.

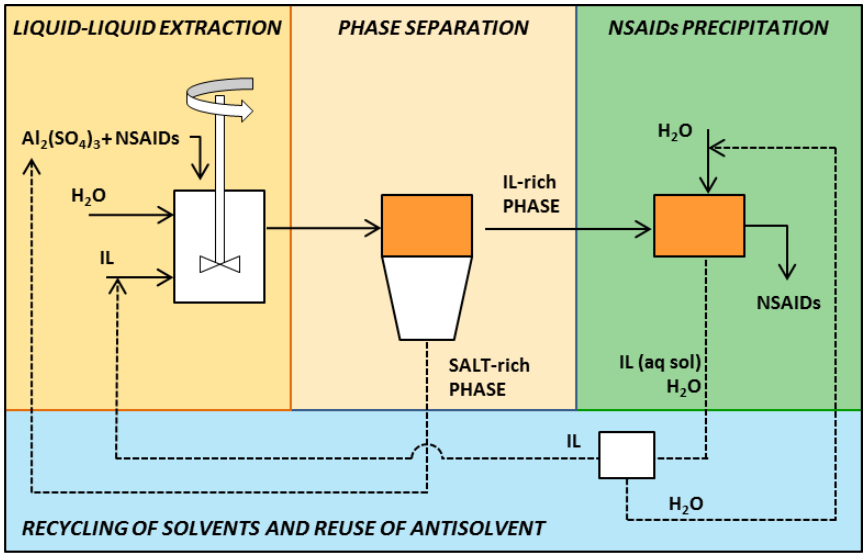

Figure 23. Representative scheme of the overall process for NSAIDs removal, comprising the NSAIDs recovery and IL recycling (bold lines and dashed lines represent the direct and indirect inputs, respectively).

5. Conclusion

A novel method to remove NSAIDs, such as diclofenac, ibuprofen, naproxen and ketoprofen, from aqueous media was here proposed. ABS composed of $Al_2(SO_4)_3$ and ILs allow extraction efficiencies of NSAIDs up to 100 % to be obtained in a single-step. Amongst the ILs investigated, phosphonium-based fluids display the best performance.

In addition to the high ability of IL-based ABS to extract an extensive number of compounds, the IL recycling and reuse remains an incomplete

approach within the scientific community dealing with IL-based ABS. Nevertheless, this step is crucial towards the development of greener and more sustainable and cost-effective IL-based processes. To overcome this main lacuna, an integrated process was proposed here and comprises: (i) the NSAIDs removal from the aqueous media; (ii) the NSAIDs recovery from the IL-rich phase by induced precipitation; and (iii) the IL recovery and reuse. Based on the high hydrophobic nature of NSAIDs, a proper choice of an anti-solvent, namely water which stands amongst the greener solvents, was used in order to precipitate NSAIDs and to "clean" the IL-rich phase, in which recovery percentages of NSAIDs up to 91 % were obtained in a single-step. The IL was then recovered (more than 94 wt%) and reused in 4 consecutive cycles, contributing to the sustainability of the proposed process and with no losses on the ABS extraction performance.

The proposed integrated process represents an improvement towards the use of IL-based ABS comprising the recyclability of the system and contributing to a circular economy, while demonstrating the relevant potential of these systems to remove pharmaceutical drugs from aqueous media and by unlocking new doors to the treatment of aqueous streams/effluents.

6. Acknowledgment

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7. Supporting Information

7.1. Phase Diagrams and Tie-Lines (TLs)

TLs were determined by the lever-arm rule through the relationship between the ionic-liquid-rich phase composition and the overall system composition, and for which the following equations (eqs. (S1) –(S4)) and unknown values ($[IL]_{IL}$, $[IL]_{salt}$, $[salt]_{IL}$, $[salt]_{salt}$) were solved:[59]

$$[\mathrm{IL}]_{\mathrm{IL}} = \mathrm{A}\exp[(\mathrm{B} \times [\mathrm{salt}]_{\mathrm{IL}}^{0.5}) - (\mathrm{C} \times [\mathrm{salt}]_{\mathrm{IL}}^{3})] \quad \text{(S1)}$$

$$[\mathrm{IL}]_{\mathrm{salt}} = \mathrm{A}\exp[(\mathrm{B} \times [\mathrm{salt}]_{\mathrm{salt}}^{0.5}) - (\mathrm{C} \times [\mathrm{salt}]_{\mathrm{salt}}^{3})] \quad \text{(S2)}$$

$$[\mathrm{IL}]_{\mathrm{IL}} = \frac{[\mathrm{IL}]_{\mathrm{M}}}{\alpha} - \frac{1-\alpha}{\alpha} \times [\mathrm{IL}]_{\mathrm{salt}} \quad \text{(S3)}$$

$$[\mathrm{salt}]_{\mathrm{IL}} = \frac{[\mathrm{salt}]_{\mathrm{M}}}{\alpha} - \frac{1-\alpha}{\alpha} \times [\mathrm{salt}]_{\mathrm{salt}} \quad \text{(S4)}$$

where IL, salt and M are the IL-rich phase, the salt-rich phase and the mixture, respectively. [IL] and [salt] correspond to the weight fraction percentage of IL and salt, respectively, and α is the ratio between the weight of the IL-rich phase and the total weight of the mixture. The system solution results in the weight fraction percentage (wt%) of the ionic liquid and inorganic salt in the IL- and salt-rich phases.

For the calculation of each tie-line length (TLL), the following equation was applied:

$$\mathrm{TLL} = \sqrt{([\mathrm{salt}]_{\mathrm{IL}} - [\mathrm{salt}]_{\mathrm{salt}})^2 + ([\mathrm{IL}]_{\mathrm{IL}} - [\mathrm{IL}]_{\mathrm{salt}})^2} \quad \text{(S5)}$$

where IL and salt represents the IL-rich phase and salt-rich phase, respectively, and [IL] and [salt] correspond to the weight fraction percentage of IL and salt.

7.2. Supporting Tables

Table S1. Weight fraction percentage (wt%) of IL ([IL]) and $Al_2(SO_4)_3$ ([salt]) in the initial mixture ($[IL]_M$, $[salt]_M$), salt-rich phase ($[IL]_{salt}$, $[salt]_{salt}$), and IL-rich-phase ($[IL]_{IL}$, $[salt]_{IL}$), tie-line length (TLL), pH values of the IL-rich phase (pH_{IL}), and extraction efficiencies (%*EE*) of the system for diclofenac. Standard deviations (σ) are included according to the results of at least three replicates.

	Diclofenac sodium salt								
	wt%						**TLL ± σ**	**pH_{IL} ± σ**	**%*EE* ± σ**
Ionic Liquid	**$[IL]_{salt}$**	**$[salt]_{salt}$**	**$[IL]_M$**	**$[salt]_M$**	**$[IL]_{IL}$**	**$[salt]_{IL}$**			
$[C_2C_1im][CF_3SO_3]$	2.69	41.77	41.44	15.15	61.41	1.43	71.6 ± 0.5	2.63 ± 0.02	94.97 ± 2.23
	2.56	42.13	42.03	14.98	61.79	1.38			
$[C_4C_1im][CF_3SO_3]$	4.17	23.90	29.97	15.02	72.10	0.53	72.4 ± 0.5	2.97 ± 0.00	99.16 ± 1.29
	4.37	23.59	30.02	14.99	73.26	0.48			
$[C_4C_1im][Tos]$	0.08	49.28	41.99	15.00	54.72	4.59	70.6 ± 0.0	2.99 ± 0.01	100.0 ± 0.00
	0.09	48.97	42.01	15.01	54.94	4.54			
$[P_{i(444)1}][Tos]$	0.08	37.01	38.02	14.99	58.21	3.28	67.0 ± 0.3	2.56 ± 0.03	100.0 ± 0.00
	0.05	37.99	37.99	15.00	57.10	3.41			
$[P_{4441}][CH_3SO_4]$	1.37	40.89	40.00	15.00	59.20	2.13	69.6 ± 0.1	2.93 ± 0.01	100.0 ± 0.00
	1.35	41.04	39.97	15.00	59.03	2.15			
$[P_{4444}]Br$	0.70	33.13	35.01	15.01	59.79	1.93	66.9 ± 0.1	2.33 ± 0.04	97.57 ± 3.12
	0.73	32.95	35.01	15.01	60.06	1.91			
$[P_{4444}]Cl$	0.27	46.96	40.05	14.97	55.01	2.95	70.3 ± 0.1	2.80 ± 0.01	99.11 ± 4.98
	0.27	46.97	40.10	15.00	55.13	2.94			

Table S2. Weight fraction percentage (wt%) of IL ([IL]) and $Al_2(SO_4)_3$ ([salt]) in the initial mixture ($[IL]_M$, $[salt]_M$), salt-rich phase ($[IL]_{salt}$, $[salt]_{salt}$), and IL-rich-phase ($[IL]_{IL}$, $[salt]_{IL}$), tie-line length (TLL), pH values of the IL-rich phase (pH_{IL}), and extraction efficiencies (%*EE*) of the system for ibuprofen. Standard deviations (σ) are included according to the results of at least three replicates.

	Ibuprofen								
	wt%						**TLL ± σ**	**pH_{IL} ± σ**	**%*EE* ± σ**
Ionic Liquid	**$[IL]_{salt}$**	**$[salt]_{salt}$**	**$[IL]_M$**	**$[salt]_M$**	**$[IL]_{IL}$**	**$[salt]_{IL}$**			
$[C_2C_1im][CF_3SO_3]$	2.69	41.77	42.00	14.99	61.41	1.43	71.6 ± 0.5	2.78 ± 0.02	97.81± 2.53
	2.56	42.13	70.90	15.02	61.79	1.38			
$[C_4C_1im][CF_3SO_3]$	3.96	24.21	30.08	14.98	70.81	0.59	71.7 ± 1.8	3.05 ± 0.02	*a*
	4.33	23.66	30.00	15.01	73.11	0.49			
$[C_4C_1im][Tos]$	0.10	48.56	42.01	15.00	55.11	4.50	70.5 ± 0.1	3.13 ± 0.01	91.26 ± 10.0
	0.09	48.91	42.03	15.00	54.99	4.53			
$[P_{i(444)1}][Tos]$	0.07	37.06	37.95	15.04	58.16	3.29	67.2 ± 0.0	2.71 ± 0.01	91.09 ± 10.6
	0.07	37.08	37.99	15.00	58.10	3.29			
$[P_{4441}][CH_3SO_4]$	1.36	40.94	40.04	15.00	59.23	2.13	70.7 ± 1.5	3.04 ± 0.02	99.83 ± 0.21
	0.36	50.53	40.04	14.99	53.81	2.67			
$[P_{4444}]Br$	0.65	33.55	35.99	15.01	61.10	1.84	68.3 ± 0.1	2.48 ± 0.01	94.95 ± 2.97
	0.65	33.51	36.03	15.03	61.30	1.83			
$[P_{4444}]Cl$	0.29	46.58	40.02	15.03	55.27	2.92	70.2 ± 0.0	3.00 ± 0.04	97.31 ± 0.35
	0.27	47.04	39.98	15.00	54.89	2.96			

[a]not performed due to quantification problems

Table S3. Weight fraction percentage (wt%) of IL ([IL]) and $Al_2(SO_4)_3$ ([salt]) in the initial mixture ($[IL]_M$, $[salt]_M$), salt-rich phase ($[IL]_{salt}$, $[salt]_{salt}$), and IL-rich-phase ($[IL]_{IL}$, $[salt]_{IL}$), tie-line length (TLL), pH values of the IL-rich phase (pH_{IL}), and extraction efficiencies (%*EE*) of the system for naproxen. Standard deviations (σ) are included according to the results of at least three replicates.

	Naproxen								
	wt%						**TLL ± σ**	**pH_{IL} ± σ**	**%*EE* ± σ**
Ionic Liquid	**$[IL]_{salt}$**	**$[salt]_{salt}$**	**$[IL]_M$**	**$[salt]_M$**	**$[IL]_{IL}$**	**$[salt]_{IL}$**			
$[C_2mim][CF_3SO_3]$	2.88	41.30	41.99	14.99	62.35	1.30	73.7 ± 2.8	2.75 ± 0.01	98.31 ± 3.26
	0.24	54.79	41.99	15.01	55.03	2.58			
$[C_4mim][CF_3SO_3]$	4.24	23.78	30.03	14.98	72.44	0.52	72.6 ± 0.8	3.04 ± 0.02	99.53 ± 0.06
	4.45	23.49	29.99	15.01	73.86	0.46			
$[C_4mim][Tos]$	0.11	48.32	41.97	15.00	55.17	4.49	70.4 ± 0.1	3.03 ± 0.01	98.53 ± 0.77
	0.10	48.61	41.99	15.01	55.08	4.51			
$[P_{i(444)1}][Tos]$	0.04	38.50	43.01	13.00	59.66	3.12	68.0 ± 1.9	1.50 ± 0.05	98.05 ± 1.02
	0.05	37.90	37.95	14.97	57.05	3.42			
$[P_{4441}][CH_3SO_4]$	1.31	41.28	39.97	15.01	58.88	2.16	69.6 ± 0.0	1.54 ± 0.01	99.99 ± 0.02
	1.24	41.73	39.97	15.00	58.52	2.19			
$[P_{4444}]Br$	0.63	33.72	35.01	15.00	58.91	1.99	66.4 ± 0.1	1.48 ± 0.02	100.0 ± 0.00
	0.67	33.41	34.93	15.01	59.21	1.97			
$[P_{4444}]Cl$	0.24	47.55	39.99	14.98	54.62	2.99	70.1 ± 0.2	2.65 ± 0.02	100.0 ± 0.00
	0.34	45.82	39.99	14.99	55.56	2.89			

Table S4. Weight fraction percentage (wt%) of IL ([IL]) and $Al_2(SO_4)_3$ ([salt]) in the initial mixture ($[IL]_M$, $[salt]_M$), salt-rich phase ($[IL]_{salt}$, $[salt]_{salt}$), and IL-rich-phase ($[IL]_{IL}$, $[salt]_{IL}$), tie-line length (TLL), pH values of the IL-rich phase (pH_{IL}), and extraction efficiencies (%*EE*) of the system for ketoprofen. Standard deviations (σ) are included according to the results of at least three replicates.

	Ketoprofen								
	wt%						**TLL ± σ**	**pH_{IL} ± σ**	**%*EE* ± σ**
Ionic Liquid	**$[IL]_{salt}$**	**$[salt]_{salt}$**	**$[IL]_M$**	**$[salt]_M$**	**$[IL]_{IL}$**	**$[salt]_{IL}$**			
$[C_2C_1im][CF_3SO_3]$	2.90	41.24	42.02	14.98	62.43	1.29	71.7 ± 0.1	2.71 ± 0.01	100.0 ± 0.00
	2.89	41.28	42.05	14.99	62.48	1.28			
$[C_4C_1im][CF_3SO_3]$	4.19	23.86	30.02	14.98	72.06	0.53	72.1 ± 0.4	2.97 ± 0.01	96.39 ± 3.13
	4.28	23.74	30.00	15.02	72.82	0.50			
$[C_4C_1im][Tos]$	0.08	49.28	42.01	15.01	54.71	4.59	70.6 ± 0.0	2.99 ± 0.02	*a*
	0.09	49.31	42.09	15.05	54.68	4.65			
$[P_{i(444)1}][Tos]$	0.07	37.09	37.98	15.00	58.06	3.30	66.9 ± 0.3	2.62 ± 0.00	96.39 ± 3.13
	0.05	37.99	37.99	15.00	57.10	3.41			
$[P_{4441}][CH_3SO_4]$	1.42	40.56	39.96	15.00	59.38	2.12	69.6 ± 0.0	2.98 ± 0.01	99.18 ± 0.98
	1.35	41.04	39.97	15.00	59.03	2.15			
$[P_{4444}]Br$	0.69	33.26	36.00	14.98	61.43	1.82	68.3 ± 0.1	2.36 ± 0.01	98.57 ± 2.86
	0.68	33.31	35.95	14.97	61.22	1.83			
$[P_{4444}]Cl$	0.24	47.52	39.95	14.97	54.56	3.00	70.2 ± 0.0	2.92 ± 0.00	100.0 ± 0.00
	0.27	47.05	39.99	14.99	54.88	2.96			

[a]not performed due to quantification problems

Table S5. Octanol-Water Partition Coefficients (logK_{ow}) of NSAIDs.

NSAID	logK_{ow}
Diclofenac	4.52[64]
Ibuprofen	3.97[74]
Naproxen	3.18[74]
Ketoprofen	3.12[63]

Table S6. Extraction efficiencies (%*EE*) of the $[P_{4441}][CH_3SO_4]$ + $Al_2(SO_4)_3$ + H_2O ABS for diclofenac, ibuprofen, naproxen and ketoprofen, and recovery percentage (%Recovery) of each NSAID by induced precipitation with water, in four sequential cycles.

Cycle	*Diclofenac*	*Ibuprofen*	*Naproxen*	*Ketoprofen*
			%*EE*	
1st Extraction	99.98 ± 0.01	99.67 ± 0.11	99.92 ± 0.05	99.81 ± 0.12
			%Recovery	
1st Precipitation	71.79 ± 3.60	88.29 ± 3.84	86.83 ± 3.56	45.91 ± 0.85
			%*EE*	
2nd Extraction	99.78 ± 0.02	99.86 ± 0.09	99.99 ± 0.01	99.99 ± 0.09
			%Recovery	
2nd Precipitation	65.86 ± 3.90	83.89 ± 2.80	92.92 ± 2.80	52.21 ± 1.90
			%*EE*	
3rd Extraction	99.99 ± 0.02	99.84 ± 0.13	99.99 ± 0.05	99.98 ± 0.11
			%Recovery	
3rd Precipitation	72.30 ± 4.19	80.53 ± 1.69	90.64 ± 1.96	49.63 ± 1.52
			%*EE*	
4th Extraction	99.81 ± 0.01	99.98 ± 0.21	99.99 ± 0.04	99.95 ± 0.10
			%Recovery	
4th Precipitation	67.20 ± 4.55	85.96 ± 2.38	89.16 ± 1.49	48.43 ± 2.89

7.3. Supporting Figures

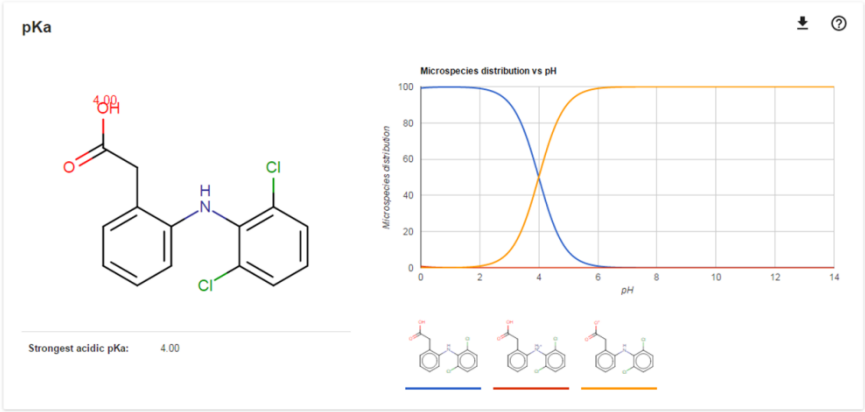

Figure S1. Speciation curves of diclofenac as a function of pH and respective acidic dissociation constants.[62]

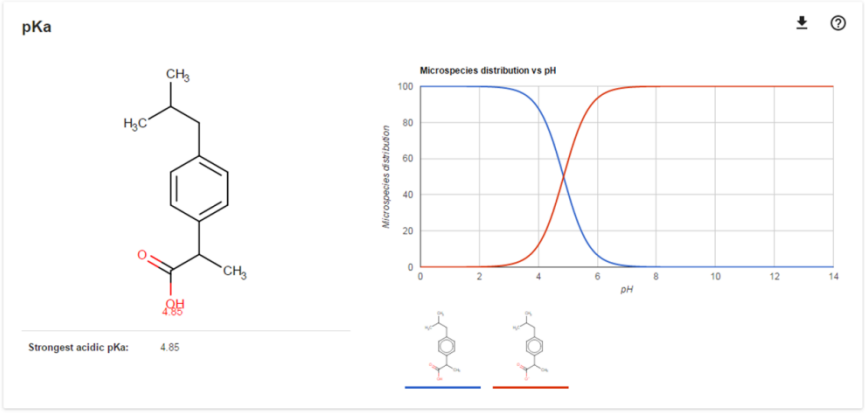

Figure S2. Speciation curves of ibuprofen as a function of pH and respective acidic dissociation constants.[62]

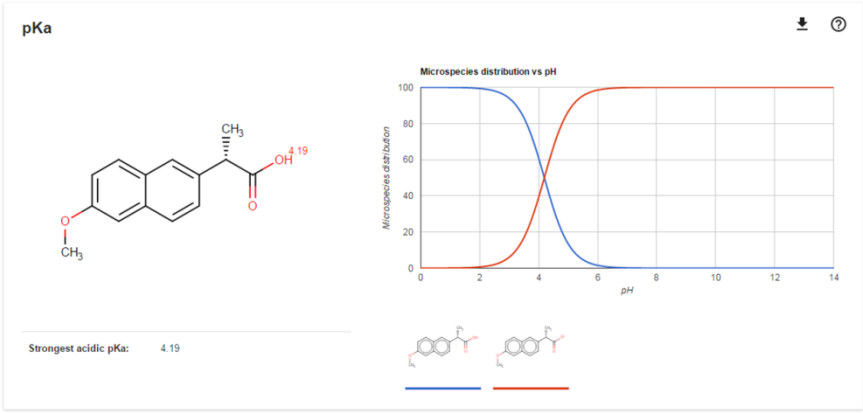

Figure S3. Speciation curves of naproxen as a function of pH and respective acidic dissociation constants.[62]

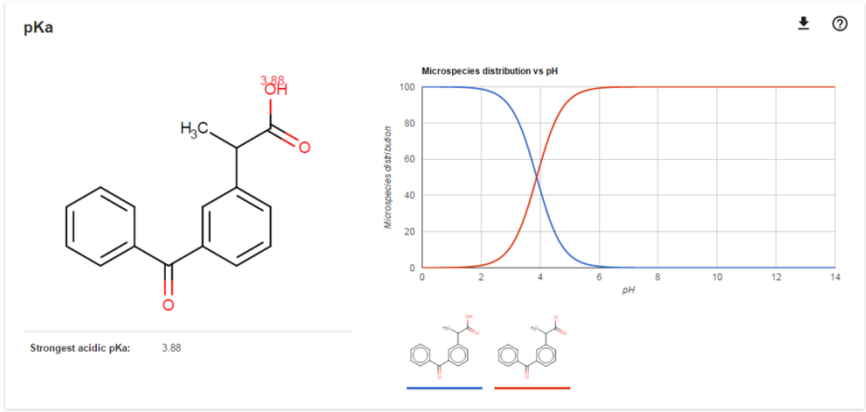

Figure S4. Speciation curves of ketoprofen as a function of pH and respective acidic dissociation constants.[62]

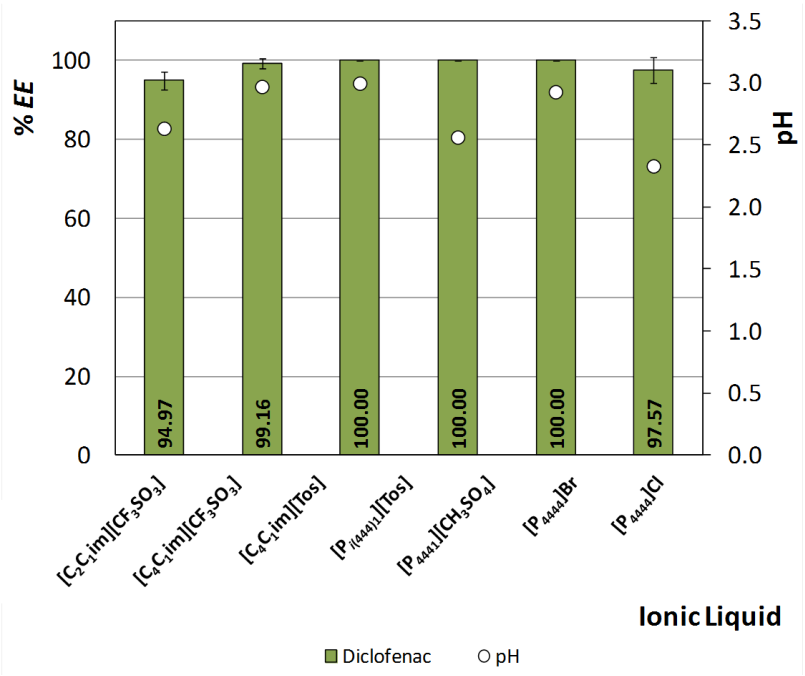

Figure S5. Extraction efficiencies of diclofenac (%*EE*) (bars) obtained with the investigated ABS at 25ºC and the corresponding pH at the IL-rich phase (symbols).

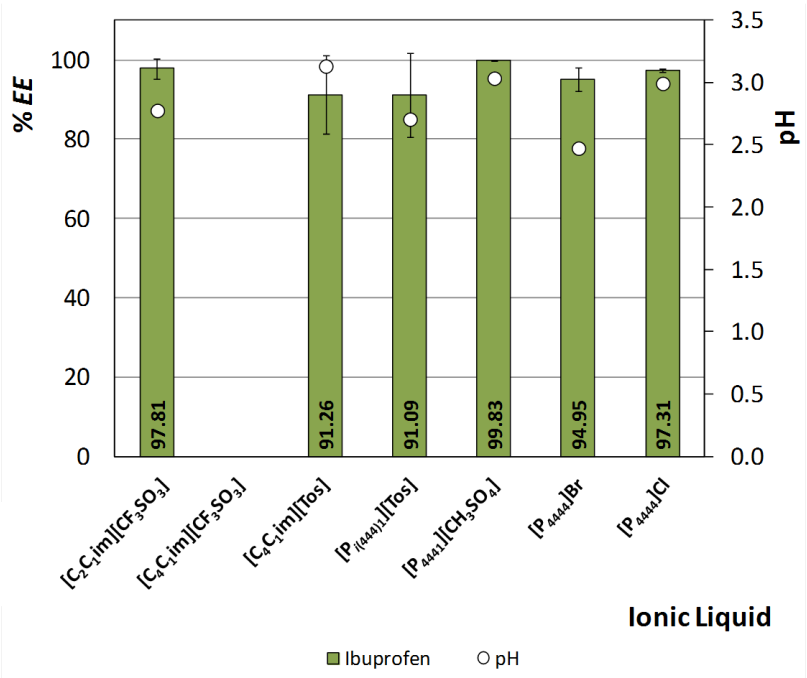

Figure S6. Extraction efficiencies of ibuprofen (%*EE*) (bars) obtained with the investigated ABS at 25 ºC and the corresponding pH at the IL-rich phase (symbols).

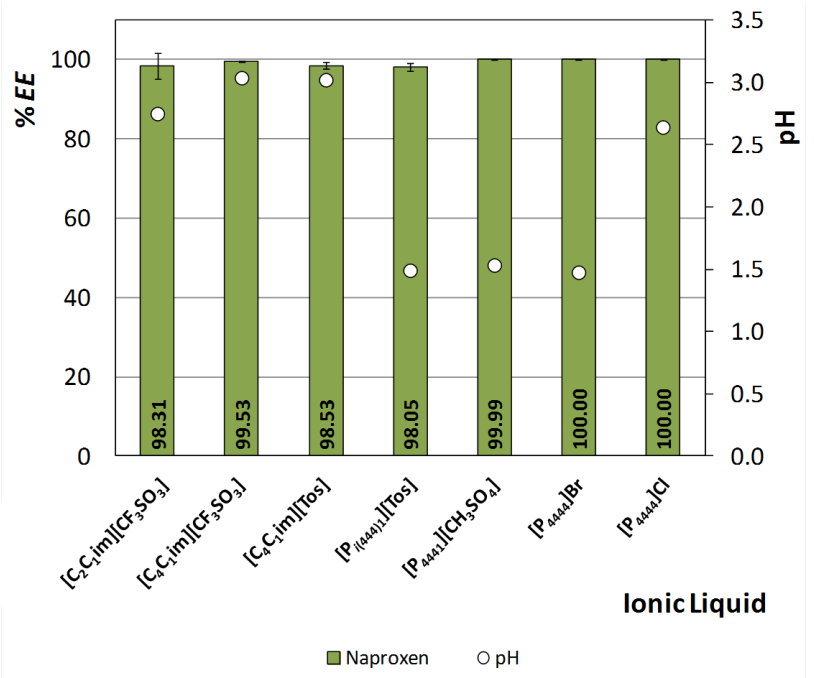

Figure S7. Extraction efficiencies of naproxen (%*EE*) (bars) obtained with the investigated ABS at 25 ºC and the corresponding pH at the IL-rich phase (symbols).

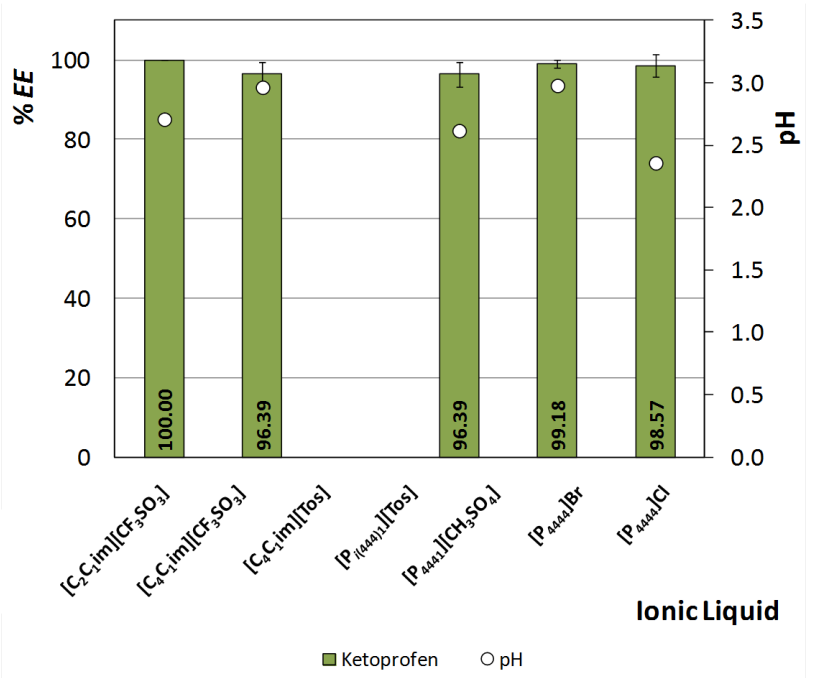

Figure S8. Extraction efficiencies of ketoprofen (%*EE*) (bars) obtained with the investigated ABS at 25 ºC and the corresponding pH at the IL-rich phase (symbols).

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Chapter 4

Improved Extraction of Fluoroquinolones with Recyclable Ionic-Liquid-based Aqueous Biphasic Systems

Adapted from: **Hugo F. D. Almeida**, Mara G. Freire and Isabel M. Marrucho; Improved extraction of fluoroquinolones with recyclable ionic-liquid-based aqueous biphasic systems; *Green Chem.*, **2016**, 18, 2717-2725.

The sample preparation for all the experiments presented herein was performed by the author. The author was also involved in all the experiments, as well as on the discussion and interpretation of the data and preparation of the manuscript.

1. Abstract

In the past few years, the improvement of advanced analytical tools confirmed the presence of trace amounts of metabolized and unchanged active pharmaceutical ingredients (APIs) in wastewater treatment plants (WWTPs) as well as in freshwater surfaces. It is known that the continuous contact with APIs, even at very low concentrations (ng/L – μg/L), leads to serious human health problems. In this context, this work shows the feasibility of using ionic-liquid-based aqueous biphasic systems (IL-based ABS) in the extraction of quinolones present in aqueous media. In particular, ABS composed of imidazolium- and phosphonium-based ILs and aluminium-based salts (already used in water treatment plants) were evaluated in one-step extraction of six fluoroquinolones (FQs), namely ciprofloxacin, enrofloxacin, moxifloxacin, norfloxacin, ofloxacin and sarafloxacin, and extraction efficiencies up to 98 % were obtained. Despite the large interest devoted to IL-based ABS as extractive systems of outstanding performance, their recyclability/reusability has seldomly been studied. An efficient extraction/cleaning process of the IL-rich phase is here proposed by FQs induced precipitation. The recycling of the IL and its further reuse without losses in the ABS extractive performance for FQs was established, as confirmed by the four consecutive removal/extraction cycles evaluated. This novel recycling strategy supports IL-based ABS as sustainable and cost-efficient extraction platforms.

2. Introduction

In the 21st century, the discovery of forthcoming pollutants in different environmental matrices, including pseudo-persistent compounds with toxic and cumulative characteristics, has gained a crucial attention. The idea of pharmaceuticals and personal care products (PPCPs) as major pollutants was firstly suggested by Daughton and Ternes[1], being afterwards classified as a rising class of potentially harmful environmental substances since cumulative side

effects go unnoticed until they lead to irreversible changes. The early detection and accurate quantification of PPCPs levels in the environment is linked to recent improvements on analytical techniques, resulting from both the development of more sensitive equipment and novel pre-treatment methods aiming at concentrating the target analytes from original matrices.[2, 3] Nowadays, the most abundant PPCPs are active pharmaceutical ingredients (APIs), which have raised serious concerns after their identification in non-negligible levels in sewage treatment plants (STPs), wastewater treatment plants (WWTPs) and surface water effluents.[4-14] Although the consumption of pharmaceuticals has led to significant improvements in human living conditions, their major drawback results from their excretion into the environment (either metabolized or as unchanged species).[7, 10, 12, 15-19] Different classes of APIs are known as mutagenic, carcinogenic and endocrine disrupters and have been detected in concentrations up to $ng.L^{-1}$ and $\mu g.L^{-1}$ in worldwide effluents of STPs and WWTPs, respectively.[7-10, 12, 20] Albeit STPs and WWTPs use advanced technologies for the removal of pollutants/contaminants, none of those methodologies was specifically designed for APIs,[7-12] explaining why some of these contaminants were already detected even in drinking water.[5]

Amongst APIs, antibiotics belonging to the quinolones group, including fluoroquinolones (FQs – Figure 24), are of particular environmental concern due to the current increasing number of antibiotic resistant bacteria.[21, 22] FQs are a family of broad spectrum systemic antibacterial agents, active against a wide range of aerobic gram-positive and gram-negative organisms, and which have been widely used in the treatment of respiratory and urinary tract infections.[22] Due to their high effectiveness, quinolones have been largely used by humans and in veterinary medicines for decades, and therefore, their entry into the environment has been continuous and silent. Molander *et al.*[23] compiled a web-database (www.wikipharma.org) where the ecotoxicity of 831 APIs is presented. Among these, FQs appear as a source for DNA-damaging or as mutagenic compounds, and in general as highly harmful to aquatic organisms. Ciprofloxacin, norfloxacin

and ofloxacin feature priority concerns amongst the top 44 APIs, where ciprofloxacin belongs to the high priority pharmaceuticals class (Class 1 – 10 pharmaceuticals).[21] Since FQS are not completely metabolized by humans and animals, and also due to their incorrect discharge,[4, 5, 7, 24] the development of a cost-effective and "greener" extraction technique for FQs is an urgent requirement – either for their removal or for their accurate monitoring in the environment.

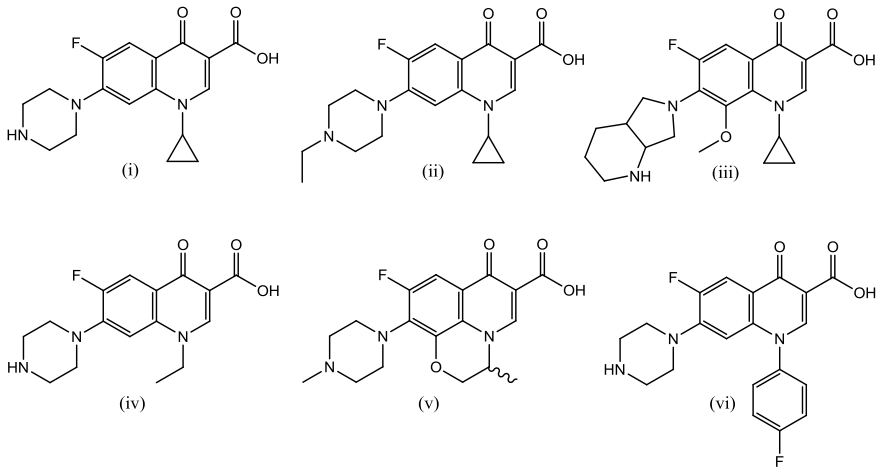

Figure 24. Chemical structures of fluoroquinolones: ciprofloxacin (i), enrofloxacin (ii), moxifloxacin (iii), norfloxacin (iv), ofloxacin (v) and sarafloxacin (vi).

Aqueous biphasic systems (ABS) fit within the liquid-liquid extraction techniques, and are constituted by two aqueous-rich phases formed by the addition of two water-soluble phase-forming components. In general, two polymers, a salt and a polymer or two salts lead to the creation of two-phase aqueous systems above given concentrations.[25, 26] ABS are formed by non-volatile solvents in a water-rich environment, and thus can be seen as an environmentally friendly approach. The partition and/or extraction of target compounds occur between the coexisting aqueous-rich phases, while the chemical nature and physical properties of both the phase-forming components and solute play a major role. Even so, the limited polarity differences between the

two phases and the restricted type of interactions between the solute and the phase forming components, aiming at tailoring the extraction and selectivity, are the major drawbacks of more conventional polymer-based ABS. To overcome this constraint, in the past few years, the functionalization of polymers and the addition of ligands have been investigated.[27] In 2003, Rogers and co-workers[28] demonstrated that the addition of a "kosmotropic" salt to an aqueous solution of a given ionic liquid (IL) leads to phase separation. After this proof of concept, in the following years a new plethora of extraction/separation routes was created, through the combined use of ILs and a large number of salts, amino acids, carbohydrates and polymers.[29] Although most ILs display some outstanding properties, namely a negligible vapor pressure, non-flammability, high thermal and chemical stabilities, and a large liquid temperature range, the major advantages of IL-based ABS *versus* conventional polymer-based ABS is due to the tailoring ability of the phases' polarities and affinities by an adequate choice of the ILs ions.[30-33] As a result, the superior performance of IL-based ABS is well illustrated by their extraction efficiencies and selectivities for the most diverse compounds, *e.g.* proteins,[34] alkaloids,[35] phenolic compounds,[36] dyes,[37] etc. The use of IL-based for the extraction of contaminants/pollutants has also been investigated.[35, 38-42] Domínguez-Pérez *et al.*[38] presented extraction efficiencies of around 85 % for ciprofloxacin and ciprofloxacin.HCl using ABS formed by 1-butyl-3-methylimidazolium triflate ($[C_4C_1im][CF_3SO_3]$) and lysine. In the same line, Shahriari *et al.*[43] proposed the use of ABS composed of cholinium-based ILs for the extraction of tetracycline and ciprofloxacin from aqueous media. The extraction of macrolide antibiotics was also investigated with ABS constituted by 1-butyl-3-methylimidazolium tetrafluoroborate ($[C_4C_1im][BF_4]$) and diverse inorganic salts, where extraction efficiencies ranging from 91.8 % to 96.2 % and 89.6 % to 92.2 % were attained for azithromycin and mydecamycin, respectively.[39] Han *et al.*[44] used an ABS formed by $[C_4C_1im][BF_4]$ and an organic salt aiming an easier identification and quantification of chloroamphenicol in water, milk and honey samples. The recovery of the antibiotics ranged between 90.4 and

102.7 %. Overall, ABS implemented with imidazolium-based ILs coupled, most of the times, to non-water stable anions, such as $[BF_4]^-$ have been largely studied for antibiotic removal from water.[45] Furthermore, the antibiotics recovery, which is an essential step for the IL-rich phase recovery and reuse, has not been previously attempted. None of these studies also considered the implementation of ABS in STPs or WWTPs.

In a simplified version of a WTTP, three different stages (mechanical, biological and disinfection treatments) are combined.[46] In this work, we designed ABS composed of $Al_2(SO_4)_3$ (as salting-out agent) for APIs removal to be introduced in the final stage of a WTTP, since this inorganic salt is already used as a flocculating agent in the purification of drinking water.[47] In particular, the extraction capacity of seven ABS composed of different ILs based on imidazolium and phosphonium cations was evaluated for six fluoroquinolones (ciprofloxacin, enrofloxacin, moxifloxacin, norfloxacin, ofloxacin and sarafloxacin – *cf.* Figure 24). It is well-known, and it is clearly demonstrated in this work, that the use of ILs as phase-forming components in ABS leads to outstanding extraction performances compared to more traditional routes. However, the IL regeneration, recycling and reuse lagged behind and still remain today a challenging task. Due to the negligible volatility of ILs, the recovery/removal of the compounds extracted to such phase is the main obstacle towards their reutilization. An FQ´s precipitation step, through pH change, here proposed, further allowing ILs to be recycled and reused, while warranting a more sustainable process

3. Experimental Section

3.1. Materials

Six fluoroquinolones, FQs, were used in this work, namely ciprofloxacin hydrochloride (CAS# 86393-32-0), enrofloxacin (CAS# 93107-08-5), moxifloxacin hydrochloride (CAS# 186826-86-8), norfloxacin (CAS# 70458-96-7), ofloxacin

(CAS# 82419-36-1) and sarafloxacin (CAS# 91296-87-6) with a quoted purity ≥ 99 wt% for moxifloxacin hydrochloride, ≥ 98 wt% for ciprofloxacin hydrochloride, enrofloxacin, norfloxacin and ofloxacin, and ≥ 91 wt% for sarafloxacin. Ciprofloxacin hydrochloride, moxifloxacin hydrochloride, norfloxacin and ofloxacin were acquired from Sigma-Aldrich, enrofloxacin was purchased from BioChemika, and sarafloxacin was acquired from LKT Laboratories, Inc. The chemical structures of the studied FQs are depicted in Figure 24.

The ionic liquids investigated were 1-ethyl-3-methylimidazolium trifluoromethanesulfonate (triflate), $[C_2C_1im][CF_3SO_3]$, purity 99 wt% (CAS# 145022-44-2); 1-butyl-3-methylimidazolium triflate, $[C_4C_1im][CF_3SO_3]$, purity 99 wt% (CAS# 174899-66-2); 1-butyl-3- methylimidazolium tosylate, $[C_4C_1im][Tos]$, purity 99 wt% (CAS# 410522-18-8); tri(isobutyl)methylphosphonium tosylate, $[P_{i(444)1}][Tos]$, purity 98 wt% (CAS# 374683-35-9); tributylmethylphosphonium methylsulfate, $[P_{4441}][CH_3SO_4]$, purity 96-98 wt% (CAS# 69056-62-8); tetrabutylphosphonium bromide $[P_{4444}]Br$, purity 95 wt% (CAS# 3115-68-2); and tetrabutylphosphonium chloride, $[P_{4444}]Cl$, purity 97 wt% (CAS# 2304-30-5). All the phosphonium-based ILs were gently supplied by Cytec Industries Inc., while the imidazolium-based fluids were purchased from Iolitec. To decrease the volatile impurities and water contents, individual samples of ILs were purified at room temperature under constant stirring under vacuum for a minimum of 24 h. However, for $[P_{4444}]Br$ and $[P_{4444}]Cl$, the temperature was raised up to 373 K and these samples were kept under vacuum for a minimum of 72 h, due their higher amount of water. The purity of each IL was checked by ^{1}H and ^{13}C NMR spectra and found to be in agreement with the purities given by the suppliers. The chemical structures of the ILs investigated are depicted in Figure 25.

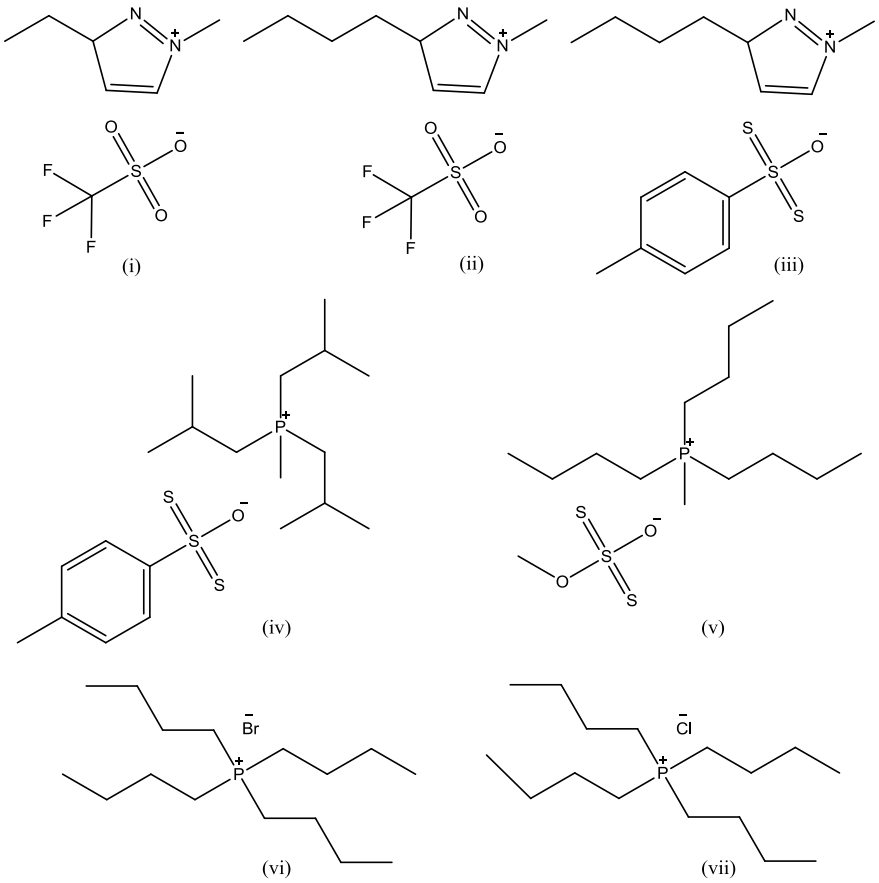

Figure 25. Chemical structures of the ILs used to form ABS: $[C_2C_1im][CF_3SO_3]$ (i), $[C_4C_1im][CF_3SO_3]$ (ii), $[C_4C_1im][Tos]$ (iii), $[P_{i(444)1}][Tos]$ (iv), $[P_{4441}][CH_3SO_4]$ (v), $[P_{4444}]Br$ (vi), and $[P_{4444}]Cl$ (vii).

The $Al_2(SO_4)_3$ (CAS# 17927-65-0) salt used in the ABS formulations was acquired from José Manuel Gomes dos Santos, LDA (purity ≥ 98.0 wt%). NaOH (CAS# 7647-14-5) was acquired from Sigma Aldrich (purity ≥ 99.5 wt%), KOH pure (CAS# 1310-58-3) was acquired from Pronolab, K_2CO_3 (CAS# 584-08-7) was acquired from Sigma Aldrich (purity = 99.995 wt %), and K_3PO_4 (CAS# 7778-53-2) was acquired from Sigma-Aldrich (purity ≥ 97.0 wt%). HCl (CAS# 7647-01-0) was acquired from Sigma Aldrich (at 37 wt% in aqueous solution). The water used in the extractions experiments was double distilled, passed across a reverse

osmosis system and further treated with a Milli-Q plus 185 water purification equipment. Buffers solutions of pH of 4.00 and 7.00, acquired from Panreac, were used for the calibration of the pH meter.

3.2. Phase diagrams and tie-lines

The liquid-liquid ternary phase diagrams used in the current work for extraction purposes were previously reported by Neves *et al.*[48] However, each tie-line (TL), corresponding to the mixture compositions used in the extraction experiments, was measured in this work through an established gravimetric method originally proposed by Merchuk *et al.*[49] Further details can be found in the in Supporting Information.

3.3. Extraction of Fluoroquinolones with IL-based ABS

The extraction of FQs was carried out using ABS composed of IL + $Al_2(SO_4)_3$ + water corresponding to ternary mixtures in the biphasic region, which were prepared gravimetrically using a Sartorius CPA225D Analytical Balance, within ± 2×10^{-5} g. The ABS were prepared in small glass ampoules (15 cm^3) by adding appropriate amounts of IL, inorganic salt and water solutions containing each of the FQs. The concentration of each FQ in the initial aqueous solutions was *circa* 0.05 $g.dm^{-3}$. The mixtures were vigorously stirred, and left to equilibrate for 24 h at (25 ± 1) ºC to reach the equilibrium and complete the separation of both phases. Afterwards, both the IL and salt-rich phases were carefully separated and weighted. The amount of each FQ in each phase was quantified through UV-spectroscopy, using a Shimadzu UV-1800, Pharma-Spec UV-VIS Spectrophotometer, at a wavelength of 276, 275, 292, 275, 291 and 277 nm for ciprofloxacin, enrofloxacin, moxifloxacin, norfloxacin, ofloxacin and sarafloxacin, respectively, which correspond to the maximum absorbance wavelengths, and using calibrations curves previously established. To eliminate possible

interferences of the IL and salt in the quantification, ternary mixtures at the same weight fraction composition were prepared, using pure water instead of the aqueous solutions containing the FQs, and used as control samples.

The extraction efficiencies of FQs (% EE_{FQs}) are defined as the ratio between the total weight of each FQ present in the IL-rich phase to that in the total mixture (both phases). At least three replicates were prepared for each mixture composition and IL-based ABS allowing the determination of the average extraction efficiency and respective standard deviation.

3.4. pH determination

The pH values (± 0.02) of the IL-rich phase were measured at (25 ± 1) ºC, using the Mettler Toledo S47 SevenMulti™ dual meter pH/conductivity.

3.5. Removal of Fluoroquinolones and Recycling of the IL

After the evaluation of the ABS performance for extraction FQs from aqueous media, the removal of FQs from the IL-rich phase and further IL recycling were addressed. The procedure was divided in three parts: i) study of pH to manipulate the speciation, and thus the solubility, of FQs in aqueous solutions; ii) test of several alkaline salts to add to manipulate the IL-rich phase pH; iii) IL recovery and reuse in four consecutive cycles. Each procedure was carried out in triplicate, accompanied by control samples, where FQs were not introduced in ABS.

The speciation curves of each FQ and respective pKa values were taken into account on the evaluation of the optimum pH which induces their maximum precipitation (minimal solubility) from aqueous solution. The speciation curves of the studied FQs are shown Figure S1-S3, in the Supporting Information.

The amount of FQs used for the preliminary solubility assays (first and second parts) was ≈ 0.01 g, while for the third part, aqueous solutions of FQs (at

circa 0.05 g.dm^{-3}) were used. The alkaline solutions used for the implementation of the described procedure were HCl (8.9 mM), NaOH (10.1 mM), $Al_2(SO_4)_3$ (1.5 wt%), KOH (40 wt%), K_2CO_3 (40 wt%), and K_3PO_4 (40 wt%). The weight of NaOH, KOH, K_2CO_3, and K_3PO_4 aqueous solutions used for increasing the pH up to the desired value ranged between 0.2 g and 0.3 g of solution for 3.0 g of the IL-rich phase. The composition of the IL-rich phases used is presented in Table S1 in the Supporting Information.

After the FQs precipitation, their removal from the IL-rich phase was achieved by vacuum filtration, using Whatman® regenerated cellulose membrane filters with a pore size of 0.2 μm. The obtained precipitate was filtered under vacuum, washed with 10 mL of deionized water, dried at 70 ºC for 24 h and weighted until constant weight was attained. For the second and third parts, the obtained precipitate was filtered under vacuum and due to the presence of a second compound (as it will be explained later), the FQ precipitated (% PP_{FQs}) was calculated indirectly through the quantification of each FQ by UV spectroscopy in the IL-rich phase before and after the precipitation step at the maximum wavelengths previously described and using the respective calibration curves.

4. Results and Discussion

4.1. Extraction of Fluoroquinolones with IL-based ABS

The composition of each system to perform the extraction of FQs was chosen to correspond to a fixed TLL (≈ 70) aiming at maintaining the difference in composition between the two phases, thus allowing a better evaluation of the IL chemical nature effect. The mixture compositions used for the several IL-based ABS ranged between 29 – 43 wt% for IL and a fixed composition, 15 wt%, was chosen for $Al_2(SO_4)_3$. Moreover, the use of a long TLL usually guaranties an increase in the extraction efficiency as well as a lower cross-contamination of

each phase by the component enriched in the opposite layer.[29] The liquid-liquid ternary phase diagrams used in the current work were previously reported by Neves *et al.*[48] However, the respective TLs (phases' compositions) for the mixture compositions used in the extraction studies were determined in this work. Data of the initial mixture compositions in addition to the composition of the two phases in equilibrium (TLs) for each extraction experiment are reported in Tables S1, in the Supporting Information. The detailed extraction efficiencies and pH of each phase are also provided in the Supporting Information. Aqueous solutions of $Al_2(SO_4)_3$ are acidic, and the pH values of the corresponding coexisting phases of IL-based ABS range between 1.16 and 3.15, according to the IL employed. Taking into account the FQs speciation curves (*cf.* Supporting Information),[50, 51] at these pH values these compounds are in their protonated form and so electrostatic interactions may play a role in the extractive performance of the investigated ABS.

Figure 26 and Figure 27 depict the one-step extraction efficiencies of different fluoroquinolones (% EE_{FQs}) using the investigated ABS composed of IL + $Al_2(SO_4)_3$ + H_2O, at 25 ºC. % EE_{FQs} for the IL-rich phase ranging between 27.6 % and 97.8 % were obtained.

In general, the % EE_{FQs} for the different FQs do not seem to be linked to the IL aromatic character (either at the cation or at the anion). $[C_2C_1im][CF_3SO_3]$, $[C_4C_1im][Tos]$ and $[P_{i(444)1}][CF_3SO_3]$ feature the highest extraction efficiencies, independently of the FQ being extracted. However, ABS composed of phosphonium-based ILs combined with the Cl^- and Br^-, also displays a good performance to extract sarafloxacin and moxifloxacin. Although FQs preferentially partition for the IL-rich phase, it should be noted that norfloxacin and ofloxacin preferentially partition to the salt-rich when ABS composed of $[C_4C_1im][CF_3SO_3]$, $[P_{4441}][CH_3SO_4]$, and $[P_{4444}]Cl$ are used. Generally, the three ILs bearing sulfonated moieties, namely $[C_2C_1im][CF_3SO_3]$, $[C_4C_1im][Tos]$ and $[P_{i(444)1}][Tos]$, present good extraction performances, above 80 %, for all the studied FQs.

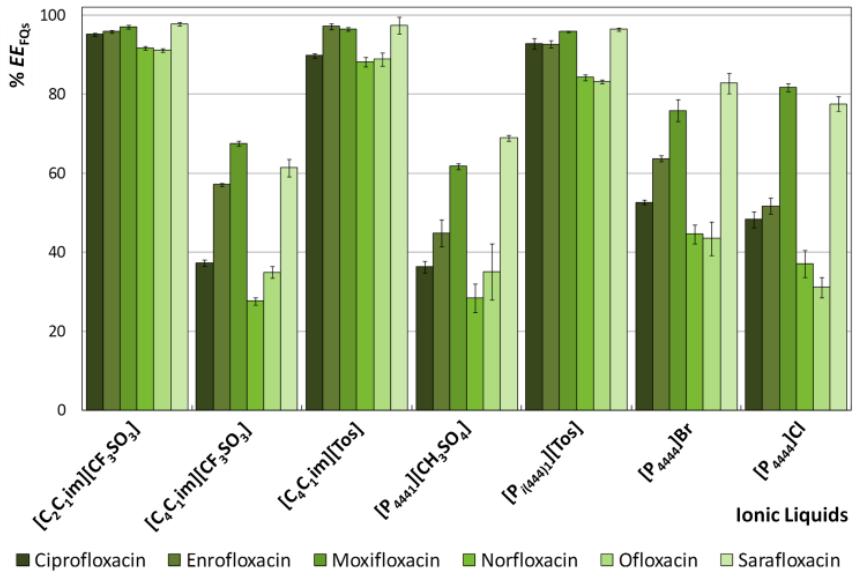

Figure 26. Extraction efficiencies of FQs (% EE_{FQs}) with ABS composed of IL + $Al_2(SO_4)_3$ + H_2O, at 25 ºC, as a function of the FQ investigated.

In order to further warrant the application of the proposed methodology to water treatment systems, the joint extraction of the six studied fluoroquinolones from aqueous solution was studied. For this purpose, ABS composed of $[C_2C_1im][CF_3SO_3]$ and $Al_2(SO_4)_3$ were used to simultaneously extract ciprofloxacin, norfloxacin, ofloxacin, enrofloxacin, sarafloxacin and moxifloxacin from the same water sample. The extraction efficiencies (Figure S4 and Table S3 - in the Supporting Information) show similar results to those obtained for the separate extraction of each fluoroquinolone, evidencing the feasibility of the application of this procedure to wastewater treatment.

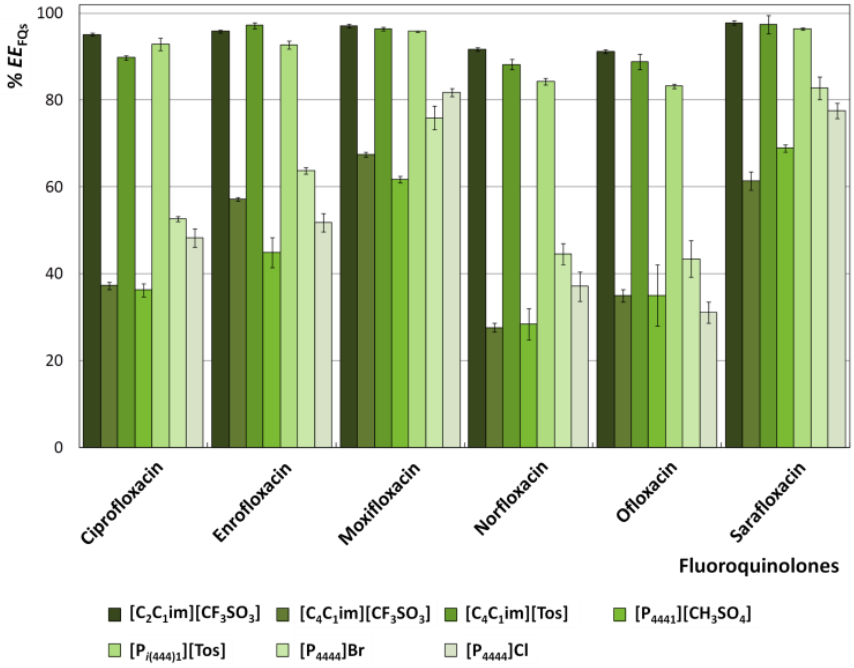

Figure 27. Extraction efficiencies of the six studied fluoroquinolones (%EE_{FQs}) with ABS composed of IL + $Al_2(SO_4)_3$ + H_2O, at 25 ºC, as a function of the IL investigated.

In general, a decrease in the extraction efficiency of all FQs with the increase of the alkyl side chain length of the imidazolium cation is observed (by comparing the results afforded by $[C_2C_1im][CF_3SO_3]$- and $[C_4C_1im][CF_3SO_3]$-based ABS). On the other hand, the direct effect of the IL anion can be evaluated taking into account the ABS formed by $[C_4C_1im][CF_3SO_3]$ and $[C_4C_1im][Tos]$. The presence of the $[Tos]^-$ anion dramatically increases the extraction efficiency of ABS for FQs. These results might be explained by the presence of the aromatic ring in this anion that seems to be favorable for increasing the affinity of FQs for the IL-rich phase. This result is further confirmed by the results provided by $[P_{i(444)1}][Tos]$ which also leads to high % EE_{FQs} (up to 96.4 % in one-step). From these results, it is clear that the aromaticity at the anion is more important than the aromaticity at the IL cation.

Amongst all ILs investigated, $[P_{i(444)1}][Tos]$ and $[C_4C_1im][Tos]$ are the most promising candidates for removing FQs from aqueous media. In addition to their remarkable extraction efficiencies, there is a lower loss of IL for the salt-rich phase (or cross-contamination) when these two ILs are used. For the mixtures investigated, the amount of $[P_{i(444)1}][Tos]$ and $[C_4C_1im][Tos]$ in the salt-rich phase is *circa* 0.10 and 0.03 wt%, respectively – *cf.* Supporting Information detailed tie-lines data. The high capability of phosphonium-based ILs to extract FQs from aqueous media, particularly by using $[P_{i(444)1}][Tos]$ can be additionally seen as a major advantage. In general, and although scarcely explored as phase-forming constituents of ABS,[48, 52] phosphonium-based ILs are less expensive and produced at a large scale. Tetraalkylphosphonium-based ILs are also thermally more stable and have no acidic protons which make them more stable towards nucleophilic and basic conditions when compared to imidazolium- and pyridinium-based ILs.[52] In summary, the proper choice of the IL leads to outstanding one-step extraction results for FQs, using IL-based ABS, and which have not yet been described in the literature.

Tetraalkylphosphonium-based ILs with long alkyl chains might also be used to extract FQs, and due to their hydrophobic character no salt needed and thus simpler IL recycling schemes could be envisaged. In order to evaluate FQs extraction efficiency of phosphonium-based hydrophobic ILs, $[P_{666(14)}]Br$, $[P_{666(14)}]Cl$ and $[P_{666(14)}][NTf_2]$ were tested in the extraction of ciprofloxacin, norfloxacin and ofloxacin. The extraction procedure has already been described in our previous articles.[53, 54] The obtained results, presented in In Table S4 and Figure S5 (in the Supporting Information), show that these hydrophobic phosphonium-based ILs present lower extraction efficiencies than that obtained for hydrophilic imidazolium- and phosphonium- based ABS, with extraction efficiencies ranging from 20.8 % to 68.6 %. The better results obtained for the implemented ABS can probably be attributed to the presence of the aluminium-salt and its salting out capacity, enabling the higher concentration of FQs in the IL rich phase. Despite the simplicity in recycling hydrophobic ILs, the obtained

results clearly show that the advantageous use of ABS containing hydrophilic ILs, in particular $[P_{i(444)1}][Tos]$, and $Al_2(SO_4)_3$ for efficient extraction of FQs from aqueous phases.

4.2. Removal of Fluoroquinolones and Recycling of the Ionic Liquid

According to the literature,[21] ciprofloxacin is one of the ten active pharmaceutical ingredients included in the high priority removal list of Global Water Research Coalition (which lists the APIs that are encountered in water supplies in high concentrations and that may have significant impact on human health). Norfloxacin and ofloxacin are also classified as priority compounds to be removed from fresh waters.[22, 55] Although IL-based ABS are remarkable systems for the removal of FQs from water media, a sustainable process can only be developed if the recovery and re-use of the IL could be additionally attained. In this section, we address the removal of ciprofloxacin and the re-use of the IL-rich phase in a subsequent ABS-mediated extraction.

Taking into account the acidic dissociation constants of ciprofloxacin (Figure S1, shown in the Supporting Information), at pH = 7.2, only the neutral and/or the zwitterionic form exist in solution.[56] Since these species are nonionic (the zwitterionic form is neutral overall although charge separation exists in the molecule), their precipitation from the highly ionic and polar IL-rich phase is bound to occur extensively, *i.e.*, the solubility of ciprofloxacin in aqueous media will decrease for the non-charged solute. Therefore, in order to evaluate the feasibility of precipitating ciprofloxacin through a pH change, this fluoroquinolone was initially dissolved in an HCl aqueous solution and then NaOH aqueous solution was added. Overall, the pH of the solution was changed in a range between pH = 5 – 9. The results obtained from this procedure are listed in Table 3. As expected, at a pH close to 7.2, the maximum amount of precipitated ciprofloxacin occurred and thus this was the target pH for the recovery of this API from the IL-rich phase.

By observing Table 3, the percentage of fluoroquinolone precipitated (% PP_{FQ}) increases from pH 5.23 to 7.23. However, when the pH increases from 7.23 to 9.53, the %PP_{FQ} decreases, demonstrating that the presence of the zwiterionic and neutral forms of ciprofloxacin are vital to control the FQ precipitation efficiency from aqueous solutions.

Table 3. Percentage of precipitated ciprofloxacin (% (PP_{FQ} ± σ)) from aqueous solutions as a function of pH (by adding HCl and NaOH).

pH ± σ	5.23 ± 0.23	6.08 ± 0.12	7.23 ± 0.19	8.36 ± 0.43	9.53 ± 0.09
% (PP_{FQs} ± σ)	*α*	6.06 ± 1.10	85.05 ± 2.32	62.28 ± 0.85	48.50 ± 1.45

α Precipitation did not occur

Subsequently, the same precipitation procedure was tested for ciprofloxacin in an aqueous solution of $Al_2(SO_4)_3$. Three different salts which provide alkaline solutions, KOH, K_3PO_4 and K_2CO_3, were used in the precipitation of ciprofloxacin and their efficiency evaluated. The objective here is not only to fix the pH around 7.2 but also to take advantage of the relative salting-out capacity of these salts. The obtained results are listed in Table 4.

Table 4. Percentage of ciprofloxacin precipitated (% (PP_{FQ} ± σ)) from an aqueous solution of $Al_2(SO_4)_3$ using different salts.

	KOH	**K_3PO_4**	**K_2CO_3**
pH ± σ	7.21 ± 0.01	7.37 ± 0.14	7.29 ± 0.16
% (PP_{FQs} ± σ)	65.79 ± 1.93	96.97 ± 0.35	85.73 ± 4.39

As expected, K_3PO_4 presents the highest precipitation efficiency, in agreement with the ranking of the anions according to the Hofmeister series.[57] Therefore, in addition to the alkaline pH provided by these salts, also the salt salting-out effect plays a major role. However, in these studies, two different types

of crystals, white and yellow were obtained, as it can be observed in Figure 28. Since the IL-rich phase contains ≈ 1.2 wt% of $Al_2(SO_4)_3$, the addition of K_3PO_4 for the precipitation purpose leads to the formation of $AlPO_4$,[58, 59] which precipitates together with the FQ. In order to further explore this aspect, K_3PO_4 was added to an aqueous solution of $Al_2(SO_4)_3$, without FQ, leading to the formation of a white precipitate only (Figure 28b). According to Gu and Karthikeyan,[60] ciprofloxacin forms binary complexes in solution with Al^{3+} at acidic and neutral pH. So, the yellow crystals probably correspond to complexed ciprofloxacin, since ciprofloxacin powder has a yellowish color. Even so, this concomitant precipitation also allows the removal of the major salting-out species from the IL-rich solution – what can be seen as a major advantage when envisaging an IL "cleaning process". Similar results were obtained during the precipitation of norfloxacin and ofloxacin (Figure S6 in the Supporting Information).

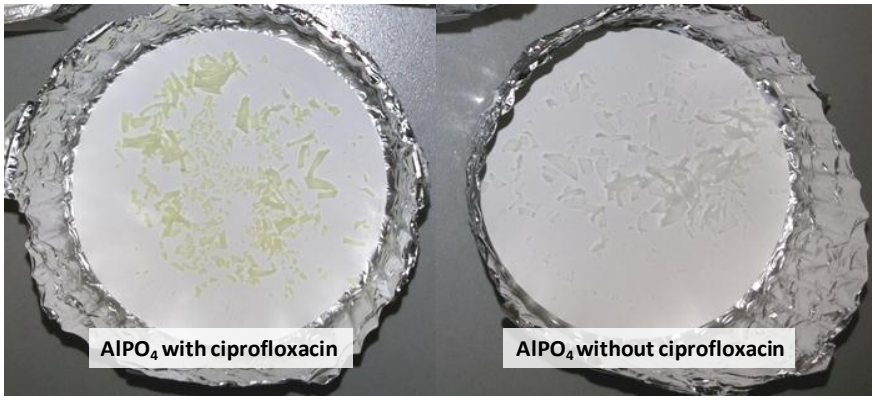

Figure 28. Precipitates obtained through the addition of K_3PO_4 to: a) an aqueous solution of $Al_2(SO_4)_3$ and ciprofloxacin; and b) an aqueous solution of $Al_2(SO_4)_3$.

Finally, the described precipitation procedure was tested in the IL-rich phases after the extraction step. To this end, $[C_2C_1im][CF_3SO_3]$, $[C_4C_1im][Tos]$ and $[P_{i(444)1}][Tos]$ were selected since these ILs are those that lead to the higher extraction efficiencies of FQs. Representative mixtures of the IL-rich phase, using these three ILs and $Al_2(SO_4)_3$ were prepared – detailed compositions are given in

the Supporting Information. Afterwards, an aqueous solution of 40 wt% of K_3PO_4 was used to increase the pH up to 7.2, so that the maximum ciprofloxacin precipitation was attained. The obtained precipitate was filtered under vacuum and the ciprofloxacin left behind in the IL-rich solution was quantified. The results obtained are displayed in Table 5.

Table 5. Percentage of ciprofloxacin precipitated (% (PP_{FQ} ± σ)) from the IL-rich phase of ABS composed of IL + $Al_2(SO_4)_3$ + water. Three ILs, namely $[C_2C_1im][CF_3SO_3]$, $[C_4C_1im][Tos]$ and $[P_{i(444)1}][Tos]$, were investigated.

	$[C_4C_1im][Tos]$	**$[C_2C_1im][CF_3SO_3]$**	**$[P_{i(444)1}][Tos]$**
pH ± σ	7.21 ± 0.05	7.34 ± 0.10	7.31 ± 0.05
% (PP_{FQs} ± σ)	82.48 ± 1.93	82.96 ± 3.39	65.38 ± 0.88

Similar results were obtained for $[C_2C_1im][CF_3SO_3]$ and $[C_4C_1im][Tos]$, with precipitation efficiencies around 82 %, while a smaller efficiency was obtained with $[P_{i(444)1}][Tos]$. The IL $[C_2C_1im][CF_3SO_3]$ was chosen to carry out the rest of our work since it presents smaller interferences in the FQ quantification and lower cross contamination of the IL and salt rich phases. To confirm the recyclability of the IL, $Al_2(SO_4)_3$ and water were added to the recycled IL so that of $[C_2C_1im][CF_3SO_3]$ + $Al_2(SO_4)_3$ + water ABS was again created and subsequently used to evaluate in the extraction of FQs in a single-step from a new aqueous solution.

Ideally, the overall recycling process includes two steps: a precipitation step, where the FQ is removed from the IL-rich phase, followed by a second step where the recovery of the IL-rich phase takes place. For that purpose, only water and $Al_2(SO_4)_3$ should be added to attain the initial mixture composition. However, and even in small amounts, K_3PO_4 will be increasingly accumulated in the IL-rich phase combined with an increase in the pH values. In order to eliminate the undesired K_3PO_4 from IL-rich phase $Al_2(SO_4)_3$ was added. Aluminium sulfate salt

is mainly used as coagulant/flocculant for the removal of phosphorus,[61, 62] organic contaminants[61] and polyvinyl chloride[63] from wastewaters. Moreover, it is known that this inorganic salt has its best flocculation/coagulation performance at pH values ranging between 6 and 8, which correspond to its lowest water solubilities.[64] In this work, the addition of $Al_2(SO_4)_3$ to the IL-rich phase where a solution of K_3PO_4 was previously added to set the pH at *circa* 7, will promote again the formation of two aqueous-rich phases, one rich in IL and the other rich in inorganic salt. However, the formation of a colloidal matter in the aqueous phase, was observed, indicating the formation of $AlPO_4$.[58, 59] Remarkably, this step can be seen as a further cleaning step of the IL from the organic phosphorous matter and therefore to a reset of pH to acidic values. After the extraction step, the IL-rich phase (upper phase) can be simple recovered and further used in the FQs removal to proceed to a new extraction cycle.

In order to evaluate the feasibility of the proposed methodology, four consecutive removal of FQS /recovery of IL-rich phase cycles were performed. The obtained data are listed in Table 6. Three priority fluoroquinolones, namely ciprofloxacin, norfloxacin and ofloxacin, were used to validate the methodology.

According to the results reported in Table 6, the ability of $[C_2C_1im][CF_3SO_3]$ to extract fluoroquinolones from aqueous solution is maintained in the four consecutive cycles, with average extraction efficiencies of 95.3, 90.9, and 91.2 % for ciprofloxacin, norfloxacin and ofloxacin, respectively. Similar conclusions can be drawn for the amount of fluoroquinolones removed, with an average of 80.8, 80.9 and 77.0 % for ciprofloxacin, norfloxacin and ofloxacin, respectively. These results ensure the reusability of $[C_2C_1im][CF_3SO_3]$ without lost in the performance.

Table 6 Extraction efficiencies (% EE_{FQs}) of three FQs, namely ciprofloxacin, norfloxacin and enrofloxacin from aqueous solutions using $[C_2C_1im][CF_3SO_3]$ + $Al_2(SO_4)_3$ + H_2O ABS, at 25 ºC, the pH for the precipitation of the fluoroquinolones (pH) and percentage of fluoroquinolones precipitated (% PP_{FQs}), in four sequential cycles.

Cycle	*Ciprofloxacin*	*Norfloxacin*	*Ofloxacin*
		% *EE*$_{FQs}$	
1st Extraction Cycle	94.9 ± 0.6	91.0 ± 0.3	91.4 ± 0.8
		pH	
$pH_{initial}$	2.75	2.74	2.74
pH_{final}	7.18	7.28	5.88
		% PP_{FQs}	
1st Recovery Cycle	81.2 ± 0.6	80.9 ± 1.2	76.3 ± 0.6
		% *EE*$_{FQs}$	
2nd Extraction Cycle	95.0 ± 0.4	91.1 ± 0.2	91.2 ± 0.5
		pH	
$pH_{initial}$	2.67	2.75	2.75
pH_{final}	7.20	7.18	7.18
		% PP_{FQs}	
2nd Recovery Cycle	80.7 ± 1.3	80.8 ± 0.4	76.9 ± 0.3
		% *EE*$_{FQs}$	
3th Extraction Cycle	95.7 ± 1.3	90.9 ± 0.6	91.0 ± 0.8
		pH	
$pH_{initial}$	2.61	2.63	2.53
pH_{final}	7.24	7.26	5.88
		% PP_{FQs}	
3th Recovery Cycle	80.5 ± 1.0	80.9 ± 1.5	77.7 ± 0.8
		% *EE*$_{FQs}$	
4th Extraction Cycle	95.5 ± 0.5	90.8 ± 0.1	91.2 ± 1.1
		pH	
$pH_{initial}$	2.69	2.68	2.63
pH_{final}	7.20	7.18	5.79
		% PP_{FQs}	
4th Recovery Cycle	81.0 ± 0.3	81.0 ± 1.3	77.1 ± 1.7

In general, and in order to proceed to a new extraction cycle, one needs to know the exact composition of the IL-rich phase, so that the amount of $Al_2(SO_4)_3$ and aqueous solution containing FQs needed for the formation of a new ABS can be calculated. This information can be obtained by direct quantification of the phases or by the ternary phase diagrams/tie-lines information (given in detail in the Supporting Information). With this information, and as shown with the four cycles investigated, similar % EE_{FQs} and % PP_{FQs} were obtained, ensuring the reusability of more than 97 wt% of the IL of the IL-rich phase without losing its outstanding performance. In fact, and despite the advantages connected to $Al_2(SO_4)_3$ as a flocculant agent already used in the treatment of drinking water,[47] the strong salting-out aptitude of this salt leads to a low cross-contamination of the phases and to low losses of IL in each cycle.

Figure 29 summarizes the procedure here proposed for the extraction of FQs from aqueous media, where the removal of FQs is attained followed by the recyclability of the IL-rich phase, thus guaranteeing the sustainability of the proposed process.

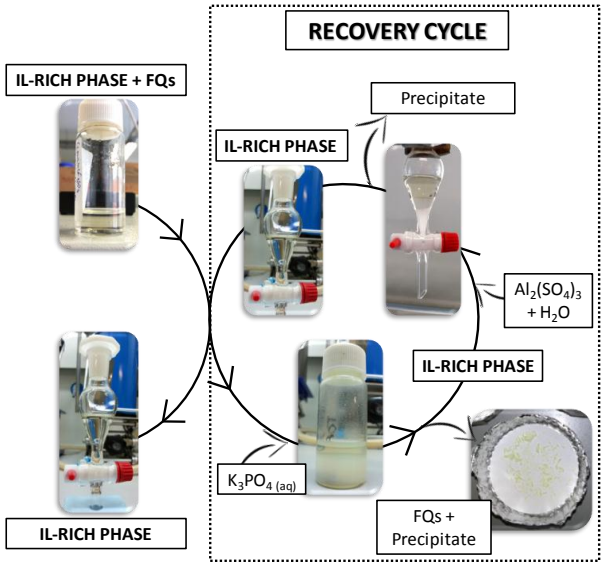

Figure 29. Representative scheme of the overall methodology here proposed comprising the IL-recovery cycle.

5. Conclusion

Recyclable IL-based ABS to extract FQs, such as ciprofloxacin, enrofloxacin, moxifloxacin, norfloxacin, ofloxacin and sarafloxacin, from water media were here proposed. ABS composed of $Al_2(SO_4)_3$ and imidazolium- or phosphonium-based ILs lead to extraction efficiencies of fluoroquinolones up to 97 %, in a single-step. In fact, the use of ILs as phase-forming components in ABS is well-known to provide outstanding extraction performances compared to more traditional approaches. Nonetheless, the IL regeneration, recycling and reuse lagged behind and still remain a challenging issue towards the development of greener and more cost-effective processes. In this context, a novel methodology was proposed aiming at removing FQs from the IL-rich phase followed by the IL recovery and reuse. A proper choice of an inorganic salt with alkaline characteristics was used to manipulate the pH of the solution, as well as taking advantage of its salting-out aptitude, in order to induce the precipitation of FQs. Precipitation rates up to 81 % were obtained in a single-step. Four removal/extraction cycles were conducted ensuring the sustainability of the proposed process and without loss of the ABS extraction performance. The results obtained support the development of IL-based ABS with a lower environmental footprint and economic impact.

This work opens new perspectives for the implementation of ABS-based processes in wastewater treatment, in particular for the extraction and recovery of FQs and other APIs.

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7. Supporting Information

7.1. Phase Diagrams and Tie-Lines (TLs)

The composition of each phase in equilibrium was determined using the following four equations (eqs. (1) – (4)) and four unknown values ($[IL]_{IL}$, $[IL]_{salt}$, $[salt]_{IL}$, $[salt]_{salt}$): [49]

$$[\mathrm{IL}]_{\mathrm{IL}} = \mathrm{A}\exp[(\mathrm{B} \times [\mathrm{salt}]_{\mathrm{IL}}^{0.5}) - (\mathrm{C} \times [\mathrm{salt}]_{\mathrm{IL}}^{3})] \quad (1)$$

$$[\mathrm{IL}]_{\mathrm{salt}} = \mathrm{A}\exp[(\mathrm{B} \times [\mathrm{salt}]_{\mathrm{salt}}^{0.5}) - (\mathrm{C} \times [\mathrm{salt}]_{\mathrm{salt}}^{3})] \quad (2)$$

$$[\mathrm{IL}]_{\mathrm{IL}} = \frac{[\mathrm{IL}]_{\mathrm{M}}}{\alpha} - \frac{1-\alpha}{\alpha} \times [\mathrm{IL}]_{\mathrm{salt}} \quad (3)$$

$$[\mathrm{salt}]_{\mathrm{IL}} = \frac{[\mathrm{salt}]_{\mathrm{M}}}{\alpha} - \frac{1-\alpha}{\alpha} \times [\mathrm{salt}]_{\mathrm{salt}} \quad (4)$$

where IL, salt and M are the IL-rich phase, the salt-rich phase and the mixture, respectively. [IL] and [salt] correspond to the weight fraction percentage of IL and salt, respectively, and α is the ratio between the mass of the IL-rich phase and the total mass of the mixture.

For the calculation of each tie-line length (TLL), the following equation was applied:

$$\mathrm{TLL} = \sqrt{([\mathrm{salt}]_{\mathrm{IL}} - [\mathrm{salt}]_{\mathrm{salt}})^2 + ([\mathrm{IL}]_{\mathrm{IL}} - [\mathrm{IL}]_{\mathrm{salt}})^2} \quad (5)$$

7.2. Supporting Tables

Table S1. Weight fraction percentage (wt%) of each compound at the coexisting phases of the ABS investigated.

	Weight fraction percentage / wt (%)					
Ionic Liquid	**$[IL]_{salt}$**	**$[salt]_{salt}$**	**$[IL]_{M}$**	**$[salt]_{M}$**	**$[IL]_{IL}$**	**$[salt]_{IL}$**
$[C_2C_1im][CF_3SO_3]$	3.29	40.36	41.94	15.03	63.02	1.21
$[C_4C_1im][CF_3SO_3]$	4.81	22.99	29.24	14.94	73.10	0.49
$[C_4C_1im][Tos]$	0.07	49.95	42.09	14.96	54.48	4.64
$[P_{4441}][CH_3SO_4]$	1.24	41.60	40.21	14.97	58.85	2.16
$[P_{i(444)1}][Tos]$	0.08	37.05	37.95	15.02	58.12	3.29
$[P_{4444}]Br$	0.88	31.98	35.91	15.05	63.55	1.68
$[P_{4444}]Cl$	0.33	46.09	39.91	15.02	55.34	2.91

Table S2. Tie-line length (TLL), pH of the IL-rich phase (pH_{IL}), and extraction efficiencies of the six studied fluoroquinolones (%EE_{FQs}), and corresponding standard deviations (σ).

	Ciprofloxacin				***Norfloxacin***		
Ionic Liquid	**TLL ± σ**	**pH_{IL} ± σ**	**% EE_{FQs} ± σ**	**Ionic Liquid**	**TLL ± σ**	**pH_{IL} ± σ**	**% EE_{FQs} ± σ**
$[C_2C_1im][CF_3SO_3]$	72.4 ± 0.1	2.78 ± 0.01	95.13 ± 0.33	$[C_2C_1im][CF_3SO_3]$	71.4 ± 0.0	2.95 ± 0.01	91.70 ± 0.40
$[C_4C_1im][CF_3SO_3]$	72.4 ± 0.4	2.15 ± 0.01	37.29 ± 0.84	$[C_4C_1im][CF_3SO_3]$	72.6 ± 0.1	2.73 ± 0.01	27.61 ± 0.97
$[C_4C_1im][Tos]$	70.5 ± 0.4	2.72 ± 0.01	87.79 ± 0.49	$[C_4C_1im][Tos]$	70.9 ± 0.1	3.09 ± 0.02	88.23 ± 1.24
$[P_{4441}][CH_3SO_4]$	69.7 ± 0.1	1.71 ± 0.03	36.26 ± 1.49	$[P_{4441}][CH_3SO_4]$	66.5 ± 0.2	3.13 ± 0.02	28.45 ± 3.59
$[P_{i\,(444)1}][Tos]$	66.4 ± 0.1	1.47 ± 0.05	92.85 ± 1.37	$[P_{i\,(444)1}][Tos]$	67.5 ± 0.0	2.66 ± 0.02	84.26 ± 0.75
$[P_{4444}]Br$	67.6 ± 0.0	1.16 ± 0.04	52.62 ± 0.56	$[P_{4444}]Br$	70.6 ± 0.4	1.30 ± 0.03	44.56 ± 2.42
$[P_{4444}]Cl$	70.4 ± 0.1	2.74 ± 0.04	48.26 ± 2.03	$[P_{4444}]Cl$	70.2 ± 0.1	2.88 ± 0.07	37.07 ± 3.35
	Enrofloxacin				***Ofloxacin***		
Ionic Liquid	**TLL ± σ**	**pH_{IL} ± σ**	**% EE_{FQs} ± σ**	**Ionic Liquid**	**TLL ± σ**	**pH_{IL} ± σ**	**% EE_{FQs} ± σ**
$[C_2C_1im][CF_3SO_3]$	71.4 ± 0.2	2.95 ± 0.01	95.87 ± 0.35	$[C_2C_1im][CF_3SO_3]$	71.7 ± 0.0	2.92 ± 0.01	91.18 ± 0.50
$[C_4C_1im][CF_3SO_3]$	71.9 ± 0.9	2.73 ± 0.01	57.16 ± 0.37	$[C_4C_1im][CF_3SO_3]$	73.9 ± 2.0	2.70 ± 0.02	34.94 ± 1.43
$[C_4C_1im][Tos]$	70.8 ± 0.1	3.11 ± 0.01	97.18 ± 0.74	$[C_4C_1im][Tos]$	70.9 ± 0.2	3.14 ± 0.08	88.85 ± 1.73
$[P_{4441}][CH_3SO_4]$	69.9 ± 0.4	3.10 ± 0.03	44.83 ± 3.45	$[P_{4441}][CH_3SO_4]$	69.8 ± 0.1	3.08 ± 0.03	35.04 ± 7.07
$[P_{i\,(444)1}][Tos]$	67.1 ± 0.1	2.73 ± 0.06	92.70 ± 0.85	$[P_{i\,(444)1}][Tos]$	67.4 ± 0.3	2.70 ± 0.02	83.19 ± 0.47
$[P_{4444}]Br$	69.6 ± 0.8	1.37 ± 0.04	63.74 ± 0.78	$[P_{4444}]Br$	70.0 ± 0.5	1.39 ± 0.01	43.46 ± 4.24
$[P_{4444}]Cl$	70.0 ± 0.0	2.84 ± 0.05	51.74 ± 2.10	$[P_{4444}]Cl$	70.2 ± 0.2	2.87 ± 0.02	31.08 ± 2.50
	Moxifloxacin				***Sarafloxacin***		
Ionic Liquid	**TLL ± σ**	**pH_{IL} ± σ**	**% EE_{FQs} ± σ**	**Ionic Liquid**	**TLL ± σ**	**pH_{IL} ± σ**	**% EE_{FQs} ± σ**
$[C_2C_1im][CF_3SO_3]$	71.7 ± 0.6	2.95 ± 0.01	97.05 ± 0.45	$[C_2C_1im][CF_3SO_3]$	71.7 ± 0.4	2.95 ± 0.01	97.76 ± 0.51
$[C_4C_1im][CF_3SO_3]$	71.0 ± 1.4	2.72 ± 0.01	67.49 ± 0.63	$[C_4C_1im][CF_3SO_3]$	72.8 ± 1.8	2.70 ± 0.01	61.37 ± 2.15
$[C_4C_1im][Tos]$	70.0 ± 1.3	3.14 ± 0.04	96.42 ± 0.46	$[C_4C_1im][Tos]$	71.0 ± 0.1	3.12 ± 0.03	97.39 ± 2.07
$[P_{4441}][CH_3SO_4]$	69.3 ± 0.6	3.13 ± 0.02	61.74 ± 0.71	$[P_{4441}][CH_3SO_4]$	69.5 ± 0.2	3.15 ± 0.04	68.91 ± 0.79
$[P_{i\,(444)1}][Tos]$	67.2 ± 0.1	2.70 ± 0.05	95.86 ± 0.19	$[P_{i\,(444)1}][Tos]$	67.3 ± 0.3	2.69 ± 0.02	96.41 ± 0.30
$[P_{4444}]Br$	69.5 ± 0.4	1.39 ± 0.04	75.92 ± 2.73	$[P_{4444}]Br$	69.6 ± 0.0	1.34 ± 0.03	82.79 ± 2.59
$[P_{4444}]Cl$	70.1 ± 0.0	2.87 ± 0.03	81.74 ± 0.96	$[P_{4444}]Cl$	70.1 ± 0.0	2.88 ± 0.02	77.53 ± 1.82

Table S3. Comparison of the extraction efficiencies of six studied fluoroquinolones (%EE_{FQs}), and corresponding standard deviations (σ), from separate samples and one mixture sample, using $[C_2C_1im][CF_3SO_3]$ + $Al_2(SO_4)_3$ ABS.

	% EE_{FQs} ± σ	
Fluoroquinolones	**Separate sample**	**Mixture sample**
Ciprofloxacin	95.1 ± 0.3	94.9 ± 0.3
Norfloxacin	91.7 ± 0.4	92.9 ± 0.2
Ofloxacin	91.2 ± 0.5	91.3 ± 0.7
Enrofloxacin	95.9 ± 0.4	95.1 ± 0.7
Sarafloxacin	97.8 ± 0.5	96.9 ± 0.9
Moxifloxacin	97.1 ± 0.5	97.5 ± 0.7

Table S4. Extraction efficiencies of three studied fluoroquinolones (%EE_{FQs}), and corresponding standard deviations (σ) using phosphonium-based hydrophobic ILs.

	% EE_{FQs} ± σ		
Ionic Liquid	**Ciprofloxacin**	**Norfloxacin**	**Ofloxacin**
$[P_{666(14)}]Br$	60.2 ± 6.5	46.1 ± 3.4	28.0 ± 5.1
$[P_{666(14)}]Cl$	60.2 ± 1.1	68.6 ± 1.9	54.7 ± 5.6
$[P_{666(14)}][NTf_2]$	49.1 ± 3.8	29.7 ± 1.5	20.8 ± 1.7

7.3. Supporting Figures

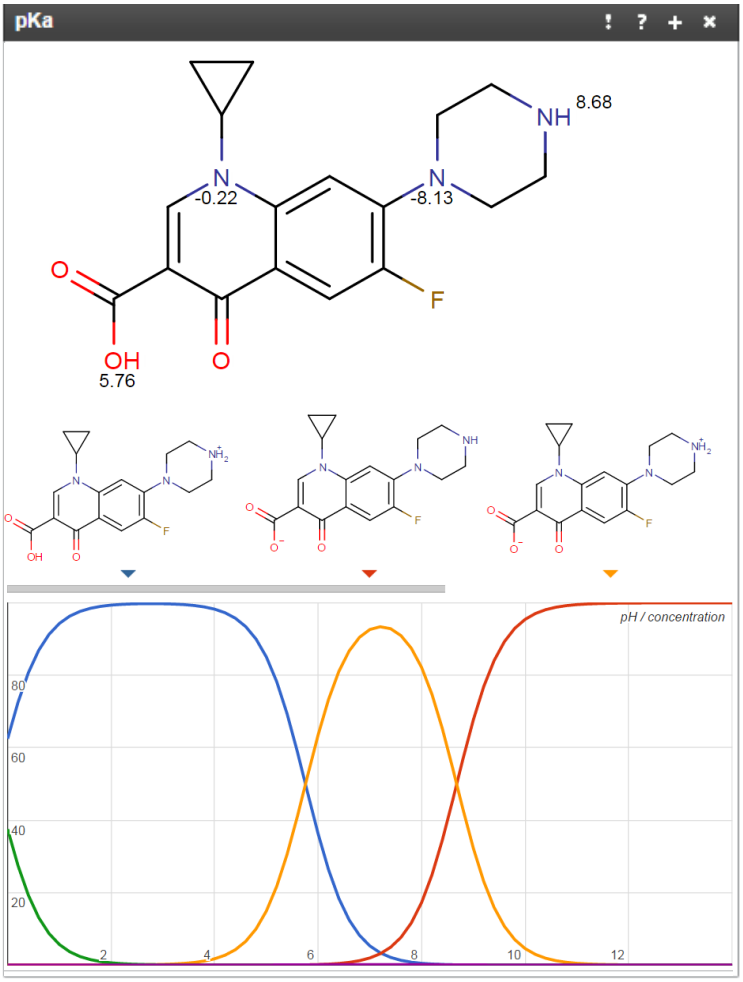

Figure S1. Acid dissociation constant of ciprofloxacin.[50]

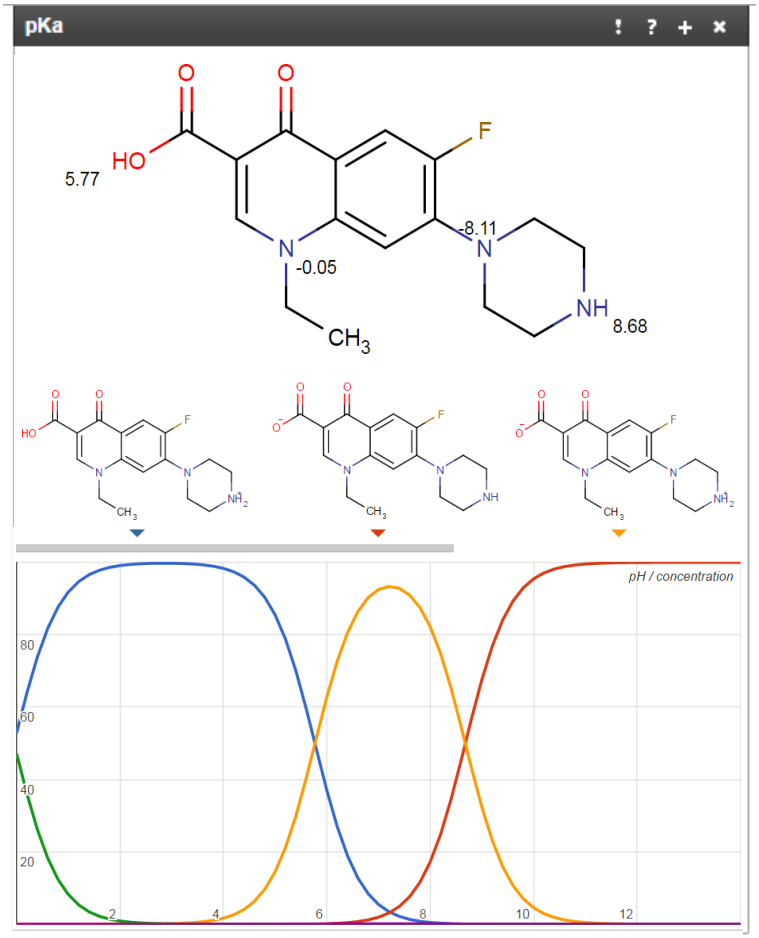

Figure S2. Acid dissociation constant of norfloxacin.[50]

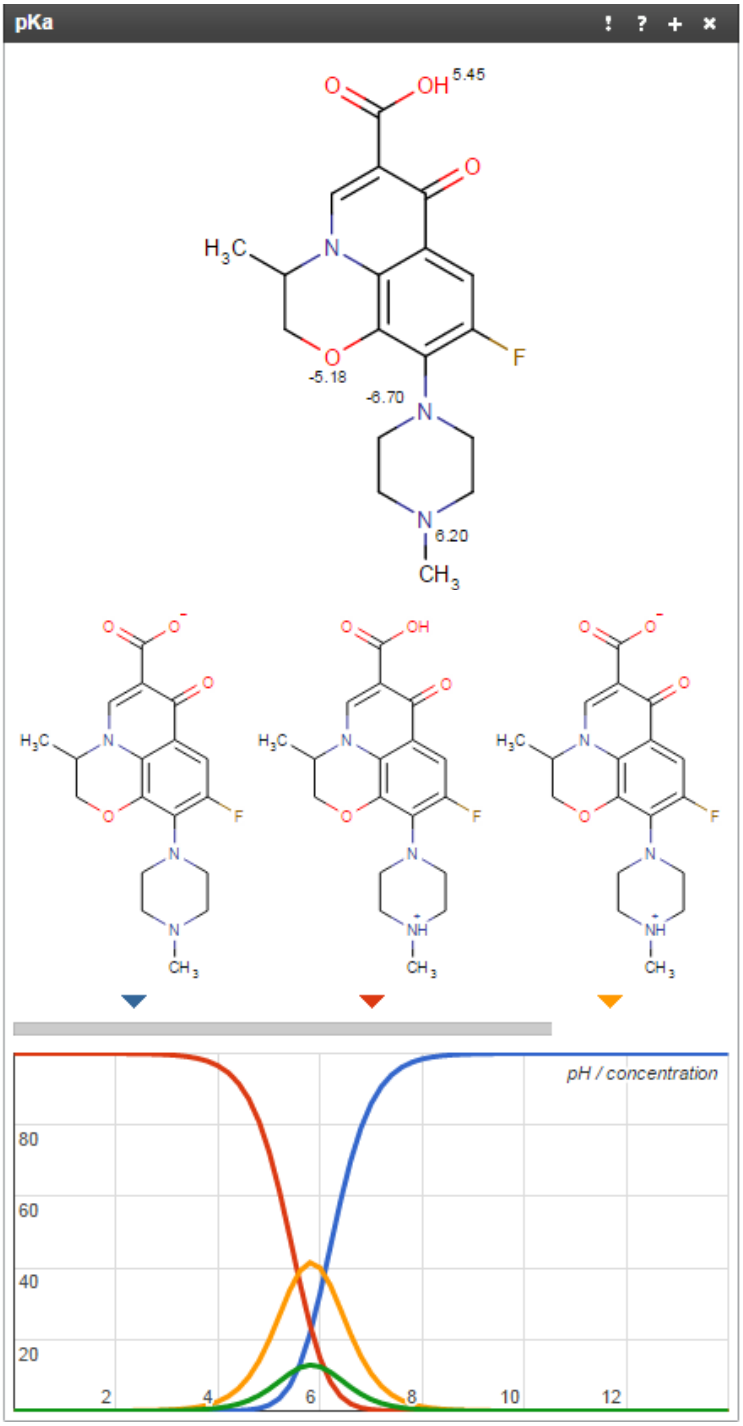

Figure S3. Acid dissociation constant of ofloxacin.[50]

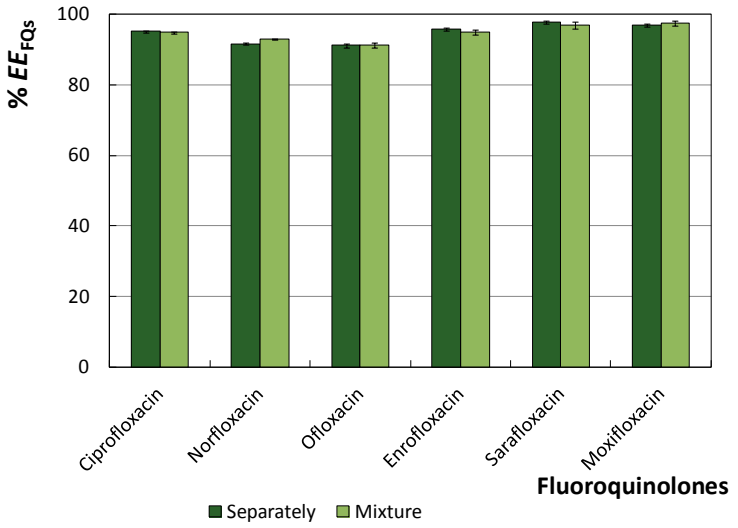

Figure S4. Extraction efficiencies of six studied fluoroquinolones (%EE_{FQs}) from separate samples and one mixture sample, using $[C_2C_1im][CF_3SO_3]$ + $Al_2(SO_4)_3$ ABS.

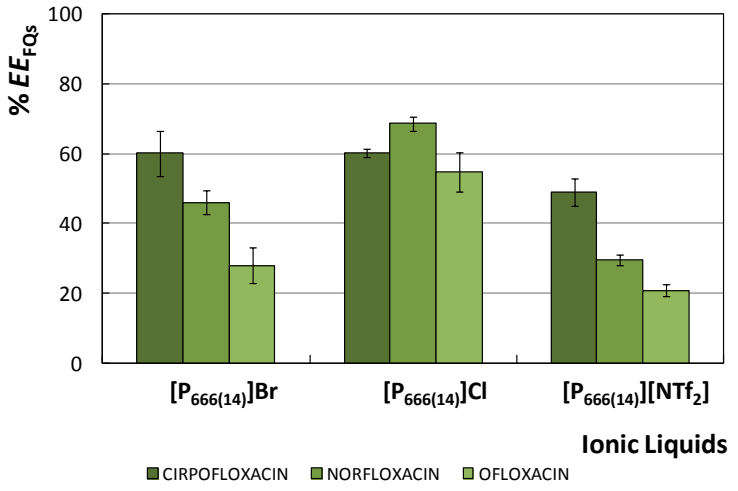

Figure S5. Extraction efficiencies of FQs (% EE_{FQs}) using hydrophobic phosphonium-based ILs, at 25 ºC.

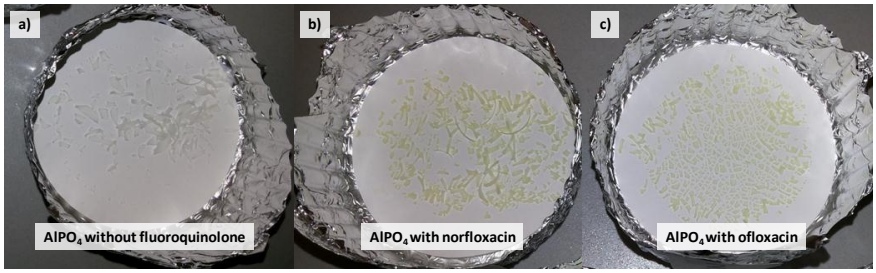

Figure S6. Precipitates obtained through the addition of K_3PO_4 to: a) an aqueous solution of $Al_2(SO_4)_3$ without fluoroquinolone; b) an aqueous solution of $Al_2(SO_4)_3$ and norfloxacin; and c) an aqueous solution of $Al_2(SO_4)_3$ and ofloxacin.

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Chapter 5

Supported Ionic Liquids as Efficient Materials to Remove Non-Steroidal Anti-Inflammatory Drugs from Aqueous Media

Adapted from: **Hugo F. D. Almeida,** Márcia C. Neves, Isabel M. Marrucho and Mara G. Freire; *Supported Ionic Liquids as Efficient Materials to Remove Non-Steroidal Anti-Inflammatory Drugs from Aqueous Media*; *in prepration*, **2017**.

The sample preparation for all the experiments presented herein was performed by the author. The author was also involved in all the experiments, as well as on the discussion and interpretation of the data and preparation of the manuscript. Scanning electron microscope, elementary analysis and nuclear magnetic resonance sections were performed by Márcia C. Neves.

1. Abstract

Due to their analgesic and antipyretic effects, as well as anti-inflammatory effects when consumed in higher doses, non-steroidal anti-inflammatory drugs (NSAIDs) are a class of active pharmaceutical ingredients (APIs). Mainly due to their large worldwide consumption, NSAIDs were already found in a wide variety of environmental aqueous samples, in concentrations ranging from ng/L to μg/L. This is due to the inability of the current technologies used in sewage treatment plant (STPs) and wastewater treatment plants (WWTPs) to remove such pollutants/contaminants, thus leading to serious environmental and public health concerns after long-term exposures. This work addresses the preparation and evaluation of novel materials based on silica modified with 1-methyl-3-propylimidazolium as alternative materials to remove NSAIDs from aqueous media. Modified silica-based materials with six different anions were prepared and chemically and morphologically characterized by elemental analysis, ^{13}C solid nuclear magnetic resonance, infrared spectroscopy, and scanning electron microscopy. Afterwards, the adsorption kinetics and adsorption isotherms of diclofenac – as one of the most worldwide consumed NSAIDs - were determined at 25°C. The adsorption equilibrium data obtained were well described either by the Langmuir or the Freundlich isotherm models, depending on the material counter ion. A maximum equilibrium concentration of diclofenac in the solid phase of 0.75 mmol (or 0.22 g) *per* g of SILP was obtained with the SILP containing chloride as the counter ion. Considering values of diclofenac in the order of $\mu g.L^{-1}$, 1 gram of the prepared material is "ideally" able to treat *ca.* 50,000 L of water. SILPs are thus promising materials for the removal of APIs and are envisioned as alternative strategies for the treatment of wastewater samples.

2. Introduction

The global occurrence of active pharmaceutical ingredients (APIs) in the environment is a current matter of serious concern.[1] APIs have been found in non-negligible levels (up to $\mu g.L^{-1}$) in sewage treatment plants (STPs), wastewater treatment plants (WWTPs) and surface water effluents,[2-6] leading to serious problems in environmental and public health[7] after long-term exposures.[8] Although the initial purpose of pharmaceuticals is to provide a better quality life and to improve humans and animals health, when released into the environment they will affect the entire biota, from primary producers and consumers to top predators, including humans.[8, 9] Most of these compounds are cytotoxic, genotoxic and/or endocrine disruptors. Since the administered doses of APIs are not completely metabolized by humans or animals, they are excreted by urine and/or feaces in either conjugated or in unchanged forms, reaching the aquatic environment.[10-14] Also, unnecessary or expired medications are recurrently straightly disposed into wastewaters.[15] Albeit WWTPs use advanced processes for water purification, such as membrane filtration, ozonation, chlorination, flocculation/sedimentation and adsorption, none of these strategies was specifically designed to remove APIs,[4, 16-18] explaining why some of these contaminants were already detected even in drinking water.[19, 20]

Within APIs and based on the Global Water Research Coalition (GWRC) criteria,[21, 22] some non-steroidal anti-inflammatory drugs (NSAIDs) are part of the top 10 persistent pollutants list, where diclofenac is the most prevalent NSAID used throughout the world.[23] As with most of the APIs, diclofenac has also a poor degradation[24] and there is a lack of efficient strategies for its removal from aqueous samples.[25] Therefore, diclofenac has been detected in rivers, sediments and sludges;[20, 25, 26] more recently, it has even been found in drinking water sources.[20, 27, 28] The physicochemical properties of diclofenac are listed in Table S1, in the Supporting Information.

The removal of diclofenac from aqueous samples has been previously addressed. Removal efficiencies of about 93 % were achieved by Betrán et al.[29] Primary treatments with coagulating and flocculating agents, such as $FeCl_3$ and $Al_2(SO_4)_3$, have also been reported; yet, they shown to be not able to completely remove the drug from wastewater samples, with a maximum of 70 % of removal.[30] Conventional activated sludge processes showed a better efficiency when compared to membrane bioreactors, where a 30-70 % of removal of diclofenac can be obtained.[31] However, the conventional WWTPs treatment techniques, such as lime clarification, dissolved air flotation, dual media filtration, ozonation, activated carbon, combined reverse-osmosis/nanofiltration and UV disinfection are highly energy-intensive and costly processes.[31, 32]

Based on the exposed, the development of cost-efficient treatment techniques able to remove diclofenac and other APIs from the aquatic environment is an urgent requirement of modern society. To this end, supported ionic liquids phases (SILPs) can be seen as an alternative removal/adsorption approach. Several SILs have been reported in the literature to separate mixtures of inorganic anions, organic anions and organic compounds,[33, 34] as well as aromatic carboxylic acids.[35] In addition to ILs covalently bonded to the supports, Myasoedova et al.[36] and Li et al.[37] reported the use of physically immobilized ILs to extract platinum (Pt(IV)) and plutonium (Pu(IV)) and five phthalates, respectively. In summary, these materials have shown to be promising in a large variety of applications due to their functionalization or impregnation with ILs. The presence of ILs in solid phases allows the preparation of materials bearing the properties of ILs. Despite the fact that ILs have a unique combination of valuable properties, the key property here is their enhanced solvation ability for a large variety of compounds. Furthermore, due to the large number of possible cation-anion combinations, these fluids are known as designer solvents, meaning that their chemical structures can be designed for target applications.[38, 39] Mainly due to this possibility, in recent years, ILs have been successfully applied in a wide

variety of fields, such as in analytical chemistry,[40, 41] synthesis,[42, 43] catalysis,[44, 45] electrochemistry,[46, 47] and separation processes.[48-50]

The combination of the ILs properties with the advantages of solid supports raised the attention on the use of SILPs as alternative materials for solid-phase extractions (SPE).[51] To the best of our knowledge, the use of SILPs as adsorbents for the removal of diclofenac from aqueous media has not been previously reported. In this work, IL-functionalized silica was prepared by chemically bonding 3-chloropropyltrimethoxysilane onto the silica surface, followed by reaction with *N*-methylimidazolium, resulting in the formation of 1-methyl-3-propylimidazolium-based supported silica material ([Si][C_3C_1im]Cl). Other five additional SILPs were prepared from [Si][C_3C_1im]Cl by anion exchange, namely [Si][C_3C_1im][SCN], [Si][C_3C_1im][$N(CN)_2$], [Si][C_3C_1im][Tos], [Si][C_3C_1im][Male] and [Si][C_3C_1im][NTf_2] – their full description is given below. All SILPs were chemically and morphologically characterized and evaluated as alternative sorbents to remove NSAIDs from aqueous media by the determination of the adsorption kinetics and isotherms of diclofenac, as a major representative of the NSAIDs class.

3. Experimental Section

3.1. Materials

Silica gel (60, 0.2-0.5 mm), used as the functionalized material, was acquired from Merck. The non-steroidal anti-inflammatory drug used was sodium diclofenac (CAS# 15307-79-6), acquired from Sigma-Aldrich. The solvents used to prepare the IL-functionalized silica ([Si][C_3C_1im]Cl) were hydrochloric acid (CAS# 7647-01-0, purity 37 %) and toluene (CAS# 108-88-3, purity 99.8 %), both acquired from Sigma Aldrich, ethanol (CAS# 64-17-5, purity 99.9 %) from Carlo Erba, methanol (CAS# 67-56-1, HPLC grade) from Chem-Lab, and (3-chloropropyl)trimethoxysilane (CAS# 2530-87-2, purity 98 %) and *N*-

methylimidazolium (CAS# 616-47-7, purity 99 %), both from Acros Organics. To obtain the remaining SILPs by anion exchange, NaSCN (CAS# 540-72-7, purity 98 %) from Sigma-Aldrich, $NaN(CN)_2$ (CAS# 1934-75-4, purity 96 %) from Alfa Aesar, NaTos (CAS# 657-84-1, purity > 90 %) from Alfa Aesar, $NaC_4H_3O_4$ (CAS# 3105-55-3, purity 99 %) from Sigma-Aldrich, and $LiNTf_2$ (CAS# 90076-65-6, purity 99 %) from Iolitec, were used. The water used in the adsorption experiments was double distilled, passed across a reverse osmosis system and further treated with a Milli-Q plus 185 water purification equipment.

3.2. Synthesis of IL-functionalized silica (SILPs)

Silica was first immersed in hydrochloric acid for 24h, and then washed with double distilled water and dried under vacuum for 24h at 105°C. This activated silica (5.0 g) was resuspended in 60 mL of dried toluene, followed by the addition of 5.0 mL of 3-chloropropyltrimethoxysilane, and 0.5 mL of triethylamine (used as a catalyst). This suspension was mechanically stirred and refluxed for 24h at 130°C. After refluxing, the reaction was stopped and cooled to room temperature, transferred to a vacuum glass filter, and washed with toluene (100 mL), an ethanol–water (1:1, v:v) mixture (200 mL), double distilled water (500 mL), and finally with methanol (100 mL). The chloropropyl silica obtained, $[Si][C_3]Cl$, was dried under vacuum at 60°C for 24h, before the reaction with *N*-methylimidazole. To this end, 5.0 g of dried chloropropyl silica was placed in a reaction vial containing 60 mL of toluene and 5.0 mL of *N*-methylimidazole. The mixture was refluxed under stirring for 24h. The reaction was stopped and the modified silica was cooled to room temperature, transferred to a vacuum glass filter, and washed with methanol (350 mL), water (300 mL), and again with methanol (150 mL). The described synthesis procedure was adapted from that previously reported by Qiu et al.[34] The chemically modified silica, $[Si][C_3C_1im]Cl$, was dried under vacuum at 50°C for 8 h prior to their characterization and determination of the kinetic and adsorption isotherms. In Figure 30 is depicted a

schematic diagram of the synthetic approach used for the preparation of [Si][C_3C_1im]Cl.

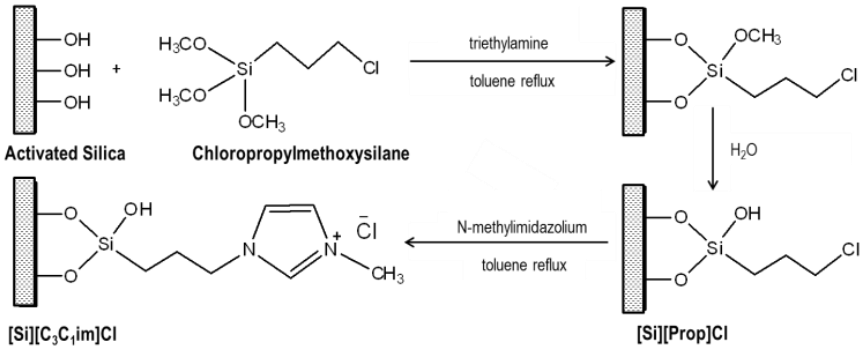

Figure 30. Preparation of silica functionalized with *N*-methylimidazolium chloride.

[Si][C_3C_1im][SCN], [Si][C_3C_1im][$N(CN)_2$], [Si][C_3C_1im][Tos], [Si][C_3C_1im][Male] and [Si][C_3C_1im][NTf_2] were prepared by an anion exchange approach from [Si][C_3C_1im]Cl. These anions were chosen to explore the effect of the counter ion towards the NSAIDs removal efficiency. 0.5 g of [Si][C_3C_1im]Cl was mixed with 0.5 g (anion in excess) of NaSCN, $NaN(CN)_2$, NaTos, $NaC_4H_3O_4$ or $LiNTf_2$, in 40 mL of double distilled water, and left under moderate stirring and temperature for 24h. The materials were then filtered and washed with double distilled water. The obtained functionalized silica was dried under vacuum at 50°C for 8 h prior to its characterization and use. In Figure 31 depicts a schematic diagram of the approach used to prepare [Si][C_3C_1im][SCN], [Si][C_3C_1im][$N(CN)_2$], [Si][C_3C_1im][Tos], [Si][C_3C_1im][Male] and [Si][C_3C_1im][NTf_2].

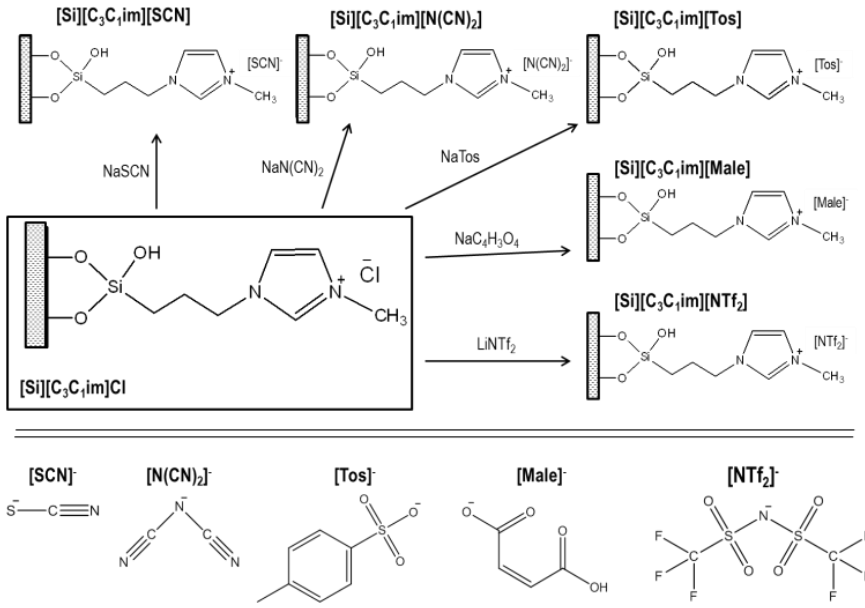

Figure 31. Preparation of supported *N*-methylimidazolium with thiocyanate ($[SCN]^-$), dicyanamide ($[N(CN)_2]^-$), tosylate ($[Tos]^-$), 3-carboxy-2-hydroxypropanoate ($[Male]^-$) and bis(trifluoromethylsulfonyl)imide ($[NTf_2]^-$) anions.

3.3. Infrared Spectroscopy

Fourier transform infrared spectroscopy (FTIR) spectra of activated silica, [Si][C_3]Cl, and [Si][C_3C_1im]Cl, were acquired using a Perkin Elmer BX Spectrometer, with resolution of 5 cm^{-1} and equipped with a horizontal Golden Gate ATR cell, in the range of 4000-500 cm^{-1}.

3.4. Solid ^{13}C Nuclear Magnetic Resonance

^{13}C nuclear magnetic resonance (NMR) spectra were recorded at 9.7 T on a Bruker Avance III - 400 MHz spectrometer (DSX model) on 4 mm BL cross-polarization magic angle spinning (CPMAS) VTN probes at 100.6 MHz, at room temperature. In order to increase the signal-to-noise ratio of the solid-state

spectra, the CPMAS NMR ^{13}C settings used were the following: $\nu_1{}^{13}$ C = 55 kHz; recycle delay, 4 s; contact time, 1-2 ms; NS = 1k; ν_R = 12 kHz. SPINAL-64 decoupling was used during data acquisition.

3.5. Elemental analysis

Carbon, hydrogen, nitrogen and sulfur contents (in weight percentage) of [Si][C_3C_1im]Cl, [Si][C_3C_1im][SCN], [Si][C_3C_1im][$N(CN)_2$], [Si][C_3C_1im][Tos], [Si][C_3C_1im][Male] and [Si][C_3C_1im][NTf_2] were determined using a TruSpec 630-200-200, with a sample size of 2 mg, a combustion furnace temperature of 1075°C, afterburner temperature of 850°C, and detection method of infrared absorption for carbon, hydrogen and sulfur, and thermal conductivity for nitrogen.

3.6. Scanning electron microscope

Scanning electron microscopy (SEM) was performed using a Hitashi SU-70, equipped with EDX Bruker model Quantax 400. A suspension of the materials was prepared by their deposition in water on a glass substrate. After evaporation of the solvent, a carbon film was deposited to turn the sample conductive.

3.7. Adsorption kinetics and isotherms

The adsorption kinetics and isotherms of the six SILPs for NSAIDs were determined using aqueous solutions of diclofenac, as major representative of the NSAIDs class, at different concentrations. For the adsorption kinetics evaluation, a solution of diclofenac with a concentration of 0.025 $g.L^{-1}$ was used, while for the adsorption isotherms, concentrations of the drug ranging from 0.001 to 0.460 $g.L^{-1}$ were employed. In these experiments, 2.5 mg of SILPs were mixed with 10 mL of diclofenac aqueous solutions in 50 mL pyrex Erlenmeyers, and then placed in an orbital shaker at 120 rpm and (25.0 ± 0.5)°C. For the adsorption kinetics evaluation, times of equilibration from 0 to 180 min were investigated, while for the

adsorption isotherms evaluation the time of equilibrium was settled as 120 min. After the equilibration assays, all samples were centrifuged at 5000 rpm for 5 min, and the amount of diclofenac in the water phase was quantified through UV-spectroscopy, using a Shimadzu UV-1800, Pharma-Spec UV-Vis Spectrophotometer, at a wavelength of 276 nm. At least, three replicates were investigated for each condition.

The equilibrium concentration of adsorbate in the solid phase (q_e / mmol.g^{-1}) was determined according to (Eq. 1):

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

where w is the weight of SILP (g), V is the volume of the diclofenac aqueous solution (L), and C_0 and C_e are the equilibrium concentrations of diclofenac before and after adsorption on SILPs, respectively (mmol.L^{-1}).

Both pseudo first-order and pseudo second-order models were applied to correlate the experimental data, namely with the linear form of the Lagergren's[52] first order rate equation (Eq. 2) and Ho's[53] second order rate equation (Eq. 3):

$$ln(q_e - q_t) = lnq_e - k_1 t \quad (2)$$

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} t \quad (3)$$

where t is the time (min), q_e is the amount of diclofenac adsorbed onto the adsorbent at equilibrium (mmol.g^{-1}), q_t is the amount of diclofenac adsorbed onto the adsorbent at different times (mmol.g^{-1}), k_1 (min^{-1}) is the rate constant of the pseudo first-order adsorption, and k_2 (gmmol^{-1}min^{-1}) is the rate constant of the pseudo-second-order adsorption.

The experimental data were also fitted with the Langmuir[54] (Eq.4), Freundlich[55] (Eq.5) and Dubinin-Radushkevich[56] (Eqs. 6 and 7) models:

$$q_e = \frac{q_{max} \times B \times C_e}{1 + B \times C_e} \quad (4)$$

$$lnq_e = lnK_f + \frac{1}{n} lnC_e \quad (5)$$

$$q_e = q_s \, exp^{(-K_{DR}\varepsilon^2)} \quad (6)$$

$$\varepsilon = RTln\left(1 + \frac{1}{C_e}\right) \quad (7)$$

where C_e is the equilibrium concentration of adsorbate (mmol.L^{-1}), q_e is the equilibrium concentration of adsorbate in the solid phase (mmol.g^{-1}), B (L.mmol^{-1}) is the Langmuir isotherm constant, q_{max} (mmol.g^{-1}) is the maximum monolayer coverage capacity, K_f (adsorption capacity) and n (adsorption intensity) are the constants of the Freundlich equation, and q_s (mgP.g^{-1}) and K_{DR} (mol^2.k^{-1}J^{-2}) are the constants in the Dubinin-Radushkevich equation, related to the adsorption capacity and free energy of adsorption. R (8.314462 J.mol^{-1}K^{-1}) and T (298. 15 K) are the gas constant and absolute temperature. The fitting of the data was performed using SigmaPlot®.

4. Results and Discussion

4.1. Characterization of the prepared SILPs

In order to confirm the silica functionalization with ILs, FTIR spectra of activated silica, chloropropyl silica, and [Si][C_3C_1im]Cl were acquired from 4000 to 500 cm^{-1}, provided in the Supporting Information (Figure S1). In general, it was observed the disappearance of the band at 960 cm^{-1}, related to the bending vibration absorption of Si–OH[57], meaning that the functionalization of silica occurred. The successful preparation of [Si][C_3C_1im]Cl was further confirmed through ^{13}C solid NMR, as shown in Figure 32. The signals at 51, 24 and 10 ppm correspond to the three carbon atoms at the imidazolium alkyl chain length (C4, C5, and C6), the peaks ranging from 120-140 correspond to the aromatic carbons

of imidazolium (C3 and C2), and the signal at 37 ppm corresponds to the carbon of the methyl chain (C1), in good agreement with previously reported results.[58]

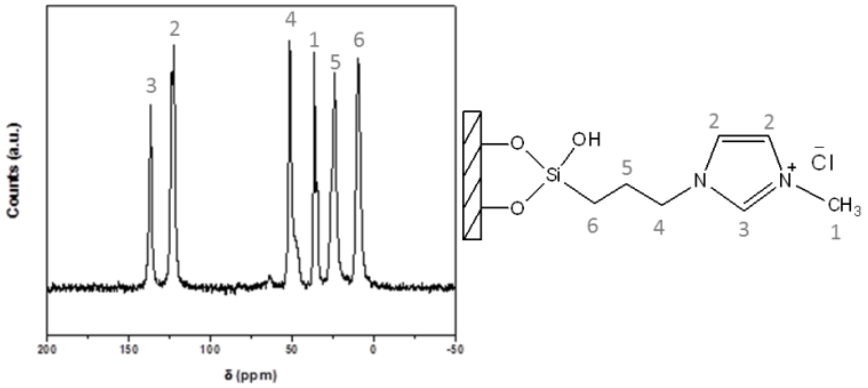

Figure 32. ^{13}C NMR solid-state spectrum of [Si][C_3C_1im]Cl.

Figure 33 depicts the SEM images of activated silica and [Si][C_3C_1im]Cl, with a magnification of 35,000-fold, showing that the IL immobilization results in some changes in the surface morphology, in agreement with conclusions provided by Su et al.[57] on SILPs with a different counter ion. The *N*-methylimidazolium-based IL material presents a less rough and more smooth or homogeneous surface. SEM images were also acquired for [Si][C_3C_1im][SCN], [Si][C_3C_1im][$N(CN)_2$], [Si][C_3C_1im][Tos], [Si][C_3C_1im][Male] and [Si][C_3C_1im][NTf_2] – shown in Figure S2 in the Supporting Information. No significant differences in morphology were observed between the six SILPs prepared, although they morphologically differ from activated silica.

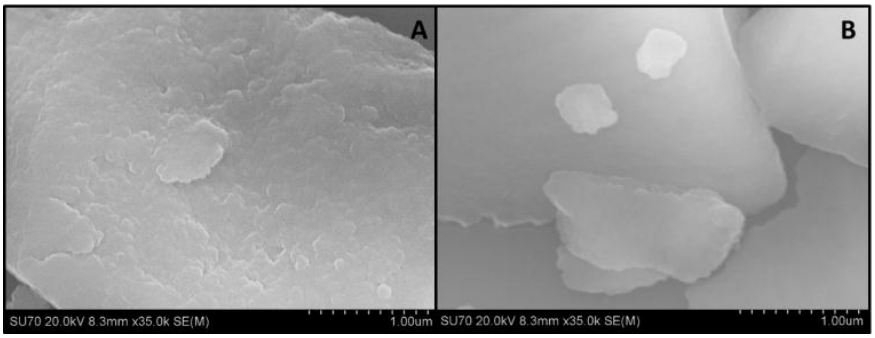

Figure 33. SEM images of: A) activated silica; and B) [Si][C_3C_1im]Cl.

Elemental analysis was carried out to quantitatively determine the carbon, hydrogen, nitrogen, and sulfur contents of the six SILPs prepared. The gathered results are provided in Table 7. The carbon and nitrogen contents (in weight fraction percentage) of the supported materials range from (8.51 ± 0.11) % to (11.12 ± 0.07) % and from (2.27 ± 0.10) % to (4.29 ± 0.02) %, respectively, depending thus on the carbon and nitrogen content also derived from the anion, whereas the carbon content of the activated silica was (0.64 ± 0.22) %. This low carbon content value of activated silica might result from absorbed air. It should be however noted that no nitrogen was detected in activated silica, supporting the absence of functional organic moieties in this starting material. According to these results, (0.0010 ± 0.0003) mol of 1-methyl-3-propylimidazolium ($[C_1C_3im]^+$) *per* 1.000 g of 1-methyl-3-propyl-imidazolium-based ILs ([Si][C_3C_1im]X) were obtained, where X represents the anion. Furthermore, sulfur was identified in [Si][C_3C_1im][SCN], [Si][C_3C_1im][Tos] and [Si][C_3C_1im][NTf_2], ranging from (0.69 ± 0.07) % to (1.53 ± 0.04) %. Likewise, (0.0003 ± 0.0001) mol as one of the most of sulfur ($[SCN]^-$, $[Tos]^-$, and $[NTf_2]^-$) *per* 1.000 g of [Si][C_3C_1im]X were obtained. These results prove that the studied ILs were immobilized on the silica surface and that the anion exchange was carried out successfully.

Table 7. Results of elemental analysis and respective standard deviations (σ) of activated silica, [Si][C_3]Cl, [Si][C_3C_1im]Cl, [Si][C_3C_1im][SCN], [Si][C_3C_1im][$N(CN)_2$], [Si][C_3C_1im][Tos], [Si][C_3C_1im][Male] and [Si][C_3C_1im][NTf_2].

	Atom content (weight fraction percentage ± σ)			
	C	H	N	S
Activated Silica	0.64 ± 0.22	1.10 ± 0.15	---	---
[Si][C_3]Cl	5.90 ± 0.16	1.54 ± 0.15	---	---
[Si][C_3C_1im]Cl	8.58 ± 0.11	1.86 ± 0.22	2.33 ± 0.12	---
[Si][C_3C_1im][SCN]	8.51 ± 0.12	1.99 ± 0.05	2.85 ± 0.09	0.88 ± 0.08
[Si][C_3C_1im][$N(CN)_2$]	9.09 ± 0.03	2.16 ± 0.15	4.29 ± 0.02	---
[Si][C_3C_1im][Tos]	10.62 ± 0.01	2.31 ± 0.10	2.32 ± 0.02	0.69 ± 0.07
[Si][C_3C_1im][Male]	11.12 ± 0.07	2.42 ± 0.09	2.27 ± 0.10	---
[Si][C_3C_1im][NTf_2]	9.18 ± 0.03	1.97 ± 0.18	2.88 ± 0.10	1.53 ± 0.04

4.2. Adsorption kinetics and isotherms of diclofenac in SILPs

The adsorption kinetic curves of diclofenac in all SILPs were determined in order to evaluate and set the appropriate contact time for further studies on the adsorption isotherms. The results obtained are depicted in Figure 34, and Tables S2 to 7 in the Supporting Information. The adsorption efficiency (%AE – Eq. S1 in the Supporting Information) of the six SILPs toward diclofenac is also shown in the Supporting Information, Figure S3. Under the conditions evaluated, it was found that a plateau in the equilibrium concentration of adsorbate in the solid phase (q_e) is reached after 15 min, and up to the total of 180 min evaluated. Moreover, a maximum equilibrium concentration of adsorbate in the solid phase of 0.310 $mmol.g^{-1}$ and an adsorption efficiency of 92.9 ± 0.4 % was found using [Si][C_3C_1im]Cl. The adsorption of diclofenac at the conditions studies is highly fast (≈ 15 min), meaning a fast non-covalent binding or anchorage of diclofenac on the

sorbent surface. According to the obtained results, the equilibrium concentration of adsorbate in the solid phase decreases in the following sequence of SILPs: [Si][C_3C_1im]Cl > [Si][C_3C_1im][SCN] > [Si][C_3C_1im][$N(CN)_2$] > [Si][C_3C_1im][Tos] > [Si][C_3C_1im][Male] > [Si][C_3C_1im][NTf_2].

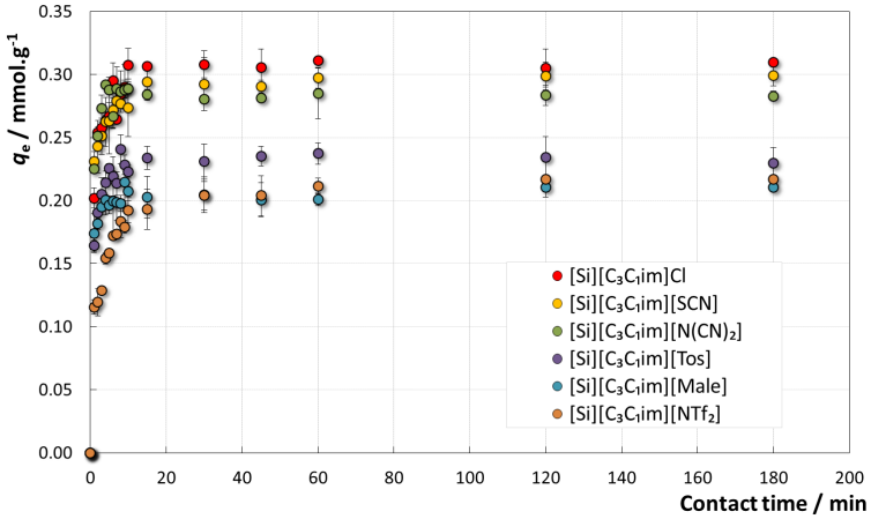

Figure 34. Adsorption kinetic curves of diclofenac in SILPs: ●, [Si][C_3C_1im]Cl; ●, [Si][C_3C_1im][SCN]; ●, [Si][C_3C_1im[$N(CN)_2$]; ●, [Si][C_3C_1im][Tos]; ●, [Si][C_3C_1im][Male]; and ●, [Si][C_3C_1im][NTf_2].

In order to explore the adsorption mechanisms of diclofenac in the prepared SILPs, pseudo first-order kinetic (Eq. 2) and pseudo second-order (Eq. 3) kinetic models were used to correlate the experimental data. The adsorption kinetic parameters are summarized in Table 8, and in Figure S4 in the Supporting Information.

Table 8. Parameters of the pseudo first-order and pseudo second-order kinetic models.

	$q_{e,exp}$ / mmol.g^{-1}	Pseudo first-order model		
		$q_{e,cal}$ / mmol.g^{-1}	k_1 / min^{-1}	R^2
[Si][C_3C_1im]Cl	0.310	0.124	0.260	0.7453
[Si][C_3C_1im][SCN]	0.299	0.033	0.040	0.8355
[Si][C_3C_1im][$N(CN)_2$]	0.292	0.063	0.320	0.7245
[Si][C_3C_1im][Tos]	0.241	0.058	0.149	0.8148
[Si][C_3C_1im][Male]	0.215	0.038	0.145	0.7578
[Si][C_3C_1im][NTf_2]	0.217	0.081	0.072	0.8186
	$q_{e,exp}$ / mmol.g^{-1}	Pseudo second-order model		
		$q_{e,cal}$ / mmol.g^{-1}	k_2 / min^{-1}	R^2
[Si][C_3C_1im]Cl	0.310	0.310	6.220	0.9999
[Si][C_3C_1im][SCN]	0.299	0.300	5.432	0.9999
[Si][C_3C_1im][$N(CN)_2$]	0.292	0.283	118.64	0.9999
[Si][C_3C_1im][Tos]	0.241	0.232	72.657	0.9998
[Si][C_3C_1im][Male]	0.215	0.210	8.772	0.9997
[Si][C_3C_1im][NTf_2]	0.217	0.219	2.514	0.9999

The correlation coefficients (R^2) obtained for the first-order kinetic model range between 0.7245 and 0.8355, while those obtained for the second-order model are higher than 0.9997, meaning that the last model better describes the adsorption experimental data. These results indicate that the adsorption process is controlled by the adsorption at the liquid-solid interface in the adsorbent.[53, 59]

The experimental data on the adsorption isotherms at 25°C were fitted by the Langmuir[54] (Eq.4), the Freundlich[55] (Eq.5) and the Dubinin-Radushkevich[56]

(Eqs. 6 and 7) models, using initial concentrations of diclofenac ranging between 0.001 and 0.460 $g.L^{-1}$. The contact time to carry out the adsorption isotherms studies was set to 120 min to guarantee that equilibrium was totally reached. The relationship between the equilibrium concentrations of diclofenac between the solid and liquid phases is shown in Figure 35 (the respective representations according to each SILP are given in Figure S5 and the detailed data are given in Table S8, in the Supporting Information).

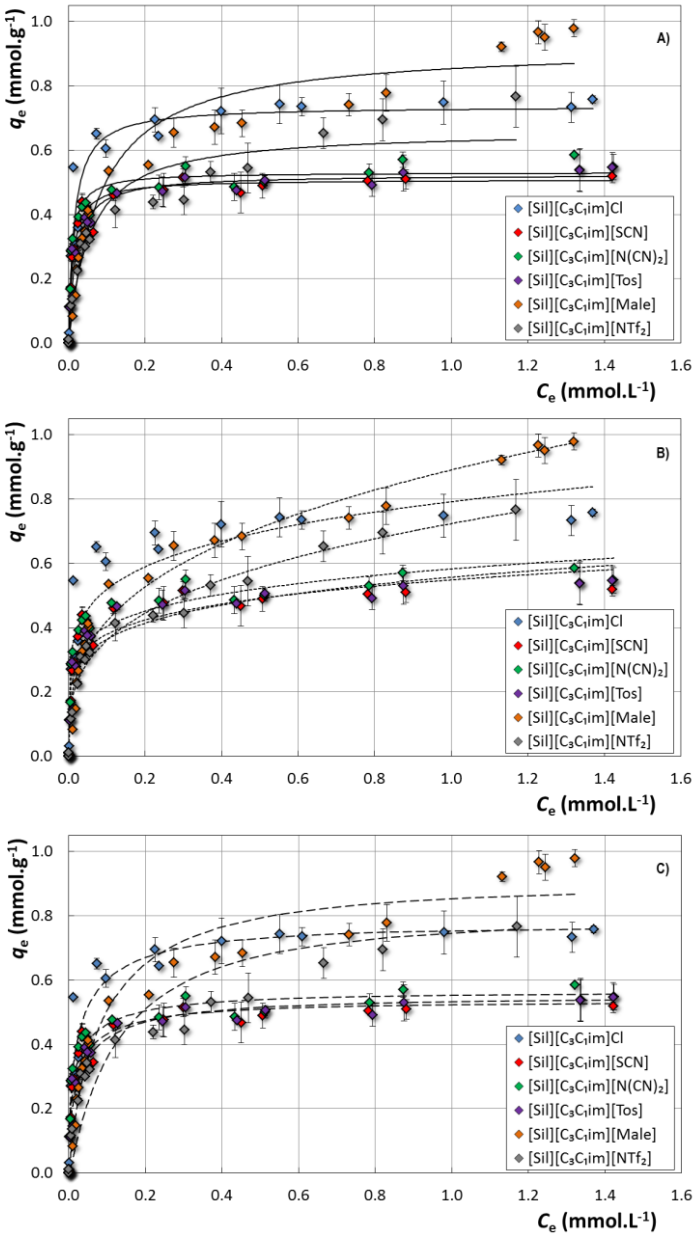

Figure 35. Adsorption isotherms of diclofenac onto SILPs at 25°C: A) Langmuir fitting (Eq. 4); B) Freundlich fitting (Eq. 5); C) and Dubinin-Radushkevich fitting (Eqs. 6 and 7); for ◆, [Si][C_3C_1im]Cl; ◆, [Si][C_3C_1im][SCN]; ◆, [Si][C_3C_1im][N(CN)$_2$]; ◆, [Si][C_3C_1im][Tos]; ◆, [Si][C_3C_1im][Male]; and ◆, [Si][C_3C_1im][NTf$_2$].

The equilibrium adsorption of diclofenac onto SILPs increases with the increase of its initial concentration until saturation, where a plateau is reached at around 0.2 mmol.L^{-1} of an equilibrium concentration of adsorbate. In Table 9 are presented the isotherms parameters and the correlation coefficients (R^2) derived from Eqs. 4 to 7. The adsorption of diclofenac onto the six SILPs can be described by the Dubinin-Radushkevich isotherm model (R^2 > 0.9); however, the Langmuir and Freundlich models are more indicated to describe the adsorption processed observed, with correlation coefficients always above 0.95. The adsorption data of diclofenac onto [Si][C_3C_1im]Cl, [Si][C_3C_1im][SCN], [Si][C_3C_1im][$N(CN)_2$] and [Si][C_3C_1im][Tos] fit better to the Langmuir model (R^2 = 0.9514, 0.9710, 0.9650 and 0.9558, respectively), while for the SILPs [Si][C_3C_1im][Male] and [Si][C_3C_1im][NTf_2] the Freundlich model (R^2 = 0.9615 and 0.9898, respectively) better describes the adsorption isotherms of diclofenac. This trend suggests that the adsorption of the drug onto [Si][C_3C_1im]Cl, [Si][C_3C_1im][SCN], [Si][C_3C_1im][$N(CN)_2$] and [Si][C_3C_1im][Tos] occurs through the formation of a monolayer adsorbate on the outer surface of the adsorbent, where no further adsorption takes place. On the other hand, the adsorption of diclofenac onto [Si][C_3C_1im][Male] and [Si][C_3C_1im][NTf_2] presents an adsorption behavior corresponding to both homogeneous and heterogeneous surfaces, where an adsorption process followed by a pore filling mechanism could be occuring. Therefore, different ionic structures as counter ions in the supported material seem to lead to different adsorption processes, and for which the functionalized silica-based materials modified with the $[C_1C_3im]^+$ cation and six anions appear as remarkable materials to remove diclofenac from aqueous media. It should be remarkable that non-functionalized silica was also tested under the same conclusions and for which no adsorption of diclofenac was found, even after long periods of time (up to 180 min).

Table 9. Correlation coefficients obtained with the Langmuir, Freundlich and Dubinin-Radushkevich models regarding the adsorption isotherms of diclofenac onto the six studied SILPs.

	Langmuir model		
SILP	**(q_{max} ± σ) / mmol.g^{-1}**	**(B ± σ) / mmol.g^{-1}**	**R^2**
[Si][C_3C_1im]Cl	0.74 ± 0.03	68.99 ± 1.29	0.9514
[Si][C_3C_1im][SCN]	0.51 ± 0.03	92.73 ± 6.62	0.9710
[Si][C_3C_1im][$N(CN)_2$]	0.53 ± 0.04	120.55 ± 7.16	0.9650
[Si][C_3C_1im][Tos]	0.52 ± 0.02	56.68 ± 4.54	0.9558
[Si][C_3C_1im][Male]	0.92 ± 0.03	12.16 ± 1.82	0.9553
[Si][C_3C_1im][NTf_2]	0.66 ± 0.07	18.64 ± 4.13	0.9223
	Freundlich model		
SILP	**(K_f ± σ)**	**(n ± σ)**	**R^2**
[Si][C_3C_1im]Cl	0.79 ± 0.04	5.46 ± 0.03	0.8409
[Si][C_3C_1im][SCN]	0.55 ± 0.03	6.23 ± 0.03	0.8404
[Si][C_3C_1im][$N(CN)_2$]	0.58 ± 0.03	6.29 ± 0.02	0.8603
[Si][C_3C_1im][Tos]	0.56 ± 0.03	5.43 ± 0.03	0.8511
[Si][C_3C_1im][Male]	0.89 ± 0.02	3.03 ± 0.03	0.9615
[Si][C_3C_1im][NTf_2]	0.72 ± 0.02	3.32 ± 0.03	0.9898
	Dubinin-Radushkevich model		
SILP	**(q_s ± σ) / mgP.g^{-1}**	**(K_{DR} ± σ) / mol^2.k^{-1}J^{-2}**	**R^2**
[Si][C_3C_1im]Cl	0.77 ± 0.03	$6.99\times10^{-9} \pm 7.65\times10^{-10}$	0.9094
[Si][C_3C_1im][SCN]	0.53 ± 0.02	$5.97\times10^{-9} \pm 6.06\times10^{-10}$	0.9284
[Si][C_3C_1im][$N(CN)_2$]	0.56 ± 0.02	$5.53\times10^{-9} \pm 5.30\times10^{-10}$	0.9367
[Si][C_3C_1im][Tos]	0.54 ± 0.02	$7.49\times10^{-9} \pm 7.40\times10^{-10}$	0.9286
[Si][C_3C_1im][Male]	0.89 ± 0.03	$1.62\times10^{-8} \pm 1.38\times10^{-9}$	0.9400
[Si][C_3C_1im][NTf_2]	0.80 ± 0.02	$2.28\times10^{-8} \pm 1.75\times10^{-9}$	0.9769

According to Table S8 in the Supporting Information, a maximum equilibrium concentration of adsorbate in the solid phase of (0.75 ± 0.07), (0.54 ± 0.07), (0.59 ± 0.01), and (0.55 ± 0.05) mmol.g^{-1} were obtained for [Si][C_3C_1im]Cl, [Si][C_3C_1im][SCN], [Si][C_3C_1im][$N(CN)_2$], and [Si][C_3C_1im][Tos]. These values correspond to a maximum equilibrium concentration ranging from 0.16 to 0.22 g of diclofenac adsorved *per* gram of material. Diclofenac was already detected in effluents from WWTP/STP in levels ranging from 460 to 3300 ng.L^{-1} in Europe, from < 0.5 to 177 ng.L^{-1} in North America, and from 8.8 to 127 ng.L^{-1} in Asia and Australia.[60] Considering the largest reported value of 3300 ng.L^{-1}, 1 gram of the prepared material will "ideally" be able to treat around 50,000 L of water.

5. Conclusion

In the present work, six supported *N*-methylimidazolium-based IL-silica materials, with 6 different anions, were synthesized and characterized, and further evaluated as novel adsorption materials to remove NSAIDs from aqueous media. The adsorption kinetics and isotherms of diclofenac, as a major representative of the NAIDs class, were determined for [Si][C_3C_1im][SCN], [Si][C_3C_1im][$N(CN)_2$], [Si][C_3C_1im][Tos], [Si][C_3C_1im][Male] and [Si][C_3C_1im][NTf_2]. The adsorption data fit well with the Langmuir model for [Si][C_3C_1im]Cl, [Si][C_3C_1im][SCN], [Si][C_3C_1im][$N(CN)_2$] and [Si][C_3C_1im][Tos], and with the Freundlich model for [Si][C_3C_1im][Male] and [Si][C_3C_1im][NTf_2], where the adsorption kinetics in all materials is better described by a pseudo-second order model. A maximum equilibrium concentration of diclofenac in the solid phase of 0.75 mmol (or 0.22 g) per g of SILP was obtained. Considering values of diclofenac in the order of μg.L^{-1}, as those found in effluents of WWTPs/STPs, it means that 1 gram of the prepared material will "ideally" be able to treat around 50,000 L of water. In summary, supported IL-modified silica can be considered as remarkable adsorption materials to remove active pharmaceutical ingredients from aqueous samples.

6. Acknowledgment

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7. Supporting Information

7.1. Supporting Equations

The adsorption efficiency (% AE) was determined according to (1):

$$\%\, AE = \left(\frac{(C_0 - C_e)}{C_0}\right) \times 100 \qquad (1)$$

where C_0 and C_e are the equilibrium concentrations of diclofenac before and after adsorption on SILPs, respectively (in $mmol.L^{-1}$).

7.2. Supporting Tables

Table S1. Physicochemical properties of diclofenac.[61, 62]

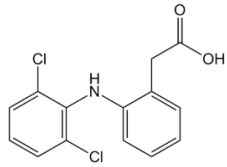
Properties	
Chemical structure	
Molecular formula	$C_{14}H_{11}Cl_2NO_2$
Molecular weight	296.16 $g.mol^{-1}$
CAS no.	15307-86-5 15307-79-6 (disodium salt)
Water solubility	2.37 $mg.L^{-1}$ (25 ºC)
Melting point	283-285 ºC
pK_a	4.15
Log K_{ow}	4.51

Table S2. Adsorption efficiency (%AE), equilibrium concentration of diclofenac after adsorption (C_e), and concentration of adsorbate in the solid phase (q_e), with respectively standard deviations (σ) of [Si][C_3C_1im]Cl with diclofenac solution.

[Si][C_3C_1im]Cl			
t / min	**% (AE_{Dic} ± σ)**	**C_e / mmol.L^{-1}**	**q_e ± σ / mmol.g^{-1}**
0	0.0 ± 0.0		
1	61.8 ± 0.3	0.033	0.202 ± 0.008
2	73.8 ± 1.1	0.022	0.254 ± 0.002
3	75.8 ± 1.2	0.021	0.258 ± 0.014
4	75.9 ± 0.3	0.021	0.264 ± 0.004
5	79.2 ± 1.5	0.018	0.267 ± 0.003
6	86.2 ± 1.4	0.012	0.295 ± 0.014
7	81.0 ± 1.9	0.016	0.264 ± 0.001
8	86.9 ± 0.8	0.011	0.285 ± 0.002
9	86.3 ± 1.7	0.012	0.290 ± 0.018
10	90.7 ± 1.4	0.008	0.307 ± 0.013
15	90.1 ± 1.6	0.008	0.306 ± 0.002
30	91.5 ± 0.9	0.007	0.308 ± 0.011
45	92.3 ± 0.7	0.007	0.306 ± 0.014
60	92.1 ± 1.0	0.007	0.311 ± 0.003
120	92.2 ± 0.8	0.007	0.305 ± 0.015
180	92.9 ± 0.4	0.006	0.310 ± 0.001

Table S3. Adsorption efficiency (%AE), equilibrium concentration of diclofenac after adsorption (C_e), and concentration of adsorbate in the solid phase (q_e), with respectively standard deviations (σ) of [Si][C_3C_1im][SCN] with diclofenac solution.

[Si][C_3C_1im][SCN]			
t / min	**% (AE_{Dic} ± σ)**	**C_e / mmol.L^{-1}**	**q_e ± σ / mmol.g^{-1}**
0	0.0 ± 0.0		
1	64.4 ± 0.8	0.031	0.231 ± 0.008
2	71.6 ± 1.8	0.025	0.243 ± 0.021
3	74.1 ± 0.2	0.023	0.251 ± 0.014
4	75.9 ± 0.8	0.021	0.263 ± 0.019
5	76.0 ± 1.2	0.021	0.263 ± 0.026
6	76.7 ± 0.8	0.020	0.272 ± 0.015
7	79.2 ± 3.1	0.018	0.279 ± 0.013
8	81.3 ± 2.5	0.016	0.277 ± 0.004
9	79.9 ± 2.9	0.018	0.288 ± 0.007
10	81.9 ± 0.6	0.016	0.274 ± 0.023
15	81.0 ± 0.8	0.017	0.294 ± 0.015
30	82.9 ± 2.4	0.015	0.292 ± 0.021
45	83.3 ± 0.9	0.015	0.291 ± 0.005
60	83.0 ± 0.3	0.015	0.297 ± 0.008
120	83.4 ± 0.5	0.015	0.299 ± 0.011
180	82.9 ± 0.4	0.015	0.299 ± 0.008

Table S4. Adsorption efficiency (%AE), equilibrium concentration of diclofenac after adsorption (C_e), and concentration of adsorbate in the solid phase (q_e), with respectively standard deviations (σ) of [Si][C_3C_1im][$N(CN)_2$] with diclofenac solution.

[Si][C_3C_1im][$N(CN)_2$]			
t / min	**% (AE_{Dic} ± σ)**	**C_e / mmol.L^{-1}**	**q_e ± σ / mmol.g^{-1}**
0	0.0 ± 0.0		
1	70.3 ± 0.8	0.026	0.225 ± 0.01
2	75.2 ± 0.5	0.022	0.251 ± 0.007
3	77.0 ± 2.6	0.020	0.273 ± 0.011
4	79.1 ± 2.3	0.018	0.292 ± 0.001
5	78.7 ± 0.3	0.019	0.288 ± 0.010
6	80.3 ± 0.3	0.017	0.267 ± 0.010
7	82.0 ± 1.3	0.016	0.289 ± 0.017
8	82.7 ± 3.3	0.015	0.286 ± 0.017
9	86.5 ± 0.8	0.010	0.288 ± 0.006
10	85.7 ± 0.3	0.010	0.289 ± 0.004
15	87.9 ± 1.1	0.009	0.284 ± 0.004
30	88.5 ± 0.9	0.008	0.280 ± 0.003
45	89.1 ± 0.7	0.008	0.281 ± 0.003
60	88.5 ± 0.5	0.008	0.285 ± 0.020
120	88.8 ± 1.3	0.008	0.283 ± 0.008
180	87.8 ± 1.7	0.009	0.283 ± 0.004

Table S5. Adsorption efficiency (%AE), equilibrium concentration of diclofenac after adsorption (C_e), and concentration of adsorbate in the solid phase (q_e), with respectively standard deviations (σ) of [Si][C_3C_1im][Tos] with diclofenac solution.

[Si][C_3C_1im][Tos]			
t / min	**% (AE_{Dic} ± σ)**	**C_e / mmol.L^{-1}**	**q_e ± σ / mmol.g^{-1}**
0	0.0 ± 0.0		
1	63.4 ± 0.5	0.026	0.164 ± 0.005
2	67.1 ± 0.6	0.023	0.191 ± 0.001
3	74.9 ± 0.8	0.018	0.205 ± 0.013
4	73.2 ± 1.1	0.019	0.214 ± 0.009
5	72.5 ± 0.9	0.021	0.225 ± 0.004
6	74.7 ± 0.1	0.020	0.219 ± 0.016
7	76.4 ± 0.6	0.018	0.214 ± 0.009
8	77.2 ± 0.8	0.018	0.241 ± 0.011
9	77.0 ± 1.7	0.018	0.228 ± 0.008
10	74.9 ± 0.8	0.019	0.223 ± 0.005
15	74.9 ± 0.8	0.019	0.234 ± 0.009
30	76.8 ± 1.3	0.018	0.231 ± 0.014
45	76.8 ± 2.2	0.018	0.235 ± 0.008
60	76.7 ± 1.4	0.018	0.237 ± 0.008
120	76.5 ± 1.7	0.018	0.234 ± 0.016
180	77.4 ± 1.4	0.017	0.230 ± 0.013

Table S6. Adsorption efficiency (%AE), equilibrium concentration of diclofenac after adsorption (C_e), and concentration of adsorbate in the solid phase (q_e), with respectively standard deviations (σ) of [Si][C_3C_1im][Male] with diclofenac solution.

[Si][C_3C_1im][Male]			
t / min	**% (AE_{Dic} ± σ)**	**C_e / mmol.L^{-1}**	**q_e ± σ / mmol.g^{-1}**
0	0.0 ± 0.0		
1	57.5 ± 1.1	0.033	0.174 ± 0.015
2	60.7 ± 0.1	0.030	0.182 ± 0.004
3	62.6 ± 2.8	0.029	0.195 ± 0.001
4	64.9 ± 0.6	0.027	0.201 ± 0.012
5	63.2 ± 1.1	0.028	0.196 ± 0.007
6	66.5 ± 1.3	0.026	0.200 ± 0.024
7	65.5 ± 2.2	0.027	0.199 ± 0.014
8	67.2 ± 0.8	0.025	0.198 ± 0.007
9	67.9 ± 0.5	0.025	0.215 ± 0.015
10	68.4 ± 0.5	0.024	0.207 ± 0.007
15	68.5 ± 0.2	0.024	0.203 ± 0.016
30	69.3 ± 0.8	0.024	0.205 ± 0.014
45	68.8 ± 0.2	0.024	0.201 ± 0.013
60	69.3 ± 0.1	0.024	0.201 ± 0.005
120	68.7 ± 0.9	0.024	0.210 ± 0.003
180	68.4 ± 1.0	0.024	0.211 ± 0.002

Table S7. Adsorption efficiency (%AE), equilibrium concentration of diclofenac after adsorption (C_e), and concentration of adsorbate in the solid phase (q_e), with respectively standard deviations (σ) of [Si][C_3C_1im][NTf_2] with diclofenac solution.

[Si][C_3C_1im][NTf_2]			
t / min	**% (AE_{Dic} ± σ)**	**C_e / mmol.L^{-1}**	**q_e ± σ / mmol.g^{-1}**
0	0.0 ± 0.0		
1	44.2 ± 1.2	0.040	0.116 ± 0.006
2	44.6 ± 0.3	0.040	0.119 ± 0.011
3	47.0 ± 1.2	0.038	0.129 ± 0.003
4	52.8 ± 2.6	0.034	0.154 ± 0.004
5	56.4 ± 0.7	0.031	0.158 ± 0.002
6	56.9 ± 0.7	0.031	0.172 ± 0.001
7	63.1 ± 2.4	0.027	0.173 ± 0.003
8	67.7 ± 0.5	0.023	0.184 ± 0.013
9	68.2 ± 0.2	0.023	0.179 ± 0.005
10	68.9 ± 0.3	0.022	0.192 ± 0.010
15	70.4 ± 0.9	0.021	0.193 ± 0.016
30	73.1 ± 0.4	0.019	0.204 ± 0.011
45	73.8 ± 0.2	0.019	0.204 ± 0.016
60	73.3 ± 0.9	0.019	0.212 ± 0.006
120	73.3 ± 0.1	0.019	0.217 ± 0.014
180	72.3 ± 1.0	0.020	0.217 ± 0.001

Table S8. Equilibrium concentration of diclofenac after adsorption (C_e), and concentration of adsorbate in the solid phase (q_e), with respectively standard deviations (σ), at 25 ºC.

[Si][C_3C_1im]Cl		[Si][C_3C_1im][SCN]		[Si][C_3C_1im[$N(CN)_2$]	
C_e / mmol.L^{-1}	q_e ± σ / mmol.g^{-1}	C_e / mmol.L^{-1}	q_e ± σ / mmol.g^{-1}	C_e / mmol.L^{-1}	q_e ± σ / mmol.g^{-1}
0.000	0.000 ± 0.000	0.000	0.000 ± 0.000	0.000	0.000 ± 0.000
0.002	0.031 ± 0.003	0.001	0.010 ± 0.000	0.001	0.010 ± 0.000
0.006	0.270 ± 0.012	0.004	0.113 ± 0.006	0.002	0.112 ± 0.004
0.006	0.168 ± 0.001	0.006	0.173 ± 0.001	0.005	0.167 ± 0.001
0.007	0.283 ± 0.000	0.01	0.266 ± 0.012	0.006	0.287 ± 0.013
0.012	0.547 ± 0.000	0.015	0.293 ± 0.002	0.011	0.324 ± 0.003
0.015	0.308 ± 0.001	0.026	0.371 ± 0.008	0.026	0.391 ± 0.006
0.026	0.358 ± 0.005	0.036	0.439 ± 0.025	0.036	0.424 ± 0.013
0.036	0.439 ± 0.023	0.046	0.422 ± 0.011	0.045	0.436 ± 0.004
0.073	0.651 ± 0.016	0.065	0.344 ± 0.003	0.056	0.398 ± 0.004
0.097	0.605 ± 0.028	0.118	0.460 ± 0.016	0.114	0.475 ± 0.006
0.226	0.695 ± 0.037	0.249	0.476 ± 0.052	0.236	0.483 ± 0.039
0.236	0.644 ± 0.003	0.299	0.514 ± 0.029	0.306	0.549 ± 0.031
0.399	0.722 ± 0.071	0.45	0.468 ± 0.064	0.433	0.486 ± 0.042
0.551	0.743 ± 0.061	0.507	0.490 ± 0.039	0.512	0.501 ± 0.019
0.610	0.736 ± 0.029	0.783	0.504 ± 0.001	0.786	0.529 ± 0.029
0.980	0.748 ± 0.068	0.882	0.509 ± 0.031	0.873	0.570 ± 0.024
1.314	0.733 ± 0.047	1.336	0.538 ± 0.067	1.322	0.585 ± 0.004
1.371	0.758 ± 0.010	1.421	0.519 ± 0.013	1.424	0.547 ± 0.040
[Si][C_3C_1im][Tos]		[Si][C_3C_1im][Male]		[Si][C_3C_1im][NTf_2]	
C_e / mmol.L^{-1}	q_e ± σ / mmol.g^{-1}	C_e / mmol.L^{-1}	q_e ± σ / mmol.g^{-1}	C_e / mmol.L^{-1}	q_e ± σ / mmol.g^{-1}
0.000	0.000 ± 0.000	0.000	0.000 ± 0.000	0.000	0.000 ± 0.000
0.003	0.004 ± 0.001	0.003	0.002 ± 0.001	0.001	0.012 ± 0.002
0.004	0.110 ± 0.000	0.011	0.082 ± 0.004	0.006	0.116 ± 0.009
0.009	0.292 ± 0.002	0.019	0.148 ± 0.005	0.009	0.136 ± 0.000
0.011	0.145 ± 0.001	0.022	0.229 ± 0.002	0.024	0.225 ± 0.005
0.019	0.278 ± 0.024	0.026	0.264 ± 0.023	0.031	0.308 ± 0.000
0.033	0.316 ± 0.008	0.035	0.326 ± 0.034	0.043	0.300 ± 0.000
0.043	0.390 ± 0.007	0.052	0.403 ± 0.023	0.047	0.343 ± 0.010
0.049	0.375 ± 0.021	0.105	0.535 ± 0.004	0.056	0.323 ± 0.008
0.058	0.373 ± 0.009	0.209	0.554 ± 0.002	0.122	0.414 ± 0.055
0.127	0.464 ± 0.009	0.275	0.654 ± 0.045	0.220	0.439 ± 0.022
0.245	0.471 ± 0.046	0.382	0.671 ± 0.053	0.302	0.446 ± 0.047
0.305	0.516 ± 0.027	0.453	0.684 ± 0.042	0.372	0.532 ± 0.034
0.439	0.474 ± 0.006	0.732	0.742 ± 0.035	0.468	0.544 ± 0.077
0.513	0.505 ± 0.002	0.831	0.778 ± 0.057	0.666	0.652 ± 0.048
0.793	0.491 ± 0.034	1.132	0.922 ± 0.015	0.820	0.695 ± 0.065
0.875	0.529 ± 0.056	1.228	0.967 ± 0.036	1.169	0.767 ± 0.095
1.335	0.537 ± 0.065	1.244	0.951 ± 0.041		
1.421	0.546 ± 0.047	1.321	0.979 ± 0.027		

7.3. Supporting Figures

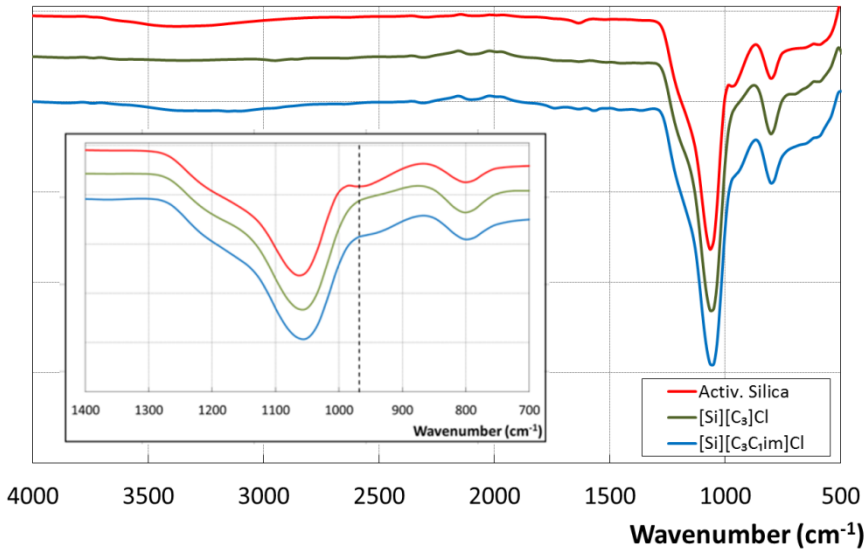


Figure S1. FTIR spectra of activated silica (red line), chloropropyl silica (green line), and supported *N*-methylimidazolium-based ionic liquid (blue line).

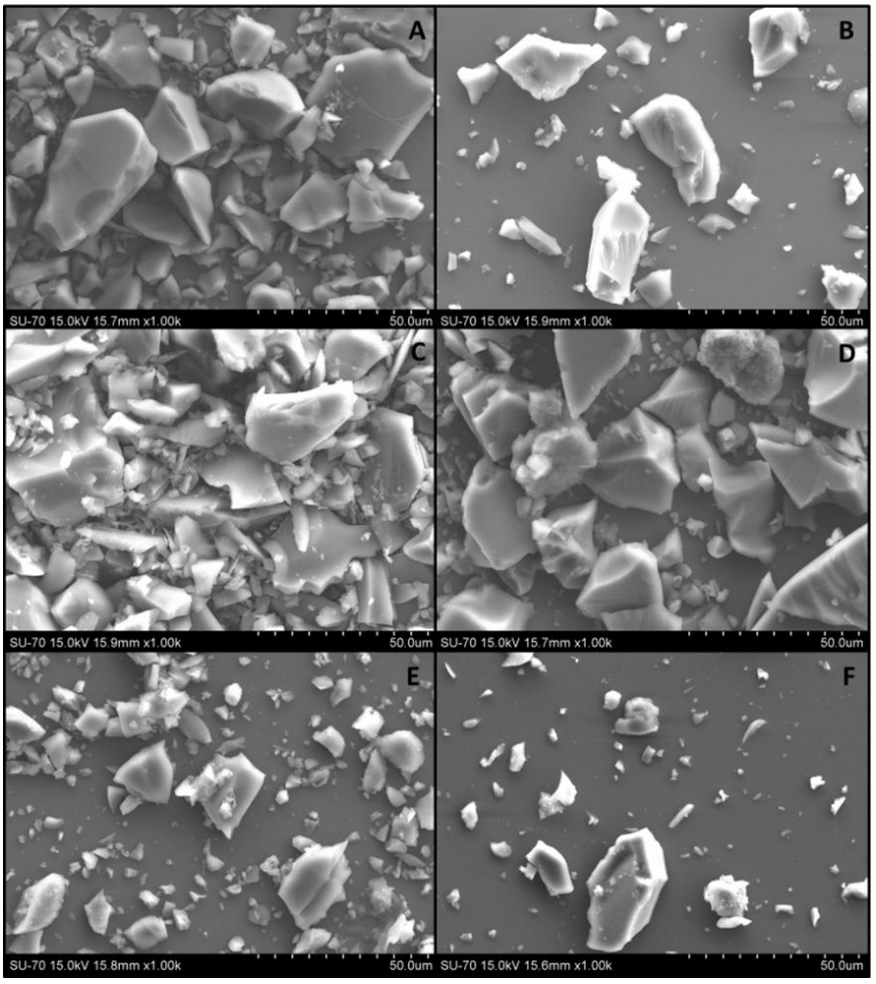


Figure S2. Scanning electron microscope of: A) [Si][C_3C_1im]Cl; B) [Si][C_3C_1im][SCN]; C) [Si][C_3C_1im][$N(CN)_2$]; D) [Si][C_3C_1im][Tos]; E) [Si][C_3C_1im][Male]; and F) [Si][C_3C_1im][NTf_2].

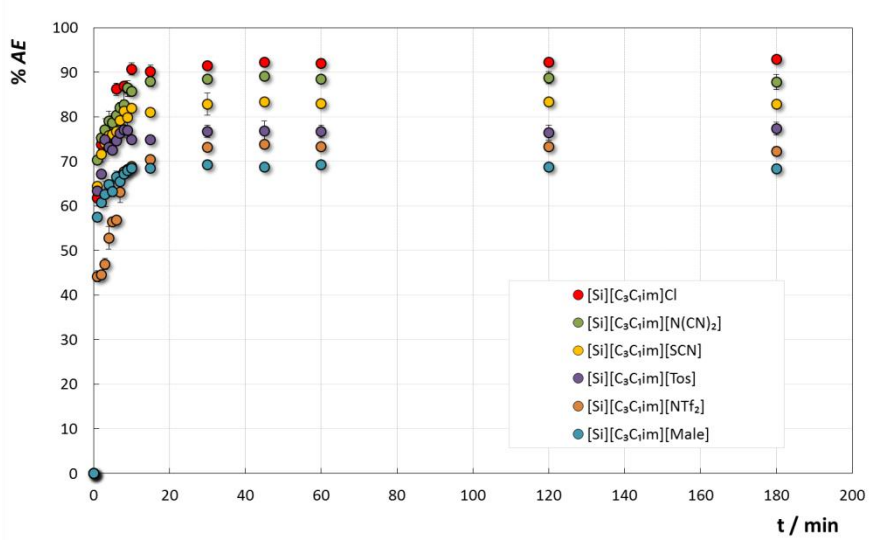


Figure S3. Adsorption efficiency of six *N*-methylimidazolium-based ionic liquid with diclofenac: ●, [Si][C_3C_1im]Cl; ●, [Si][C_3C_1im][SCN]; ●, [Si][C_3C_1im[$N(CN)_2$]; ●, [Si][C_3C_1im][Tos]; ●, [Si][C_3C_1im][Male]; and ●, [Si][C_3C_1im][NTf_2].

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Chapter 6

Adsorption of Non-Steroidal Anti-Inflammatory Drugs from Aqueous Samples using Ionic-Liquid-Silica-based Materials

Adapted from: **Hugo F. D. Almeida,** Sara Azevedo, Isabel M. Marrucho and Mara G. Freire; *Adsorption of Non-Steroidal Anti-Inflammatory Drugs from Aqueous Samples using Ionic-Liquid-Silica-based Materials*; *in prepration*, **2017**.

The sample preparation for all the experiments presented herein was performed by the author. The author was also involved in all the experiments, as well as on the discussion and interpretation of the data and preparation of the manuscript. Active pharmaceutical ingredients concentration, synthesis of SILPs and elementary analysis sections were performed by Sara Azevedo.

1. Abstract

Non-steroidal anti-inflammatory drugs (NSAIDs) are a class of active pharmaceutical ingredients, for whose prescription is not needed, and thus object of an excessive consumption. This large consumption further leads to their significant excretion into the aquatic environment, representing a matter of serious environment and health care. This work addresses the use of silica-based materials functionalized with ionic liquids (ILs) to remove NSAIDs from aqueous media. In particular, silica modified with 1-methyl-3-propylimidazolium chloride ([Si][C_1C_3im]Cl) was prepared and investigated as an adsorption material for diclofenac, naproxen and ketoprofen, as three representative drugs of the NSAIDs class. The adsorption kinetics and adsorption isotherms were determined at 25 ºC. The adsorption kinetics of the three NSAIDs are well described by a pseudo second-order model, whereas the adsorption isotherms of diclofenac is well described by the Langmuir model. Aiming at developing a continuous removal method, a packed column with [Si][C_3C_1im]Cl was also prepared. Finally, the recovery of NSAIDs and the supported ionic liquid phase (SILP) reusability were evaluated and it was found that [Si][C_3C_1im]Cl could be recycled and reused without a significant reduction in its adsorption efficiency after a “washing” step with aqueous solutions of pH 4. SILPs can thus be envisioned as promising and reusable materials to remove active pharmaceutical ingredients from aqueous environmental matrices.

2. Introduction

The detection of emerging contaminants in various environmental matrices has been a focal point of debate in the last decade.[1-3] Daughton and Ternes[4] were the first recommending the classification of pharmaceuticals and personal care products (PPCPs) as relevant pollutants, which are currently classified as emerging contaminants according to the United Nations

Environmental Program (UNEP).[5] Active pharmaceutical ingredients (APIs) are known as mutagenic, carcinogenic, and endocrine disruptors and fit within the PPCPs class, and have raised serious apprehensions in more recent years after their identification in non-negligible levels in aquatic environmental samples.[6-10] Concentrations of APIs up to $\mu g.L^{-1}$ were found in worldwide effluents of sewage treatment plants (STPs), wastewater treatment plants (WWTPs), freshwaters (rivers and lakes) and estuarine/marine waters.[7, 10-15] The consumed APIs and/or PPCPs are mainly excreted through urine (in either metabolized or unchanged forms),[7, 16-21] being one of the major reasons for their identification in aquatic environmental samples. Their identification in an increasing number of environmental matrices has been possible due to advances in pre-treatment strategies and analytical techniques.[22-24] Although WWTPs and STPs use membrane filtration, ozonation, chlorination, flocculation/sedimentation and adsorption processes for water purification and decontamination, none of these procedures were target-designed to remove APIs.[7, 12, 21, 25]

According to extensive criteria, the Global Water Research Coalition (GWRC) selected ten priority APIs,[26, 27] which includes antibiotics, anti-epileptics, anti-inflammatory drugs, β-blockers and lipid regulators. Among APIs, the non-steroidal anti-inflammatory drugs (NSAIDs) are the most studied,[16, 28-30] where diclofenac, naproxen and ibuprofen are part of the top ten persistent pollutants.[27] Some strategies have been tested for the removal of NSAIDs.[31, 32] For instance, the coagulation of ibuprofen, naproxen, diclofenac, carbamazepine and diazepam was investigated by the addition of several salts, wherein the best results were obtained for diclofenac with 50 % of removal efficiency.[31] Kahn et al.[32] studied dissolved air flotation, lime clarification, activated carbon, dual media filtration, ozonation, combined reverse-osmosis/nanofiltration, and UV disinfection units, for the removal and/or degradation of NSAIDs. The authors[32] showed that reverse osmosis is the most effective process for removing a series of pharmaceuticals, although it is highly energy-intensive. Consequently, the development of cost-

efficient removal techniques for NSAIDs is a critical requirement to improve water quality and life standards of the human society.

In the past years, ionic liquids (ILs) have been immobilized onto silica and polymer supports, known as supported ionic liquid phases (SILPs), fitting within the solid-phase extraction (SPE) methods.[33-35] ILs are salts composed of organic cations (e.g., imidazolium, pyridinium, pyrrolidium, ammonium and phosphonium) and inorganic or organic anions (e.g., Cl^-, $[PF_6]^-$, $[BF_4]^-$, $[NTf_2]^-$, $[CH_3CO_2]^-$, etc.), and display some extraordinary properties, namely a negligible vapor pressure, non-flammability, high thermal and chemical stabilities, and a large liquid temperature range.[36-39] One important feature of ILs is their tailoring ability by an appropriate choice of their ions - a property that is transferrable to SILPs.[40] Therefore, taking the advantage of the ILs chemical diversity, SILPs can be considered as a new class of adsorptive materials, with new functional groups, and with tailoring ability. ILs are good candidates for use in conventional liquid-liquid extractions, while SILPs can be used as stationary phases in adsorptive extractions.[33, 40-44] Several studies have been published on the application of ILs in analytical chemistry, mainly used as stationary phases in separation techniques (gas chromatography (GC), liquid chromatography (LC) and capillary electrochromatography (CEC)),[43, 45] and as solvents or modifiers in liquid-extraction techniques (liquid-liquid extraction (LLE), liquid-phase microextraction (LPME), single-drop microextraction (SDME), solid-phase microextraction (SPME) and solid-phase extraction)).[43, 46-49]

Jiang and co-workers[50] were pioneers in the use of SILPs as an adsorption technique, where a solid-phase microextraction (SPME) with an IL coating was developed for the headspace extraction and further identification of benzene, toluene, ethylbenzene, and xylenes in paints. Particularly regarding the APIs removal from aqueous samples, Fontanals et al.[33] proposed the use of a crosslinked polymer-supported imidazolium trifluoroacetate salt (IL-CF_3COO^-) for the removal of salicylic acid, 4-nitrophenol, carbamazepine, nalidixic acid, flumequine, naproxen, fenoprofen, sodium diclofenac, ibuprofen and gemfibrozil

from different aqueous samples (ultrapure, tap and river). The favorable combination of the ILs properties and advantages of a solid support allowed the complete removal of the studied compounds. Two SILPs containing a common polymer-supported imidazolium and different anions, namely trifluoromethanesulphonate ($[CF_3SO_3]^-$) and tetrafluoroborate ($[BF_4]^-$), were evaluated as SPE adsorbents for salicylic acid, carbamazepine, antipyrine, trimethoprim, metoprolol, naproxen, fenoprofen, diclofenac, ibuprofen, and gemfibrozil from aqueous samples under strong anion-exchange conditions, resulting in a complete and effective cleanup of the aqueous sample.[44] Based on the remarkable results achieved with the use of polymer-IL materials as SPE adsorbents for the extraction of pharmaceuticals or APIs,[33, 44] in this work we propose the use of SILPs based on silica to adsorb/remove NSAIDs (diclofenac and naproxen – included in the top 10 list of current persistent pollutants[27] – and ketoprofen) from aqueous environments. In a simplistic version of a WTTP,[51] three different stages, namely mechanical, biological and disinfection treatments, are combined, where the SILPs strategy proposed is this work for the NSAIDs removal is envisioned to be introduced in the final stage. In order to develop a more sustainable technique, the recovery of the drugs and the reuse of the SILP-based material were also evaluated.

3. Experimental Section

3.1. Materials

The non-steroidal anti-inflammatory drugs investigated were diclofenac sodium salt (2-[(2,6-dichlorophenyl)amino]benzene acetic acid sodium salt, CAS# 15307-79-6), naproxen ((S)-(+)-2-(6-methoxy-2-naphthyl)propionic acid, CAS# 22204-53-1) and ketoprofen ((RS)-2-(3-benzoylphenyl)propionic acid, CAS# 22071-15-4), with a purity level ≥ 99 % for diclofenac, and ≥ 98 % for naproxen and ketoprofen. All NSAIDs were acquired from Sigma-Aldrich and used as

received. The chemical structures of the NSAIDs investigated are depicted in Figure 36.

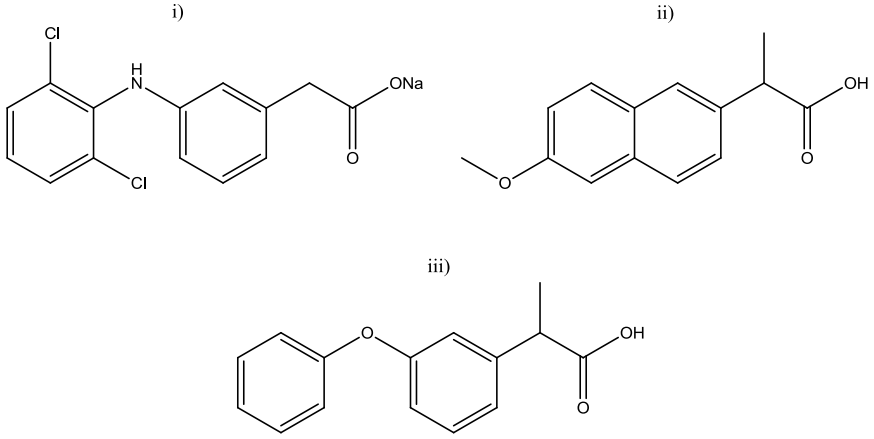

Figure 36. Chemical structures of the NSAIDs investigated: diclofenac sodium salt (i), naproxen (ii), and ketoprofen (iii).

The water applied was doubled distilled, passed across a reverse osmosis system and further treated with a Milli-Q plus 185 water purification equipment. Buffers solutions used for the pH meter equipment calibration, with a pH of 4.00 and 7.00, were acquired from Panreac. For preparation of solutions with ph = 2, 4, and 10, HCl (CAS# 7647-01-0, purity 37 %) NaOH (CAS # 1310-73-2, purity 98.0 %), both acquired from Sigma Aldrich, were used. The starting material for preparing the IL-functionalized silica (*N*-methylimidazolium functionalized silica, [Si][C_3C_1im]Cl) was silica gel (60, 0.2-0.5 mm), acquired from Merck. The solvents used in the silica functionalization were hydrochloric acid (CAS# 7647-01-0, purity 37 %) and toluene (CAS# 108-88-3, purity 99.8 %), both acquired from Sigma Aldrich, ethanol (CAS# 64-17-5, purity 99.9 %) from Carlo Erba, methanol (CAS# 67-56-1, HPLC grade) from Chem-Lab, and (3-chloropropyl)trimethoxysilane (CAS# 2530-87-2, purity 98 %) and *N*-methylimidazolium (CAS# 616-47-7, purity 99 %), both from Acros Organics.

3.2. Preparation of SILPs ([Si][C_3C_1im]Cl)

The synthesis of [Si][C_3C_1im]Cl was carried out according to the experimental procedure described in Chapter 5. Briefly, the starting silica was immersed in hydrochloric acid, washed with double distilled water, dried under vacuum at 105°C, and suspended in dried toluene. 3-chloropropyltrimethoxysilane was then added, followed by the addition of triethylamine as a catalyst, and mechanically stirred and refluxed at 130 ºC. After refluxing, the reaction was stopped and the modified silica was cooled to room temperature, transferred to a vacuum glass filter, and washed with toluene, ethanol–water (1:1, v/v), double distilled water, and methanol. The obtained chloropropyl silica, [Si][C_3]Cl, was dried under vacuum at 60 °C, and then subjected to a reaction with *N*-methylimidazole in toluene, and kept under reflux with stirring. The reaction was stopped and the modified silica was cooled down to room temperature, and washed with methanol, water, and again with methanol. The obtained SILP, [Si][C_3C_1im]Cl, was dried under vacuum at 50°C for 8 h. The synthesis procedure was adapted from Qiu et al.[52]

3.3. Elemental analysis

Carbon, hydrogen, nitrogen and sulfur contents (in weight fraction percentage) of activated silica, [Si][C_3]Cl and [Si][C_3C_1im]Cl, were performed on a TruSpec 630-200-200, with a sample size of 2 mg, combustion furnace temperature of 1075 ºC, afterburner temperature of 850 ºC and detection method of infrared absorption for carbon, hydrogen and sulfur, and thermal conductivity for nitrogen.

3.4. Adsorption kinetics and isotherms

The adsorption kinetics of NSAIDs onto [Si][C_3C_1im]Cl were determined with aqueous solutions of diclofenac, naproxen and ketoprofen at concentrations of 0.0232, 0.0103 and 0.0103 g.L^{-1}, respectively. 10 mL of aqueous solutions of each NSAID and 2.5 mg of [Si][C_3C_1im]Cl were equilibrated in 50 mL pyrex Erlenmeyers and placed in an orbital shaker at 120 rpm, at 25 ºC. For the adsorption kinetics evaluation, times of equilibration from 0 to 180 min were investigated, while for the adsorption isotherms evaluation the time of equilibrium was settled as 120 min. After each time of agitation, the sample was centrifuged at 5000 rpm for 5 min, and the amount of diclofenac, naproxen and ketoprofen in the water phase was quantified through UV-spectroscopy, using a Shimadzu UV-1800, Pharma-Spec UV-VisSpectrophotometer, at a wavelength of 276, 230 and 256 nm, respectively, using calibration curves previously established. At least 3 individual samples for each condition were prepared.

The equilibrium concentration of adsorbate in the solid phase (q_e / mmol.g^{-1}) and adsorption efficiency (%*AE*) were determined according to Eq. 1 and Eq. 2:

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

$$\%AE = \left(\frac{(C_0 - C_e)}{C_0}\right) \times 100 \quad (2)$$

where *w* is the weight of SILP (g), *V* is the volume of the diclofenac, naproxen and ketoprofen aqueous solutions (L), and C_0 and C_e are the equilibrium concentrations of diclofenac, naproxen and ketoprofen before and after adsorption, respectively (mmol.L^{-1}).

The pseudo first-order and pseudo second-order adsorption kinetic models were applied to the experimental data, with the linear forms of the

Lagergren's[53] first order rate (Eq. 3) and the Ho's[54] second order rate (Eq. 4) equations:

$$ln(q_e - q_t) = lnq_e - k_1 t \quad (3)$$

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} t \quad (4)$$

where t is the time (min), q_e is the amount of diclofenac, naproxen and ketoprofen adsorbed onto the adsorbent at equilibrium (mmol.g^{-1}), q_t is the amount of diclofenac, naproxen and ketoprofen adsorbed onto the adsorbent at any time t (mmol.g^{-1}), k_1 (min^{-1}) is the rate constant of the pseudo first-order adsorption, and k_2 (g.mmol^{-1}min^{-1}) is the rate constant of the pseudo-second-order adsorption.

The experimental isotherms data were fitted with the Langmuir[55] (Eq. 5) and Freundlich[56] models (Eq. 6):

$$q_e = \frac{q_{max} \times B \times C_e}{1 + B \times C_e} \quad (5)$$

$$lnq_e = lnK_f + \frac{1}{n} lnC_e \quad (6)$$

where C_e is the equilibrium concentration of adsorbate (mmol.L^{-1}), q_e is the equilibrium concentration of adsorbate in the solid phase (mmol.g^{-1}), B (L.mmol^{-1}) is the Langmuir isotherm constant, q_{max} (mmol.g^{-1}) is the maximum monolayer coverage capacity, and K_f (adsorption capacity) and n (adsorption intensity) are the constants of the Freundlich equation. The fitting of the data was performed using SigmaPlot®.

3.5. Removal of NSAIDs using columns filled with SILPs

In addition to the batch experiments, the removal of APIs from aqueous media was performed on a lab-scale solid-phase column (120 mm x 14 mm) packed with 85 mg (dry weight) of [Si][C_3C_1im]Cl, where two solid-phase porous

disks were introduced at the bottom and top of the SILP material. Afterwards, the column was wet-packed with 100 mL of double distilled water, through a vacuum filtration process. Figure S1, in the Supporting Information, depicts the prepared lab-scale solid-phase columns packed with [Si][C_3C_1im]Cl. This column contains a silicone tube connected to a peristaltic pump, allowing to maintain a constant flow rate of 5 mL.min^{-1} of the NSAIDs aqueous solutions.

100 mL of diclofenac aqueous solutions, of different concentrations (0.2 g.L^{-1} and 0.02 g.L^{-1}), were passed through the column packed with [Si][C_3C_1im]Cl, and the concentration of diclofenac was quantified at each 10 mL through UV-spectroscopy, using a Shimadzu UV-1800, Pharma-Spec UV-Vis Spectrophotometer, at a wavelength of 276 nm.

The recovery of NSAIDs and regeneration of the SILPs used were finally evaluated. To this end, 100 mL of a diclofenac aqueous solution with a concentration of 0.02 g.L^{-1} were passed through the column packed with [Si][C_3C_1im]Cl. The concentration of diclofenac in the aqueous solutions after passing the columns were determined at 10, 60 and 100 mL through UV-spectroscopy, using a Shimadzu UV-1800, Pharma-Spec UV-Vis Spectrophotometer, at a wavelength of 276 nm, allowing to determine the adsorption efficiency. Then, 10 mL of aqueous solutions with pH values of 2, 4 and 10 (using HCl and NaOH solutions) were passed through the column and the concentration of diclofenac was quantified in these “washing” solutions, allowing to conclude on the best pH to recover diclofenac and regenerate the SILP/column. Finally, 100 mL of the same diclofenac aqueous solution were passed through the recovered column and its adsorption efficiency again determined. The pH values (± 0.02) were measured at (25 ± 1) ºC, using a Mettler Toledo S47 SevenMulti™ dual meter pH/conductivity. The calibration of the pH meter was beforehand performed with two buffers solutions, with pH values of 4.00 and 7.00.

4. Results and Discussion

4.1. Characterization of [Si][C_3C_1im]Cl

The prepared *N*-methylimidazolium-based SILP ([Si][C_3C_1im]Cl) was previously characterized by us[57] by infrared spectroscopy, solid ^{13}C nuclear magnetic resonance, and scanning electron microscopy. Since a new batch of the material was prepared, elemental analysis was performed to appraise if the functionalization was properly carried out. The elemental analysis results of activated silica, [Sil[C_3]Cl and [Si][C_3C_1im]Cl, are presented in Table 10. According to the elemental analysis results, the carbon and nitrogen weight fraction percentage of the supported material, [Si][C_3C_1im]Cl, are (8.58 ± 0.11) % and (2.75 ± 0.03) %, respectively, whereas the carbon content of the activated silica is (0.64 ± 0.22) %. The carbon content in the activated silica could arise from air absorbed during the elemental analysis measurements, since no nitrogen was found in this material. In general, there are 0.001 mol of $([C_1C_3im])^+$ *per* gram of SILP, in agreement with our previous results.[57]

Table 10. Elemental analysis results (C, H, and N weight fraction percentage and respective standard deviations (σ)) of activated silica, [Si][C_3]Cl, and [Si][C_3C_1im]Cl.

	Elemental analysis (weight fraction percentage ± σ)		
	C	**H**	**N**
Activated Silica	0.59 ± 0.24	1.23 ± 0.15	---
[Si][C_3]Cl	6.01 ± 0.18	1.47 ± 0.03	---
[Si][C_3C_1im]Cl	8.72 ± 0.11	2.07 ± 0.06	2.75 ± 0.03

4.2. Adsorption kinetics of NAIDs onto [Si][C_3C_1im]Cl

The adsorption kinetics curves of the three NSAIDs onto the studied SILP were established in order to evaluate the time required for adsorption and to set the contact time for which diclofenac, naproxen and ketoprofen reach the equilibrium between the liquid and the solid phases. The results obtained are depicted in Figure 37, whereas detailed data are provided in Tables S1 to 3, in the Supporting Information. Also in the in the Supporting Information, in Figure S1, is depicted the adsorption efficiency of the supported material for diclofenac, naproxen and ketoprofen.

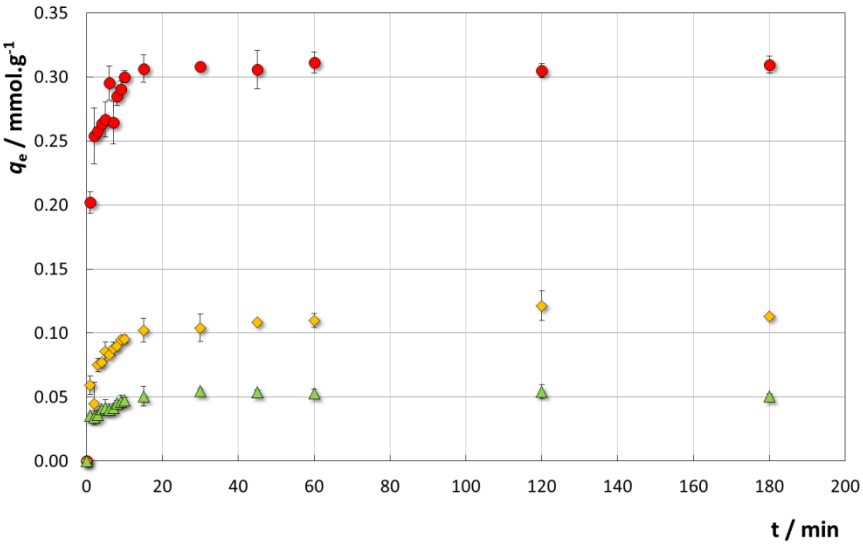

Figure 37. Adsorption kinetics curves of diclofenac (●), naproxen (◆) and ketoprofen (▲) onto [Si][C_3C_1im]Cl.

A plateau is reached in the equilibrium concentration of adsorbate in the solid phase (q_e) at around 15 min for all NSAIDs. These times correspond to maximum equilibrium concentrations of adsorbate in the solid phase of (0.306 ± 0.002), (0.102 ± 0.009), and (0.051 ± 0.008), mmol.g^{-1}, and adsorption efficiencies of the material of (90.1 ± 1.6), (56.6 ± 2.3) and (28.0 ± 1.1) %, for

diclofenac, naproxen and ketoprofen, respectively (Figure S2. and Tables S1 to 3 in the Supporting Information). The initial high adsorption rate could be attributed to the initial non-covalent binding of diclofenac, naproxen and ketoprofen onto the sorbent surface, and after achieving q_e, the supported material gets saturated by each NSAID.

According to the kinetic curves results, the maximum equilibrium concentration of adsorbate in the solid phase decreases in the following sequence: diclofenac > naproxen > ketoprofen. This trend correlates with the octanol-water partition coefficients ($\log K_{ow}$) of NSAIDs (diclofenac = 4.52,[58] naproxen = 3.18,[59] and ketoprofen = 3.12[60]), meaning that the prepared SILP is more efficient for the removal of more hydrophobic APIs.

In order to explore the adsorption mechanisms of diclofenac, naproxen and ketoprofen, pseudo first-order kinetic and pseudo second-order kinetic models were used to correlate the experimental data, where the adsorption kinetic parameters are summarized in Table 11 (and in Figure S3. in the Supporting Information).

Table 11. Pseudo first-order and pseudo second-order kinetic parameters corresponding to the fitting of the experimental kinetic data.

[Si][C_3C_1im]Cl	$q_{e,exp}$ / mmol.g^{-1}	Pseudo first-order model		
		$q_{e,cal}$ / mmol.g^{-1}	k_1 / min^{-1}	R^2
Diclofenac	0.311	0.073	0.036	0.8442
Naproxen	0.121	0.044	0.027	0.7562
Ketoprofen	0.055	0.016	0.051	0.8416
[Si][C_3C_1im]Cl	$q_{e,exp}$ / mmol.g^{-1}	Pseudo second-order model		
		$q_{e,cal}$ / mmol.g^{-1}	k_2 / min^{-1}	R^2
Diclofenac	0.311	0.308	7.2122	0.9998
Naproxen	0.121	0.114	4.239	0.9999
Ketoprofen	0.055	0.052	25.32	0.9986

The correlation coefficients (R^2) obtained with the second-order model are higher than 0.9984 for the 3 studied NSAIDs, against the R^2 values ranging between 0.7562 and 0.8442 for the pseudo first-order model, indicating that the second-order model better correlates the adsorption kinetics of diclofenac, naproxen and ketoprofen onto [Si][C_3C_1im]Cl and that the adsorption process is controlled by the adsorption of each NSAID at the liquid-solid interface.[54, 61]

The experimental data on the adsorption isotherms at 25 ºC were fitted by the Langmuir[55] (Eq. 5) and the Freundlich[56] (Eq. 6) models, using initial concentrations of diclofenac, naproxen and ketoprofen ranging between 0.001 and 0.464 g.L^{-1}, 0.001 and 0.158 g.L^{-1}, and 0.001 and 0.046 g.L^{-1}, respectively. In this type of experiments, the contact time was set to 120 min to ensure that no variations in q_e occur and that the equilibrium was attained. The correlation of the equilibrium concentrations of diclofenac, naproxen and ketoprofen between the

solid and liquid phases is depicted in Figure 38. The equilibrium adsorption of diclofenac onto [Si][C_3C_1im]Cl increases with the increase of the NSAID initial concentration until the material saturation, displayed by a plateau in q_e. However, the equilibrium adsorption of naproxen and ketoprofen onto [Si][C_3C_1im]Cl does not reach a plateau, which is due to these NSAIDs low water solubility[62] and unviability of preparing aqueous solutions of higher concentration. Therefore, the Langmuir isotherm constant (B (L.mmol^{-1})), maximum monolayer coverage capacity (q_{max} (mmol.g^{-1})) and constants of the Freundlich equation (K_f and n), were not determined for these two NSAIDs.

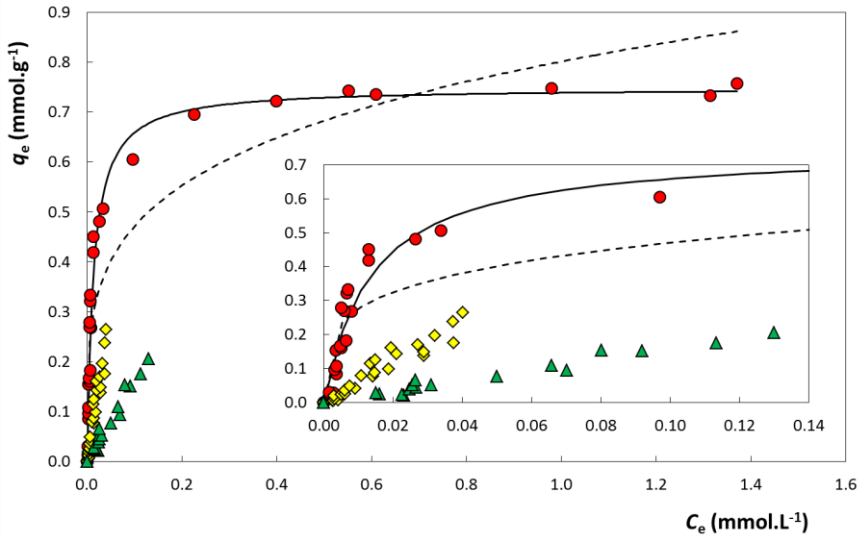

Figure 38. Adsorption isotherms of diclofenac (●), naproxen (◆) and ketoprofen (▲) onto [Si][C_3C_1im]Cl, at 25 ºC, and the Langmuir fitting (solid line, Eq. 5) and Freundlich fitting (dashed line, Eq. 6).

The most suitable isotherm model for diclofenac onto [Si][C_3C_1im]Cl is the Langmuir model, as shown by the higher correlation coefficient obtained (0.9702) and with a maximum equilibrium concentration of adsorbate in the solid phase of (0.75 ± 0.04) mmol.g^{-1}, a Langmuir isotherm constant of (73.27 ± 6.66) L.mmol^{-1}, and an equilibrium concentration of adsorbate (C_e) of *circa* 0.2 mmol.L^{-1}.

In the case of the Freundlich model (R^2 = 0.8603), and adsorption capacity (K_f) and adsorption intensity (n) of (0.80 ± 0.04) and (4.33 ± 0.02) were obtained, respectively. These results are in agreement with those previously shown by us.[57] These findings suggest that the adsorption of diclofenac onto [Si][C_3C_1im]Cl occurs through the formation of a monolayer on the outer surface of the adsorbent, where no further adsorption takes place, with a saturation point at *circa* 0.2 mmol.L^{-1} for the equilibrium concentration of adsorbate (C_e). Furthermore, the maximum equilibrium concentration of adsorbate in the solid phase of (0.75 ± 0.04) mmol.g^{-1} corresponds to an adsorption of 0.22 g of diclofenac *per* g of SILP. Although it was not possible to apply the Langmuir and Freundlich models to naproxen and ketoprofen, since their low water solubility hampered the determination of the material saturation, it is however possible to infer that the studied SILP is able to remove naproxen and ketoprofen up to 0.026 and 0.021 mmol.g^{-1} (0.06 and 0.05 g.g^{-1}), respectively. These values show that [Si][C_3C_1im]Cl is a remarkable material for the removal of NSAIDs from aqueous media.

4.3. Removal of NSAIDs using a [Si][C_3C_1im]Cl packed column

In order to evaluate a continuous removal method of NSAIDs, a packed column with [Si][C_3C_1im]Cl was prepared and used, as depicted in Figure S2 in the Supporting Information. Diclofenac aqueous solutions were used in this type of assays, since it corresponds to the most detected NSAID in aquatic environmental samples.[27] 100 mL of an aqueous solution of diclofenac at 0.02 and 0.2 g.L^{-1} were passed through 0.085 g of material, with a controlled flow rate of 5 mL.min^{-1}. In Figure 39 are depicted the %*AE* of the material for diclofenac at the two concentrations. Since [Si][C_3C_1im]Cl contains chloride as a counter-ion and APIs may be negatively charged at higher pH values, there is the possibility of ion exchange. To address this possibility it was carried out the quantification of chloride in the several collected fractions of aqueous solutions after the column adsorption assays, where no chloride ions were found, further demonstrating the

absence of ion exchange. Further details on the experimental procedure and method used are given in the Supporting Information.

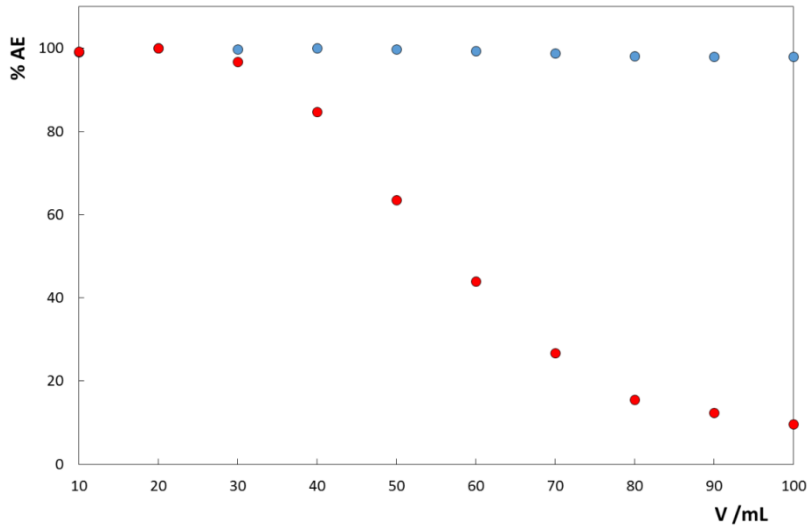

Figure 39. Adsorption efficiency (%AE) of [Si][C_3C_1im]Cl for diclofenac: ●, diclofenac solution at 0.02 g.L^{-1}; ●, diclofenac solution at 0.2 g.L^{-1}.

The adsorption of diclofenac is complete throughout the volume of 100 mL when using an aqueous solution with a concentration of 0.02 g.L^{-1}. However, when a higher concentration of diclofenac is used (0.2 g.L^{-1}), is starts with a complete adsorption, but by increasing the volume passed through the solid-phase column packed with [Si][C_3C_1im]Cl, the adsorption efficiency starts decreasing, indicative of the material saturation by diclofenac. Taking into account the volume of 30 mL of an aqueous solution of diclofenac at 0.2 g.L^{-1}, and the weight of silica used (0.085 g), it means that the material reached saturation at 0.071 g.g^{-1}, which is below the maximum q_e value dicussed above (0.22) g.g^{-1}. These results confirm that the available SILP surface area may play a significant role since higher values of equilibrium concentration of adsorbate in the solid phase are obtained in batch experiments.

According to the literature, the highest detected average concentration values of diclofenac in WWTPs effluents in Europe was of 3.3 μg.L^{-1}.[63] Therefore, according to the results obtained, by using solid-phase columns packed with [Si][C_3C_1im]Cl, it is possible to treat around 1800 L of water contaminated with diclofenac using 0.085 g of SILP. In a simplified version of a WTTP, three different stages (mechanical, biological and disinfection treatments) are combined,[51] where this packed column with [Si][C_3C_1im]Cl is envisioned to be introduced in the final stage of a WTTP.

4.4. Recovery of NSAIDs and SILP reusability

In order to investigate the reusable performance of the studied SILP, aqueous solutions of different (pH = 2, 4 and 10; no NSAID added) were used for regenerating [Si][C_3C_1im]Cl after passing 100 mL of a diclofenac aqueous solution with a concentration of 0.02 g.L^{-1}. 10 mL of these controlled pH aqueous solutions were passed through the SILP and again 100 mL of a diclofenac aqueous solution with a concentration of 0.02 g.L^{-1} were passed. In Figure 40 is presented the adsorption efficiency of [Si][C_3C_1im]Cl for diclofenac, in the first use and after regeneration at several pH values.

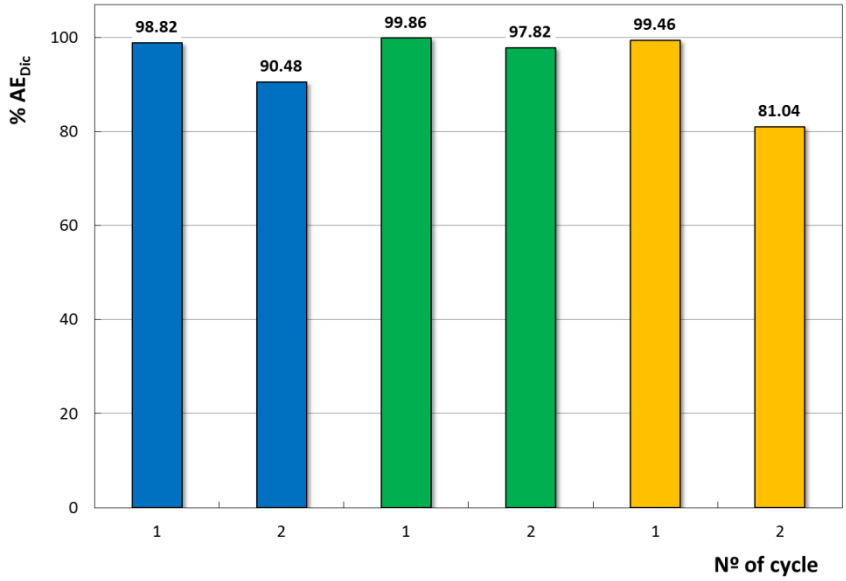

Figure 40. Adsorption efficiency of [Si][C_3C_1im]Cl for diclofenac, in new material (cycle 1) and after regeneration (cycle 2). Blue bars, pH = 2; green bars, pH = 4; and orange bars, pH = 10.

[Si][C_3C_1im]Cl could be recycled and reused without a significant reduction in its adsorption efficiency. Although the "washing" step with aqueous solutions of pH 2 and 10 lead to lower adsorption efficiencies after regeneration, the adsorption efficiency of the regenerated material could reach 97.82 % if "washed" with an aqueous solution of pH 4. These results suggest diclofenac is better removed from the SILP with aqueous solutions with a pH close to its *pKa*.[62, 64] Moreover, with aqueous solutions of more extreme pH values it might exist the block of the adsorption sites of [Si][C_3C_1im]Cl with the ions of HCl and NaOH which are at higher concentrations.

5. Conclusion

In the current work, the performance of IL-modified silica ([Si][C_3C_1im]Cl) to remove non-steroidal anti-inflammatory drugs (diclofenac, naproxen and ketoprofen) from aqueous solutions was evaluated. It was found that the adsorption kinetics of the three NSAIDs tested is well described by a pseudo second-order model. Additionally, the adsoption isotherms of diclofenac are well described by the Langmuir model. However, the equilibrium adsorption of naproxen and ketoprofen onto [Si][C_3C_1im]Cl did not reach a plateau, due to these NSAIDs low water solubility and unviability of preparing aqueous solutions of higher concentration. The maximum equilibrium concentration of diclofenac in the SILP is (0.75 ± 0.04) mmol.g^{-1} (0.22 g.g^{-1}). Although the material saturation could not be determined for naproxen and ketoprofen, the studied SILP is able to remove naproxen and ketoprofen up to 0.026 and 0.021 mmol.g^{-1} (0.06 and 0.05 g.g^{-1}), respectively.

In order to evaluate a continuous removal method of NSAIDs, a packed column with [Si][C_3C_1im]Cl was also prepared and used. In this type of approach, the material reaches saturation at 0.071 g.g^{-1}, which is below the maximum value of 0.22 g.g^{-1} corresponding to batch experiments, confirming that the available SILP surface area plays a significant role through adsorption. Finally, the recovery of NSAIDs and the SILP reusability were appraised, where it was found that [Si][C_3C_1im]Cl could be recycled and reused without a significant reduction in its adsorption efficiency after a “washing” step with aqueous solutions of pH 4. In summary, IL-modified silica SILPs can be considered as promising materials to be applied in the removal of active pharmaceutical ingredients from aqueous environmental matrices

6. Acknowledgment

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7. Supporting Information

7.1. Ionic exchange evaluation

Since [Si][C_3C_1im]Cl contains chloride as a counter-ion and APIs may be negatively charged at higher pH values, there is the possibility of ion exchange. To address this possibility it was carried out the quantification of chloride in the several collected fractions of aqueous solutions after the column adsorption assays, using a Metrohm 904 Titrando ion chloride electrode and a previously established calibration curve. The calibration curve was established with standard solutions at appropriate concentrations of chloride ions (between 0.1×10^{-3} mol.L^{-1} and 100×10^{-3} mol.L^{-1}). All measurements were performed at fixed ionic strength through the addition of a Total Ionic Strength Adjustment Buffer (TISAB) solution to all the standards and samples. The TISAB solution was prepared by mixing aqueous solutions at 0.1 mol.L^{-1} of KNO_3, CH_3COOH, and $NaC_2H_3O_2$.

7.2. Supporting Tables

Table S1. Adsorption efficiency (%AE), equilibrium concentration of diclofenac after adsorption (C_e), and concentration of adsorbate in the solid phase (q_e), with respectively standard deviations (σ) of [Si][C_3C_1im]Cl with diclofenac solution.

[Si][C_3C_1im]Cl + Diclofenac			
t / min	**% (AE_{Dic} ± σ)**	**C_e / mmol.L^{-1}**	**q_e ± σ / mmol.g^{-1}**
0	0.0 ± 0.0		
1	39.3 ± 1.4	0.047	0.12 ± 0.01
2	48.6 ± 6.7	0.040	0.15 ± 0.02
3	50.4 ± 1.5	0.039	0.16 ± 0.01
4	52.8 ± 1.3	0.037	0.16 ± 0.01
5	56.8 ± 2.6	0.034	0.18 ± 0.02
6	59.3 ± 2.5	0.032	0.19 ± 0.01
7	60.5 ± 4.1	0.031	0.18 ± 0.02
8	61.0 ± 2.2	0.030	0.18 ± 0.01
9	58.3 ± 2.5	0.033	0.16 ± 0.01
10	60.0 ± 3.2	0.031	0.19 ± 0.01
15	69.2 ± 1.9	0.024	0.22 ± 0.01
30	80.5 ± 2.2	0.015	0.24 ± 0.02
45	84.7 ± 1.0	0.012	0.24 ± 0.01
60	87.4 ± 0.3	0.011	0.26 ± 0.01
120	90.1 ± 0.2	0.009	0.26 ± 0.01
180	89.7 ± 2.2	0.029	0.27 ± 0.01

Table S2. Adsorption efficiency (%AE), equilibrium concentration of diclofenac after adsorption (C_e), and concentration of adsorbate in the solid phase (q_e), with respectively standard deviations (σ) of [Si][C_3C_1im]Cl with naproxen solution.

[Si][C_3C_1im]Cl + Naproxen			
t / min	**% (AE_{Dic} ± σ)**	**C_e / mmol.L^{-1}**	**q_e ± σ / mmol.g^{-1}**
0	0.0 ± 0.0		
1	34.3 ± 5.8	0.029	0.06 ± 0.01
2	36.6 ± 6.0	0.028	0.04 ± 0.02
3	44.9 ± 2.3	0.025	0.08 ± 0.01
4	43.5 ± 1.7	0.025	0.08 ± 0.01
5	48.9 ± 1.7	0.023	0.09 ± 0.01
6	50.2 ± 0.4	0.022	0.08 ± 0.02
7	49.5 ± 0.4	0.022	0.09 ± 0.01
8	51.7 ± 1.7	0.022	0.09 ± 0.01
9	52.1 ± 3.6	0.021	0.09 ± 0.02
10	51.4 ± 3.4	0.022	0.10 ± 0.01
15	56.6 ± 2.3	0.019	0.10 ± 0.02
30	61.3 ± 3.8	0.017	0.10 ± 0.01
45	66.2 ± 2.1	0.015	0.11 ± 0.01
60	66.2 ± 0.9	0.015	0.11 ± 0.01
120	67.2 ± 0.2	0.015	0.12 ± 0.01
180	66.2 ± 0.1	0.015	0.11 ± 0.02

Table S3. Adsorption efficiency (%AE), equilibrium concentration of diclofenac after adsorption (C_e), and concentration of adsorbate in the solid phase (q_e), with respectively standard deviations (σ) of [Si][C_3C_1im]Cl with ketoprofen solution.

[Si][C_3C_1im]Cl + Ketoprofen			
t / min	**% (AE_{Dic} ± σ)**	**C_e / mmol.L^{-1}**	**q_e ± σ / mmol.g^{-1}**
0	0.0 ± 0.0		
1	19.9 ± 0.4	0.03	0.04 ± 0.01
2	22.3 ± 0.4	0.03	0.03 ± 0.02
3	23.3 ± 1.2	0.03	0.04 ± 0.01
4	24.0 ± 1.4	0.03	0.04 ± 0.02
5	25.3 ± 0.4	0.03	0.04 ± 0.01
6	24.9 ± 1.1	0.03	0.04 ± 0.01
7	26.6 ± 0.9	0.03	0.04 ± 0.01
8	24.7 ± 0.3	0.03	0.05 ± 0.01
9	25.7 ± 1.0	0.03	0.05 ± 0.02
10	28.1 ± 0.1	0.03	0.05 ± 0.01
15	28.0 ± 0.2	0.03	0.05 ± 0.02
30	30.3 ± 0.1	0.03	0.05 ± 0.01
45	29.6 ± 0.1	0.03	0.05 ± 0.01
60	31.1 ± 1.0	0.03	0.05 ± 0.01
120	32.2 ± 0.3	0.03	0.05 ± 0.02
180	31.1 ± 0.4	0.03	0.05 ± 0.01

7.3. Supporting Figures

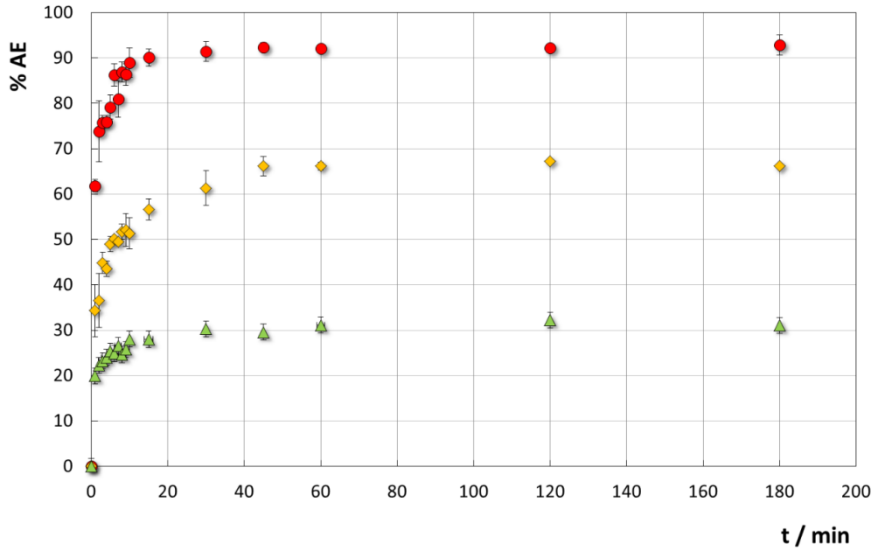

Figure S1. Adsorption efficiency of of supported *N*-methylimidazolium-based ionic liquid with diclofenac (●), naproxen (◆) and ketoprofen (▲).

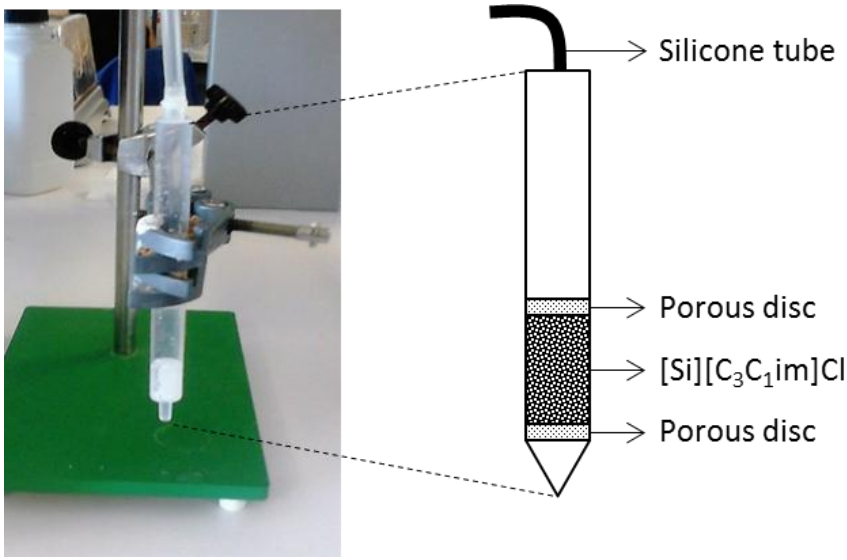

Figure S2. Lab-scale solid-phase column packed with [Si][C_3C_1im]Cl**.**

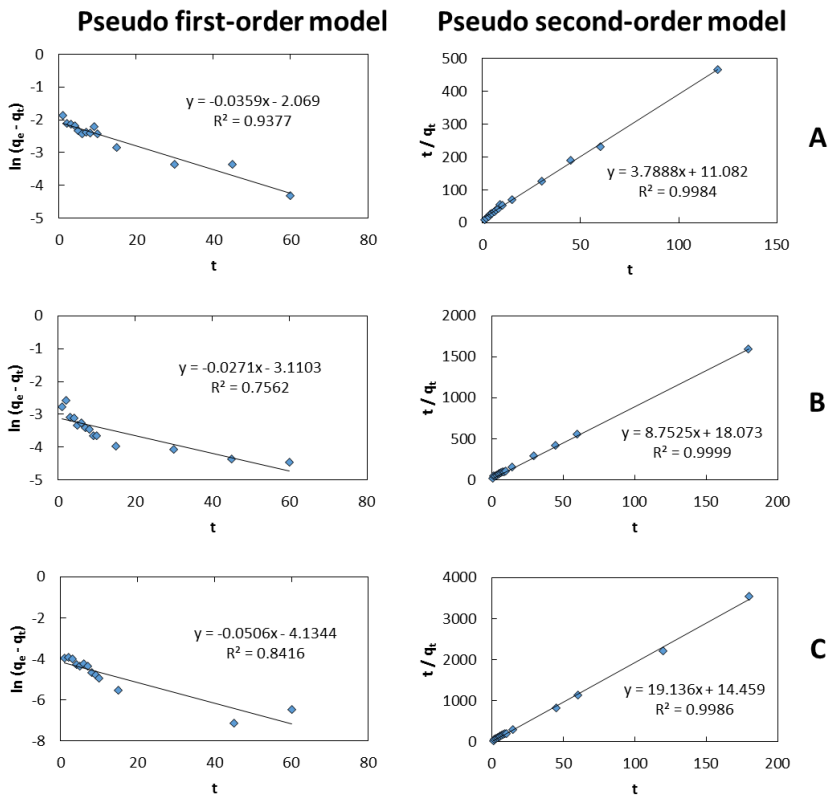

Figure S3. Parameters of the pseudo first-order and pseudo second-order kinetic models of [Si][C_3C_1im]Cl with: A) diclofenac; B) naproxen; and C) ketoprofen.

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Chapter 7

Concluding Remarks and Outlook

The introduction of active pharmaceutical ingredients (APIs) into the environment through the release of contaminated effluents from waste water treatment plants (WWTPs) is a matter of serious concern. Therefore, the development of new and cost-effective technologies that could enable the removal of APIs from WWTPs is a top priority. Two ionic-liquid-based separation technologies were exploited in the extraction, concentration and removal of active pharmaceutical ingredients from aqueous media: aqueous biphasic systems and supported ionic liquid phases. The most important results obtained during this PhD project are highlighted in the following sub-chapter. Finally, an outlook for future research within this topic is presented.

1. Towards the use of ionic liquids in active pharmaceutical ingredients removal processes

Ionic liquids (ILs) are a class of solvents extensively studied in both academic and industrial fields. Their exceptional properties, such as negligible vapour pressures, and the possibility of tailoring their properties for a specific task by an adequate combination of their ions, makes them exceptional candidates for a wide range of applications. In this work, ILs were used in two different types of separation techniques namely liquid-liquid and solid-phase extractions, in order to develop efficient separation techniques which could allow the detection, removal and recovery of APIs. In particular, ionic-liquid-based aqueous biphasic systems (IL-based ABS) and supported ionic liquid phases (SILPs) were investigated for the extraction of fluoroquinolones (FQs) and non-steroidal anti-inflammatory drugs (NSAIDs), two important classes of APIs found in the aquatic environment. .

Two different approaches, with two different objectives, were explored using IL-based ABS: i) the concentration of FQs and NSAIDs envisaging a more accurate detection and quantification; and ii) the extraction/removal/recovery of FQs and NSAIDs using reusable systems. In what concerns the first approach,

extraction efficiencies of FQs and NSAIDs up to 100 % were obtained using IL-based ABS composed of $[N_{4444}]Cl$ + $C_6H_5K_3O_7$, with pH values in the range of 7.4 to 9.9 (Chapter 2). It was found that the solutes partitioning is mainly ruled by the strong salting-out effect exerted by the salt employed. Furthermore, it was observed that the FQs and NSAIDs solubility in the IL-rich phase is much higher than their solubility in water. For instance, ciprofloxacin and diclofenac present solubility values at (25 ± 1) ºC of 1.95 ± 0.16 $g.L^{-1}$ and 5.43 ± 0.20 $g.L^{-1}$ in the IL-rich phase, respectively, representing a solubility *circa* 1500 times higher than that in water. This fact allowed very high concentration factors of FQs and NSAIDs to be achieved, up to 1000-fold in a single-step, by the appropriate manipulation of the phase-forming components compositions to tailor the volumes of the coexisting phases. The proposed methodology allowed to increase the concentration of APIs up to levels that can be identified and quantified by high-performance liquid chromatography.

The extraction of NSAIDs (Chapter 3) and FQs (Chapter 4) from aqueous streams was carried out using IL-based ABS composed of $[P_{4441}][CH_3SO_4]$ + $Al_2(SO_4)_3$ (salt already used in WWTP as floculant agent), now with the aim of evaluating their performance for the removal of APIs from aqueous media. Extraction efficiencies for NSAIDs and FQs up to 98 and 100 %, respectively, were obtained. The use of $Al_2(SO_4)_3$ warrants pH values of the IL-rich phase in the range of 1.2 – 3.2 which allowed the development of continuous extraction and recovery cycles for FQS, by precipitation through pH change, and for NSAIDs, by precipitation with water addition.

In order to overcome the cross contamination of the phases that occur in IL-based ABS, solid-phase extraction methods were finally evaluated for the removal of NSAIDs and FQs from aqueous media. Supported IL-silica materials were prepared, characterized and evaluated in what concerns their performance to remove NSAIDs (Chapter 5). Six outstanding and novel supported ionic liquids materials, based on 1-methyl-3-propylimidazolium combined with different anions

were successfully synthesized it was found that a pseudo second-order adsorption kinetic model is more suitable to describe the diclofenac adsorption, whereas the adsorption isotherms are well described by the Langmuir model. The most promising material was then evaluated to remove additional NSAIDs (Chapter 6), and a self-packed column of [Sil][C_3C_1im]Cl was tested as a continuous removal process. The recovery of the NSAIDs and the reusability of material also were appraised. In summary, the obtained results demonstrate that it is possible to functionalize a low cost and nontoxic material, such as silica, using ILs in order to obtain new efficient materials for the removal of active pharmaceutical ingredients from aqueous media.

In summary, highly effective methodologies, either based on liquid-liquid extraction or solid-liquid extraction, were developed; these are envisioned to be used in a final stage of WWTPs for the removal of APIs.

2. Outlook

This thesis provides promising and innovative results for the extraction, concentration and removal of active pharmaceutical ingredients from aqueous streams. The work developed herein proves the remarkable potential of ILs as tailored solvents. IL-based ABS as concentration platforms were also tested with real WWTPs effluents. However, a more in depth study of IL-based ABS and supported-IL materials with real effluent and influent samples is still required. Since the main goal is to use this technology in a final stage of a WWTP, additional investigations on the technologies scale-up also are needed. An overall sustainability study of the two proposed methodologies, encompassing technological, economic and environmental perspectives, should be performed in order to come up with the best overall solution.