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

Hugo F. D. Almeida[a,b], Mara G. Freire[b] and Isabel M. Marrucho*[a]

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 treatement 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.

Introduction

In the 21st century, the discovery of forthcoming pollutants in different environmental matrices, including pseudo-persistent compounds with toxic and accumulative 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 μg.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 – Fig. 1), 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

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[a] *Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, 2780-157 Oeiras, Portugal. E-mail: imarrucho@itqb.unl.pt*

[b] *CICECO – Aveiro Institute of Materials, Chemistry Department, University of Aveiro, 3810-193 Aveiro, Portugal.*

Electronic Supplementary Information (ESI) available: Supporting figures and tables. See DOI: 10.1039/x0xx00000x 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 discharged,[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.

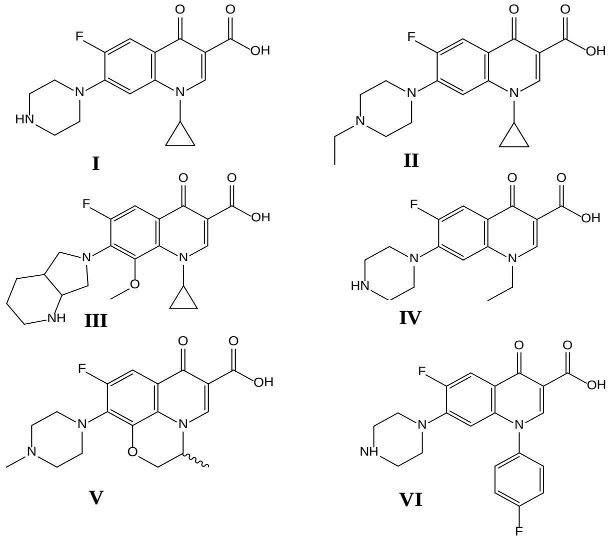

Fig. 1 Chemical structures of fluoroquinolones: I) ciprofloxacin, II) enrofloxacin, III) moxifloxacin, IV) norfloxacin, V) ofloxacin and VI) sarafloxacin.

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.* Fig 1). 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.

Experimental Section

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 Fig. 1.

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 ^1H 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 Fig. 2.

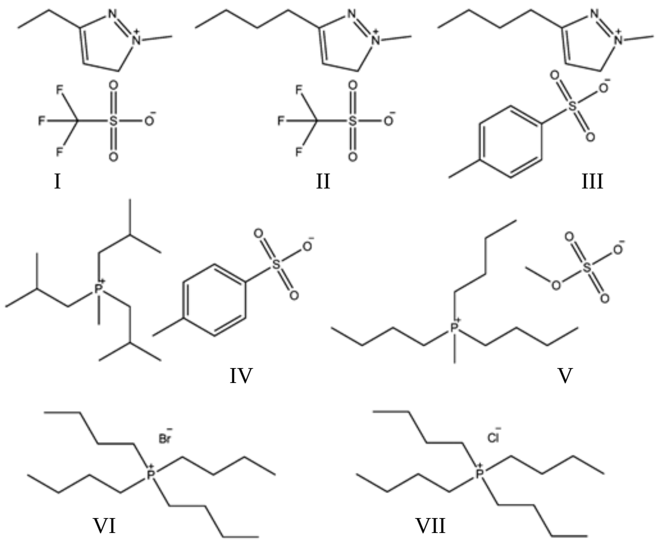

Fig. 2 Chemical structures of the ionic liquids used: I) $[C_2C_1im][CF_3SO_3]$, II) $[C_4C_1im][CF_3SO_3]$, III) $[C_4C_1im][Tos]$, IV) $[P_{i(444)1}][Tos]$, V) $[P_{4441}][CH_3SO_4]$, VI) $[P_{4444}]Br$, and VII) $[P_{4444}]Cl$.

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.

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 ESI†.

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.

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.

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 S.I. 1-3, in the ESI†.

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 S.I. 1 in the ESI†. 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.

Results and Discussion

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 S.I. 1, in the ESI†. The detailed extraction efficiencies and pH of each phase are also provided in the ESI†. 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.* ESI†),[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.

Fig. 3 and Fig. 4 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.

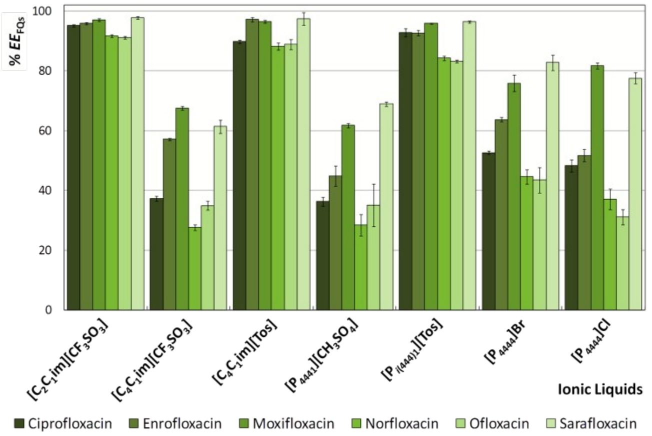

Fig. 3 Extraction efficiencies of FQs (% EE_{FQs}) with ABS composed of IL + $Al_2(SO_4)_3$ + H_2O, at 25 ºC, as a function of the FQ investigated.

In general, the % EE_{FQs} for the different FQs does 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.

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 S.I. 4 and Table S.I. 3 - in the ESI†) 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.

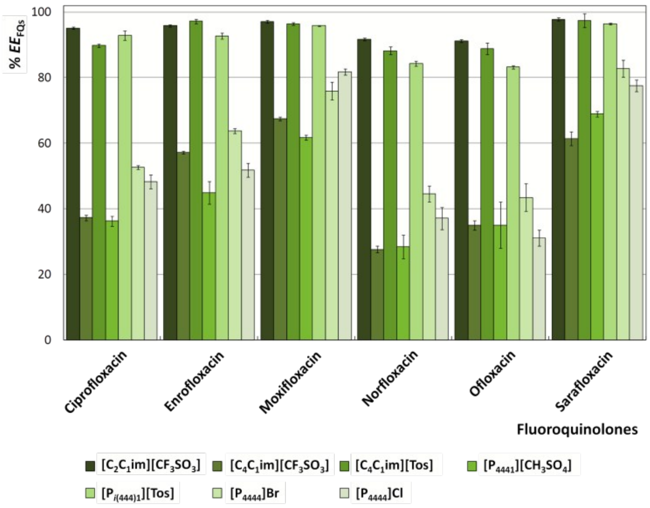

Fig. 4 Extraction efficiencies of the six studied fluoroquinolones (%EE_{FQs}) with ABS composed of IL + $Al_2(SO_4)_3$ + H_2O, at 25 ºC, as a function of the IL investigated.

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.* ESI† 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 S.I. 4 and Figure S.I. 5 (in the ESI†), 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.

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 S.I. 1, shown in the ESI†), 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 1. 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 1, 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 1 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 2.

Table 2 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 Fig. 5. 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 (Fig. 5b). 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 S.I. 6 in the ESI†).

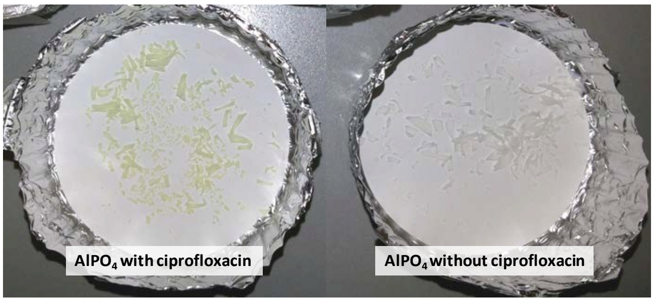

Fig. 5 Precipitates obtained through the addition of K_3PO_4 to: a) an aqueous solution of $Al_2(SO_4)_3$ and ciprofloxacin; and b) an aqueous solution of $Al_2(SO_4)_3$.

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 ESI†. 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 3.

Table 3 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 precipitation efficiencies (around 82%) were obtained for $[C_2C_1im][CF_3SO_3]$ and $[C_4C_1im][Tos]$, 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 4. Three priority fluoroquinolones, namely ciprofloxacin, norfloxacin and ofloxacin, were used to validate the methodology.

According to the results reported in Table 4, 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 4 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

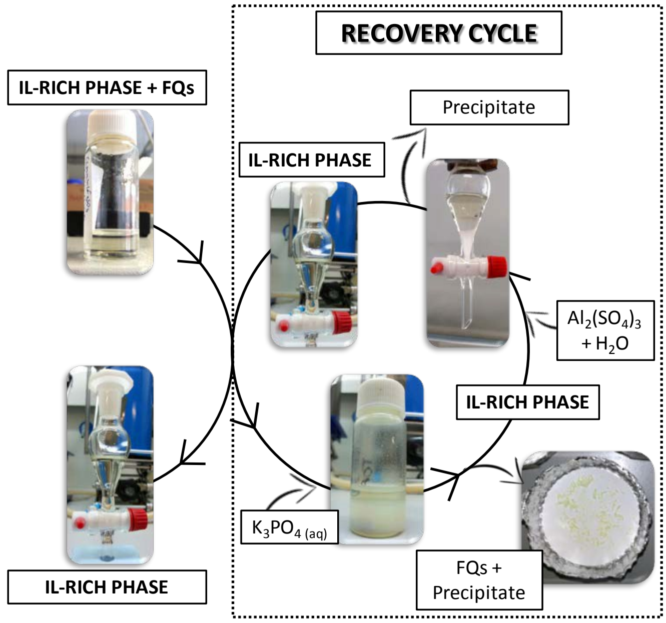

Fig. 6 Representative scheme of the overall methodology here proposed comprising the IL-recovery cycle.

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 ESI†). 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.

Fig. 6 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.

Conclusions

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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Notes and references

† Electronic Supplementary Information (ESI) available: Weight fraction percentage (wt%) composition of the coexisting phases (tie-lines, TLs); Tie-line lengths (TLL); pH values of the IL-rich phases (pH_{IL}); and extraction efficiencies of FQs ($\%EE_{FQs}$).

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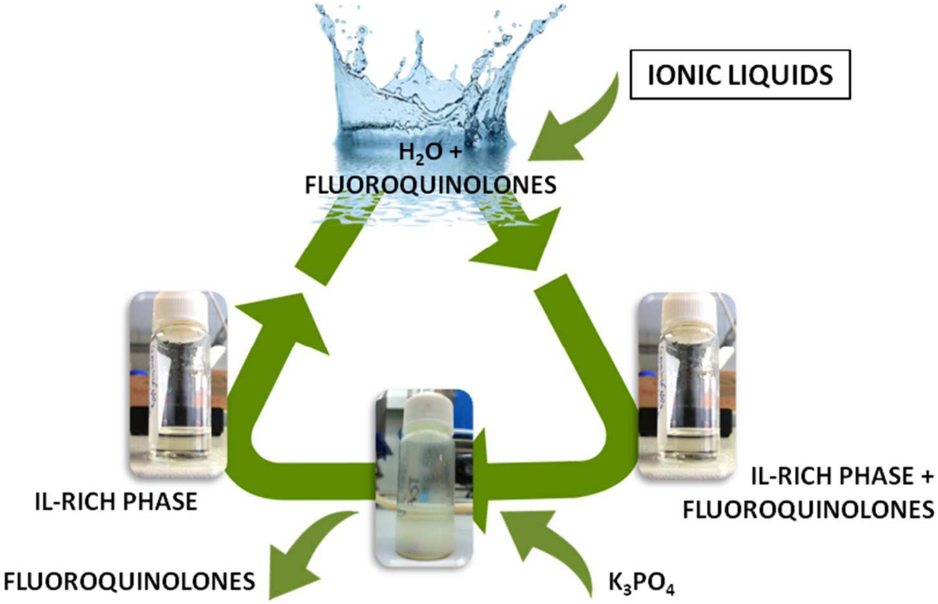

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