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New non-toxic biocompatible dianionic ionic liquids that enhance the solubility of oral drugs from BCS class II

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ABSTRACT

New dianionic ionic liquids (ILs) based on carboxylic anions and ammonium cations were prepared and characterized. They were used as excipients to increase the solubility of two model oral drugs of BCS class II, ibuprofen and piroxicam. With only 0.2 mol% (≈100 mM) of $[N_{4\,1\,1\,2OH}]_2[C_4H_4O_4]$, the solubility increases over 40-fold and 2-fold for ibuprofen, when compared with the parent drug for water and phosphate buffer solution (PBS) 0.01M pH=7.4, respectively. With only 0.02 mol% of $[N_{4\,1\,1\,2OH}]_2[C_4H_4O_4]$ it is possible to achieve a water solubility of ≈600 mg/L, in only 5 min at 37°C, corresponding to one dose of ibuprofen that an adult can take. Piroxicam also showed an increase of 20-fold and 1.5-fold for water and PBS respectively, with $[N_{4\,1\,1\,2OH}]_2[C_4H_4O_4]$ and $[N_{4\,1\,1\,2OH}]_2[C_5H_6O_4]$.

The lipophilicity (logP) of both drugs decreased in the presence of these compounds. The cytotoxicity profile of several of these ILs was determined, and all except $[N_{4\,1\,1\,2OH}]_2[C_3H_2O_4]$ have an IC_{50} higher than 100 mM for fibroblasts L929 cells.

1. Introduction

The use of ionic liquids (ILs) as "green solvents" in pharmaceutical industry have several advantages when compared with common organic solvents (Mizuuchi et al., 2008). ILs have important characteristics such as negligible vapour pressure, good chemical and thermal stability and solvation interactions with organic and inorganic compounds (Ho et al., 2014; Le Bideau et al., 2011; Zhou, 2005). There use as co-solvents is useful to dissolve drug molecules that are sparingly soluble in water and the interactions between drug and IL strongly depends on the combination of cation and anion of the ionic liquid (Lotfi et al., 2017; Md Moshikur et al., 2020; Moniruzzaman et al., 2010; Moniruzzaman and Goto, 2011; Williams et al., 2014). The dissolution of a drug is a key step to its therapeutic effect, so low water solubility of drugs affects their bioavailability (Martinez and Amidon, 2002; Sahbaz et al., 2015; Tiwari et al., 2009). The bioavailability of a drug is defined as the rate and extent to which a dissolved drug is absorbed and becomes available at its target site of action. The bioavailability depends on the solubility and the membrane permeability of the compound (Dahan et al., 2016, 2009; Loh et al., 2015; Varma and Panchagnula, 2005).

The number of lipophilic drugs discovered continuous to increase and suitable formulations for these drugs must be investigated (Dahan and Hoffman, 2007; Patel et al., 2018; Pouton, 2006). According to the biopharmaceutical classification system (BCS), the drugs with low solubility and high permeability are classified as class II drugs, which dissolution step is the rate-determining factor in drug absorption. So new approaches are needed to facilitate and enhance the solubility and thus dissolution rate of BCS class II drugs. ILs are promising candidates in the pharmaceutical industry, so the discovery of new, unique, and effective IL-based drug formulations are requested (Adawiyah et al., 2016; Huang et al., 2020).

The major reason why the use of ILs in medical applications is still limited concerns the toxicity and biodegradability of these compounds (Alawi et al., 2015; Gomes et al., 2019). In general, an increase of the alkyl chain length causes a drastic increase of the toxicity and the introduction of oxygenated groups provokes a decrease on IL toxicity (Mena et al., 2020). In contrast, the IL cations have a small effect on the solvation capability, whereas the anion plays a significant role in the solubility of drug molecules. The dissolution of drugs in ILs involves the formation of hydrogen bonds between the IL anions and the drug molecules (Balk et al., 2015).

We synthesized monoanionic ILs based on cholinium cation with anions based on acetyl amino acids and alkyl sulfonates, that increased the aqueous solubility of paracetamol and sodium diclofenac (Jesus et al., 2019, n.d.). Very recently, we designed new dianionic ionic liquids based on phosphonate anions and ammonium cations, which contributed to a good increment in the solubility of two poorly oral drugs in water, piroxicam and ibuprofen, that are classified as a class II drugs. However, several of those ILs had an IC_{50} lower than 100 mM

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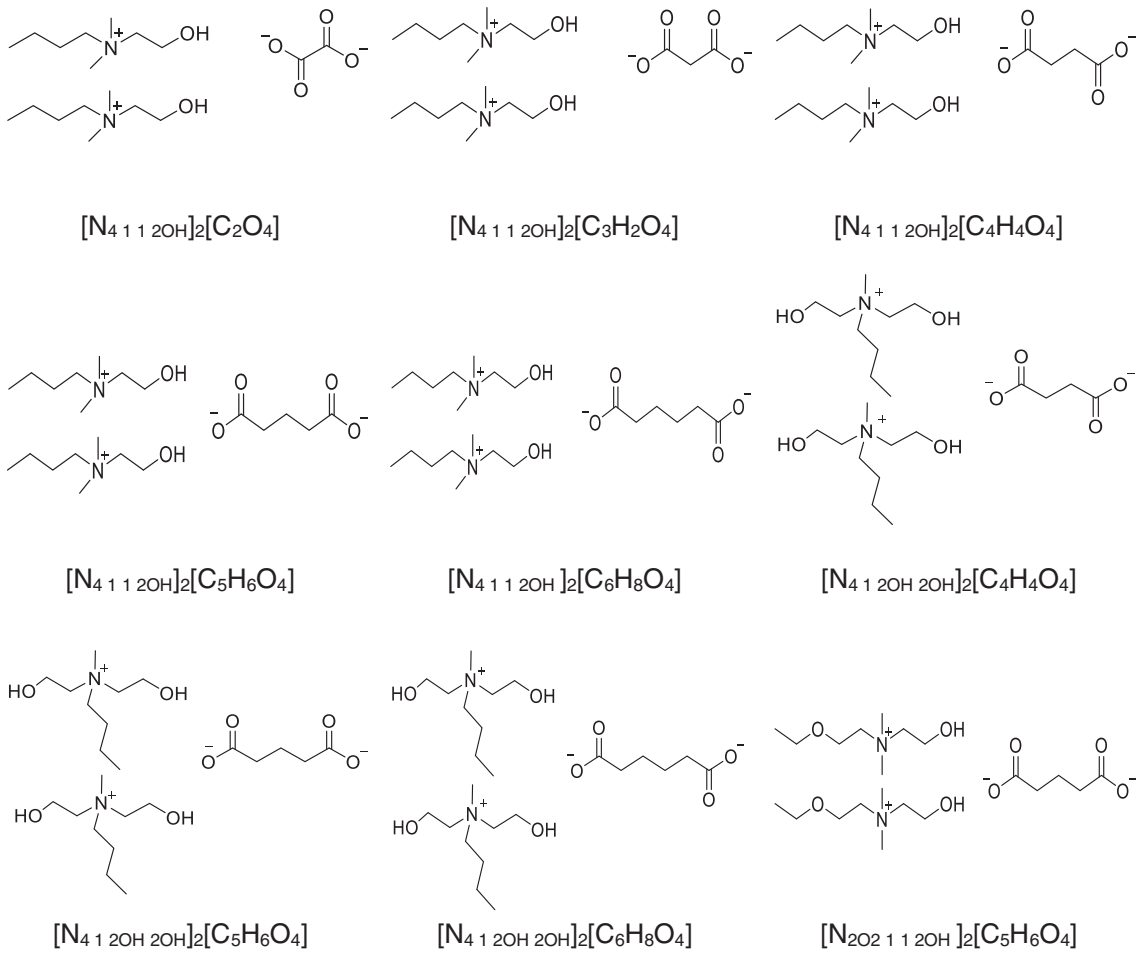

Fig. 1. Chemical structure of dianionic ionic liquids.

(Agostinho et al., 2021). Following this, we decided to synthesize another class of dianionic ILs with non-toxic, biodegradable, and biocompatible ions, using a combination of cholinium and carboxylate ions. In this work, 9 cholinium based ionic liquids with different dicarboxylic acids were prepared, which are represented in Figure 1. To better understand the effect of the anion in IL toxicity, different alkyl chains of dicarboxylic acids were used. The IL toxicity was evaluated using fibroblasts L929 cells.

These new dianionic ILs will be used as excipients in two model oral drugs of BCS class II, ibuprofen and piroxicam, which structure is represented in Figure 2.

The application of these new nontoxic dianionic ILs is reported and the bioavailability of model drugs at the target site of action is evaluated. The possibility of solubilizing active pharmaceutical ingredient (API) in biocompatible ILs can provide new perspectives for drug delivery and treatment approaches (Marrucho et al., 2014).

2. Materials and methods

2.1. Materials

N-Methyldiethanolamine (≥ 98.0%), succinic acid (99.0%), adipic acid (99.0%), 1-octanol (99.0%), Amberlite™ IRN-78, 4-isobutyl-α-methylphenylacetic acid (ibuprofen) (99.0%) and piroxicam (97.0% min.) were purchased from Alfa Aesar. 1-chlorobutane (≥ 99.0%), 1-bromobutane (≥ 98.0%), 2-dimethylaminoethanol (≥ 99.5%), 2-bromoethyl ethyl ether (90.0%), malonic acid (99.0%), oxalic acid (98.0%), glutaric acid (99.0%) and phosphate buffered saline tablet were acquired from Sigma-Aldrich. Diethyl ether (≥ 99.8%) was obtained from Honeywell – Riedel-de-Haën. Ethanol absolute anhydrous and ethyl acetate (99.8%) were purchased from Carlo Erba. Deuterium oxide (99.9%), dimethyl sulfoxide-d_6 (99.8%) and acetonitrile-d_3 (99.8%) were purchased from Eurisotop and hexane from Valente e Ribeiro Lda., respectively.

2.2. General procedure for the synthesis of $[N_{4\ 1\ 2OH\ 2OH}]Cl$, $[N_{4\ 1\ 1\ 2OH}]Br$ and $[N_{2O2\ 1\ 1\ 2OH}]Br$

N-Methyldiethanolamine (100 mmol) was mixed with the alkylating agent, 1-chlorobutane (4.0 equiv.). 2-dimethylaminoethanol (100 mmol) was mixed with the alkylating agent, 1-bromobutane or 2-bromoethyl ethyl ether (1.1 equiv.), respectively. N-hexane was added as solvent in a 100 mL pressure reaction vessel. The mixture was kept at 80-90 °C for 4 days for $[N_{4\ 1\ 2OH\ 2OH}]Cl$ and 24 h for the another precursors ILs. The ionic liquids were thoroughly washed with diethyl ether to remove the unreacted alkylating agent. Ionic liquids were then dried in vacuum for 1-2 days, and their purity confirmed by ^1H NMR. The yield of preparation of these ILs was 95-99%.

2.3. General procedure for the metathesis reaction

The procedure is already described in the literature (Agostinho et al., 2021). Briefly, an aqueous solution of *N*-alkyl derivative cholinium bromide or chloride (20 mmol) was slowly passed through an excess of an-

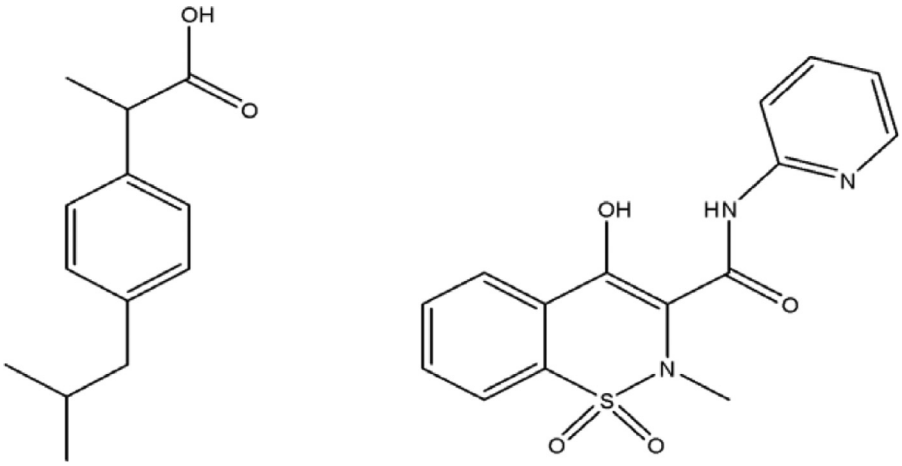

Fig. 2. Chemical structure of Ibuprofen (left) and Piroxicam (right).

ion resin exchange column Amberlite™ IRN-78. Then, the corresponding hydroxide solution was slowly added to a solution of dicarboxylic acid (0.5 equiv.). The reaction mixture was stirred at room temperature for 2-3 h prior to water removal by evaporation. Ionic liquids were washed with ethyl acetate (2 × 20 mL) and diethyl ether (1 × 20 mL), obtained in quantitative yield, and dried in high vacuum for 2 days, at 50 ˚C, to guarantee minimum water content. Coulometric Karl-Fischer titrations yielded final water contents below 1500 ppm depending on the IL. Moreover, $AgNO_3$ test was used to confirm the absence of halogen presence in the final IL, to guarantee that all halogen anion was converted to hydroxyl group.

^1H and ^{13}C of all dianionic ionic liquids synthesized are shown in Figures S1-S9.

2.4. Differential scanning calorimetry (DSC)

Approximately 8 mg of white solid IL was crimped into an aluminium TA Tzero pan and hermetic lid. The DSC cell was continuously purged with nitrogen gas at 50 mL/min. The procedure followed was dependent on the melting point of the sample. For $[N_{4\,1\,1\,2OH}]_2[C_2O_4]$ the procedure consisted of 3 consecutive cycles of: i) cooling ramp to -50˚C at 2˚C/min; ii) heating ramp at 2˚C/min up to 100˚C. For $[N_{4\,1\,1\,2OH}]_2[C_4H_4O_4]$ the procedure was repeated 3 times: i) cooling ramp to -90˚C at 5˚C/min; ii) heating ramp at 5˚C/min to 150˚C. Glass transition (Tg) and melting (Tm) temperatures (peak) were determined.

2.5. Determination of solubility limit of Ibuprofen and Piroxicam

The procedure has been previously described elsewhere (Agostinho et al., 2021). Briefly, aqueous or phosphate buffer saline solutions pH =7.4 (2 mL) of ionic liquids were prepared and ibuprofen or piroxicam were added to each sample guaranteeing that solutions are above its solubility limit. In the case of ibuprofen, an aqueous or phosphate buffer solution of IL at the same concentration was used as blank sample, because these ILs absorb in the region 190-230 nm. All solutions were stirred for 24h at 37˚C until equilibrium was reached. A calibration curve was prepared for each drug, in water or PBS 0.01 M, at their maximum absorbance wavelength, by UV-Vis VWR® spectrophotometer model UV-6300PC, 222 nm for ibuprofen and 353 nm for piroxicam, at 25 ˚C, see Figure S10 and S11. Absorbance values were kept below 1. The drug concentration was analysed following the same procedure. Triplicates were prepared for each sample and maximum absorbance was measured at 222 nm and 353 nm to ibuprofen and piroxicam, respectively. The pH in aqueous solutions was checked during the assay and no variation was observed.

2.6. Determination of partition coefficient and log P

The K_{ow} and log P values of ibuprofen and piroxicam in the presence of ionic liquids were determined using the shake-flask method reported in our previous works (Agostinho et al., 2021; Jesus et al., 2019, n.d.). Briefly, an octanol-saturated aqueous solution was used to prepare the ionic liquid solutions at 0.2 mol%. Known amounts of the drug were dissolved in 1 g of IL solution. Afterwards, the solution was mixed with 1 g of a water-saturated octanol solution. The solutions were stirred vigorously for 18-24 h and then stabilized for some hours until full separation of the organic and the aqueous layers. Three independent experiments were performed. The drug concentration in the water-rich phase was analysed in a UV-Vis VWR® spectrophotometer, model UV-6300PC, using a previously prepared calibration curve, see Figure S12. The concentration of drug in the octanol-rich phase was directly calculated by subtracting the amount in the water-rich phase to the initial amount dissolved.

2.7. Permeability

Permeability studies were conducted using a glass Franz-type diffusion cell (PermeGear) with a 10 mL reactor compartment with an effective mass transfer area of 1 cm^2 (Duarte et al., 2017; Silva et al., 2014).

The membrane used was a polyethersulfone (PES-U), with 150 μm thickness and 0.45 μm pore size (Sartorius Stedim Biotech, Germany), which was placed between the two compartments and held with a stainless-steel clamp. The receptor compartment (8 mL) was filled with PBS 0.01 M pH 7.4 and donor compartment (2 mL) loaded with an IL phosphate buffer solution (0.2 mol%) containing the oral drug (40 mg), which was filtered after being stirred 24 h at 37 ˚C. Aliquots of 200 μL were collected from the receptor compartment at predetermined time periods (0, 5, 15, 30 min and 1, 2, 3, 4, 6, 8 h) and fresh PBS 0.01 M was added to complete the volume. The concentration of the drug in the receptor compartment was analysed in a microplate reader (VICTOR Nivo™ PerkinElmer, USA) at 222 nm for ibuprofen and 353 nm for piroxicam. The experiments were performed at 37 ˚C and triplicates were performed.

The permeability, P (cm s^{-1}), the diffusion coefficient, D (cm^2 s^{-1}) and partition coefficient, K_d was calculated according to equations reported in the literature (Agostinho et al., 2021).

2.8. Cytotoxicity evaluation

2.8.1. Cell culture

Fibroblasts L929 cells (L929 DSMZ - German Collection of Microorganisms and cell culture GmbH) were cultivated in MEM (Eagle's Minimum Essential Medium) supplemented with 10% of FBS and 1%

Table 1
Physical appearance and DSC data.

IL	Appearance	T_g(°C)	T_m(°C)
$[N_{4\,1\,1\,2OH}]_2[C_2O_4]$	white solid	—-	88.3
$[N_{4\,1\,1\,2OH}]_2[C_4H_4O_4]$	white solid	-41.7	108.1

penicillin-streptomycin at 37 °C and 5% CO_2. The supplements and MEM were obtained from Corning, USA.

2.8.2. Viability assays

Fibroblasts were incubated for 24h at 37 °C and 5% CO_2 in a 96-well plate at a density of 1.0×10^4 cells/well with different concentrations of IL, ranging from 25 to 500 mM. Control cells were incubated with complete media only. After 24 h, cell monolayers were washed with PBS and cell viability was evaluated using the CellTiter 96® AQueous One Solution Cell Proliferation Assay (Promega), which is based on tetrazolium active component ((3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, MTS). The amount of formazan product formed was measured by spectroscopy, in a microplate reader (VICTOR Nivo™ PerkinElmer, USA) at 490 nm, as absorbance is directly proportional to the number of viable cells in culture. Cell viability was expressed as percentage of cells exposed to extracts *vs* control cells.

2.8.3. Statistical analysis

Graphpad Prism 7 was used for statistical analysis and calculate IC_{50} values. Statistical significant differences were calculated through One-Way Analysis of Variance (ANOVA) by Tukey's multiple comparisons tests and a p-value < 0.05 was considered significant. Data is expressed as average ± standard deviation from at least three independent experiments.

3. Results and discussion

3.1. ILs synthesis

Following the experimental procedure described previously we have been able to synthetise 9 ionic liquids in good yields (95-99%) and purity (≥99%). All ILs were liquids at room temperature, except for $[N_{4\,1\,1\,2OH}]_2[C_2O_4]$ and $[N_{4\,1\,1\,2OH}]_2[C_4H_4O_4]$ which are white solids.

3.2. Differential scanning calorimetry (DSC)

The bioavailability of API can also be reduced by polymorphism (Marrucho et al., 2014; Santos et al., 2019), but this phenomenon can be decreased to a certain extent or even eliminated. The use of an active drug in liquid form can avoid some of the polymorphism problem associated with solids. Piroxicam (Sheth et al., 2004) and ibuprofen (Oparin et al., 2019) are polymorphic drugs and both contain two polymorphic forms. Physical instability as polymorphism may challenge the pharmaceutical quality of drug (Saedtler and Meinel, 2019), so the polymorph with the lowest crystal energy is typically selected for pharmaceutical development.

Table 1 lists the physical appearance and DSC data for the 2 solid ILs synthesized.

The DSC spectrum for $[N_{4\,1\,1\,2OH}]_2[C_4H_4O_4]$ and $[N_{4\,1\,1\,2OH}]_2[C_2O_4]$ are presented in Figure 3 and Figure S13, respectively. In $[N_{4\,1\,1\,2OH}]_2[C_4H_4O_4]$ a cold crystallization occurred on heating before the melting point, that are different in 2nd and 3rd cycles, due to metastability of IL. $[N_{4\,1\,1\,2OH}]_2[C_4H_4O_4]$ also shows a glass transition. In the case of dianionic ionic liquid $[N_{4\,1\,1\,2OH}]_2[C_2O_4]$, the melting peak is clear, but there is no observable glass transition.

3.3. Solubility assays

In this work, the solubility of ibuprofen and piroxicam in aqueous media and phosphate saline buffer solution PBS 0.01 M pH=7.4, was determined at 37°C to mimic body temperature. At this temperature, their solubility in water was 60 mg/L and 32 mg/L, respectively, which is consistent with literature data (Agostinho et al., 2021; Sintra et al., 2018). In PBS, the solubilities increase significantly to 1509 mg/L and 583 mg/L for ibuprofen and piroxicam which is also consistent with literature values (A-sasutjarit et al., 2005; Mönkäre et al., 2011). Phosphate´s presence in the buffer modified drugs hydration, because provides co-solvency, so the values of solubility in PBS for both oral drugs are much higher than in water (Chatani et al., 2019).

The effect of a small quantity of IL, as excipient, to promote the solubility of both drugs was evaluated in both biological systems. The dianionic ionic liquids were tested as a co-solvent, for ibuprofen, with the solubility results presented in Figure 4.

In IL aqueous or IL PBS solutions (0.2 mol% IL), the dianionic ionic liquids induced an increment in solubility for ibuprofen. In both biological systems, the $[N_{4\,1\,1\,2OH}]_2[C_4H_4O_4]$ promote the highest solubility. In IL aqueous solutions the increment is over 40-fold, while in the IL-PBS solution the increment is only 2-fold. PBS is already a co-solvent, so the effect in increment in solubility is not so pronounced when 0.2 mol% of IL is presented. Interestingly, the anion counterpart has a big effect in solubility, being the succinic acid with two methylene groups between the carboxylic groups, the one that give better results. T. Jeliński et al. (Jeliński and Cysewski, 2018) reported that the use of carboxylic acids with two carbonyl groups and a main chain consisting of two and three methylene groups give the best solubilities, for rutin oral drug. Elongation of the aliphatic chain to four methylene's between carbonyl groups (adipic acid) reduces the acidity of the dicarboxylic acid with decrement in solubility for ibuprofen, as can be seen in Figure 4.

The effect of the cation is small as can be observed for ILs with the same anion, $[C_5H_6O_4]^{2-}$ but with the different cations $[N_{4\,1\,1\,2OH}]^+$, $[N_{4\,1\,2OH\,2OH}]^+$ and $[N_{\,2O2\,1\,1\,2OH}]^+$. The modifications in cation part of IL, changing a methyl to hydroxyethyl group or a butyl group to an ether group (hydrophobic to a more hydrophilic group), don't affect the solubility for ibuprofen.

The solubility of ibuprofen increases with increasing concentration of IL, as can be seen for ILs $[N_{4\,1\,2OH\,2OH}]_2[C_5H_6O_4]$ and $[N_{4\,1\,1\,2OH}]_2[C_4H_4O_4]$ in Figure 5. However, the increment is not proportional to the quantity of IL added. In the case of $[N_{4\,1\,1\,2OH}]_2[C_4H_4O_4]$, when the quantity of IL aqueous solution raises from 0.2 mol% to 0.35 mol%, the increment in solubility changes from 40-fold to 50-fold and in $[N_{4\,1\,2OH\,2OH}]_2[C_5H_6O_4]$ increases from 28-fold to 35-fold. In PBS solutions the increment is not as pronounced, when the quantity of IL increased from 0.2 mol% to 0.35 mol%.

Ibuprofen is taken in 6-8 h intervals and the mean onset of action per 400 mg dose is 45 minutes, which can be critical in an acute pain situation where a fast relief is critical (Santos et al., 2019). So, we decided to decrease the time in assays to 5 minutes and the quantity of ionic liquid used, down to 0.02 mol%.

As can be seen in Figure 6, with only 0.02 mol% and 0.05 mol% of $[N_{4\,1\,1\,2OH}]_2[C_4H_4O_4]$, in aqueous solutions, it is possible to achieve a solubility of ≈600 mg/L and ≈1200 mg/L in just 5 min corresponding to one dose and maximum daily intake for an adult.

The effect in the IL aqueous solutions or IL phosphate buffer saline solutions in the solubility of piroxicam is represented in Figure 7.

For both 0.2 mol% of IL solutions, the dianionic ionic liquids $[N_{4\,1\,1\,2OH}]_2[C_5H_6O_4]$ and $[N_{4\,1\,1\,2OH}]_2[C_4H_4O_4]$ give the better results for piroxicam. The increment in aqueous solutions is around 20-fold and for phosphate buffer solutions is 1.5-fold, for the two compounds. For this drug, the changes in the cation and/or anion influenced the solubility. In the cation moiety, the replacement of a methyl group by a second hydroxyethyl group leads to a significant decrease in the solubility, which is even more pronounced when we replace a butyl group

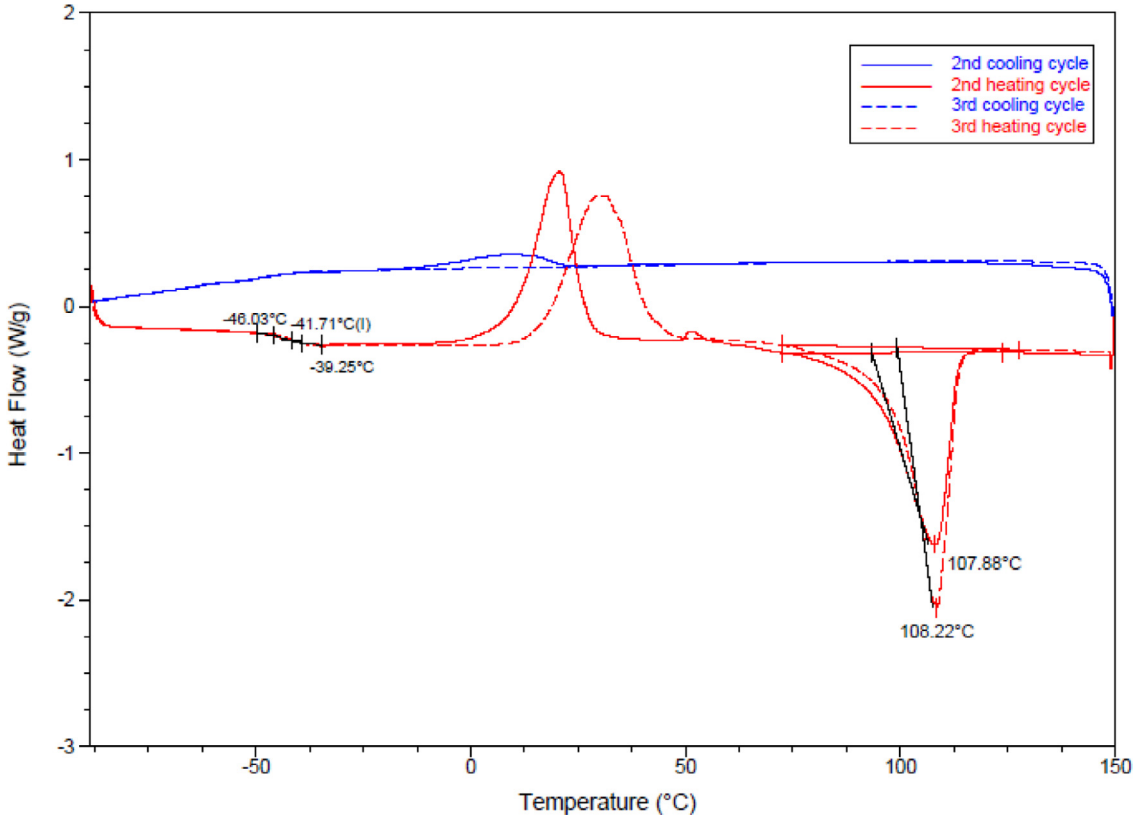

Fig. 3. DSC thermogram of $[N_{4\,1\,1\,2OH}]_2[C_4H_4O_4]$, N_2=50 mL/min, ramp 5°C/min.

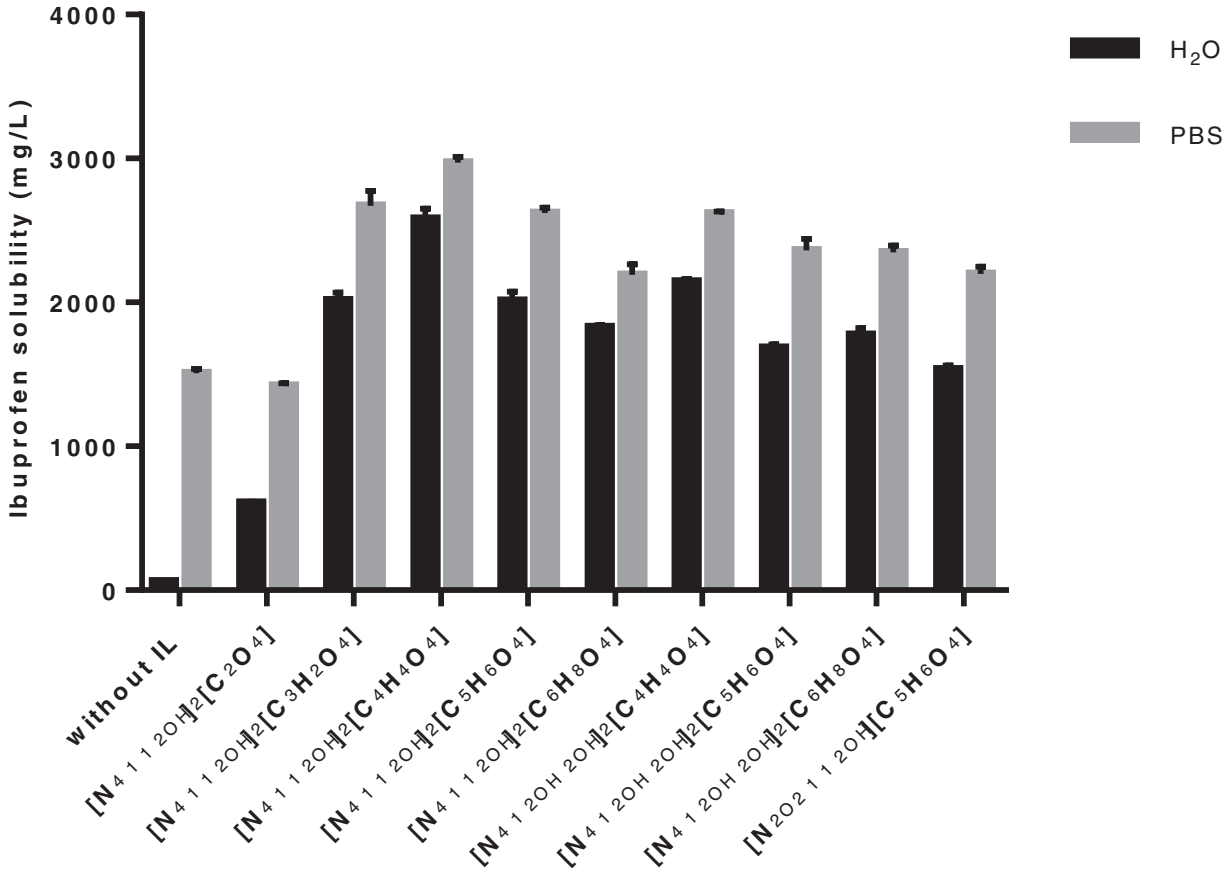

Fig. 4. Solubility of Ibuprofen in aqueous and PBS solutions with 0.2 mol% of dianionic ionic liquids. The results were expressed as the mean ± SD of the three independent experiments. All results demonstrated statistical significant differences with $p<0.0001$.

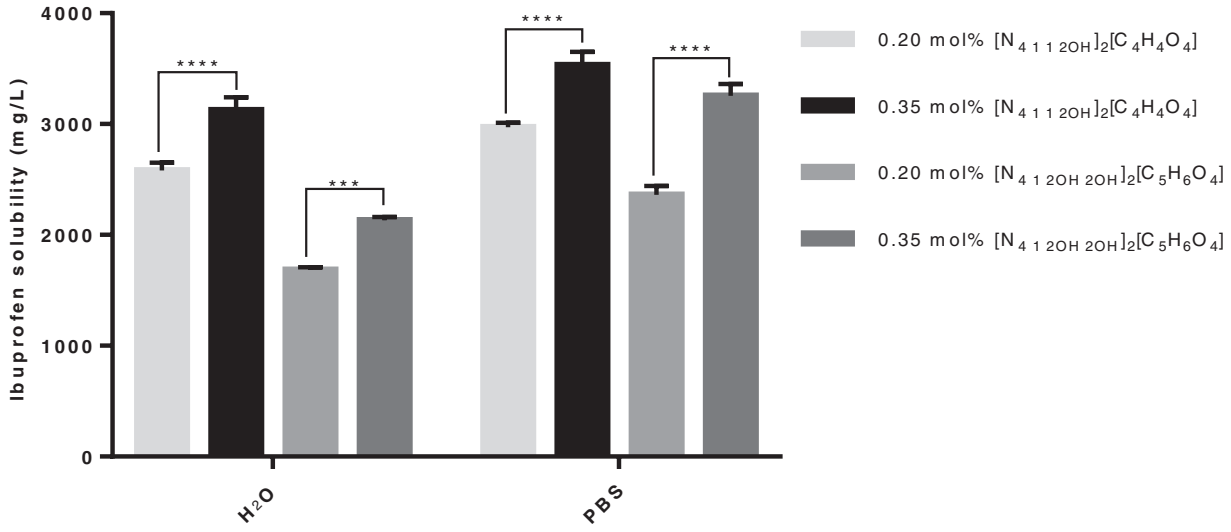

Fig. 5. Solubility of Ibuprofen in aqueous and PBS solutions with 0.2 mol% and 0.35 mol% of dianionic ionic liquids $[N_{4\,1\,1\,2OH}]_2[C_4H_4O_4]$ and $[N_{4\,1\,2OH\,2OH}]_2[C_5H_6O_4]$. The results were expressed as the mean ± SD of the three independent experiments. Statistical significant differences are represented in asterisks: **** p<0.0001 and *** p=0.0003.

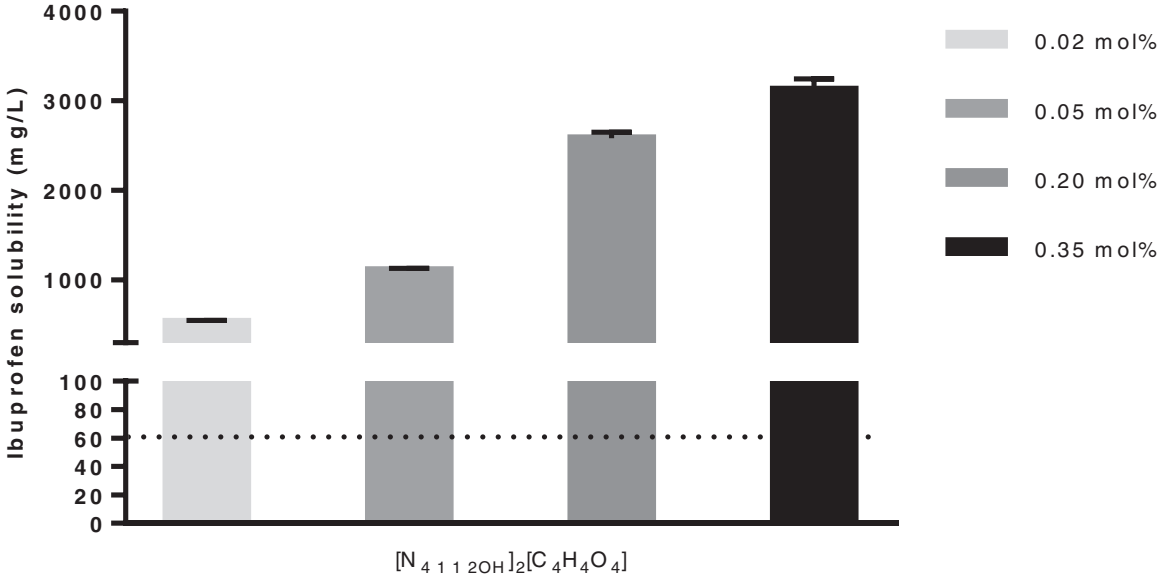

Fig. 6. Solubility of Ibuprofen in aqueous solutions with different mol percentages of [N4 1 1 2OH]2[C4H4O4] ionic liquid. The dashed line indicates Ibuprofen solubility in water and is use for comparison purposes. The results were expressed as the mean ± SD of the three independent experiments. Statistical significant differences are represented in asterisks: **** p<0.0001.

by an ether group, for ILs with the anion $[C_5H_6O_4]^{2-}$, in both solutions. Piroxicam is a weak acid due to proton donation from phenolic group attached to aromatic ring. The cation of these new dianionic ionic liquids are ammonium based, that are components with basic character that helps to promote direct heteromolecular contacts and enhance solubility. In the case of piroxicam that a hydroxyl group is connected directly to aromatic ring this effect is pronounced, which does not happen with ibuprofen. Is reported in publication (Sintra et al., 2021) that a replacement of a methyl group by a benzyl group in the cholinium cation is favourable for hydrotropy as it increases the size of the apolar moiety of the hydrotrope.

Comparing the results for ILs with the same cation $[N_{4\,1\,1\,2OH}]^+$ we can explore the effect of changes in the anion. In this case, the effect of increasing the methylene groups between the carboxylic groups from 0 to 3 had a positive effect in solubility of piroxicam in aqueous solutions. However, in PBS solutions, increasing the alkyl chain length between dicarboxylic acids, does not have an effect in solubility. When the alkyl chain in the anion increases to 4 methylene groups $[C_6H_8O_4]^{2-}$, there is a pronounced decrease in solubility in both biological fluids, for the reason explained for ibuprofen.

For piroxicam, an increase of IL concentration from 0.2 mol% to 0.35 mol%, increased the solubility as can be seen for $[N_{4\,1\,1\,2OH}]_2[C_4H_4O_4]$ in Figure S13. While, in aqueous solutions, the increase of IL from 0.2 to 0.35 mol%, duplicated the solubility from 17-fold to 33-fold, in PBS solution this effect was not observed.

3.4. Two dimension ^1H-^1H NOESY/ROESY

The intermolecular interaction between the drugs and ionic liquids was investigated by NMR, the two-dimensional $^1H/^1H$ nuclear overhauser effect spectroscopy (NOESY/ROESY). A sample of ibuprofen with 0.2 mol% of $[N_{4\,1\,1\,2OH}]_2[C_4H_4O_4]$, was prepared in dmso-$d_6$ to observe

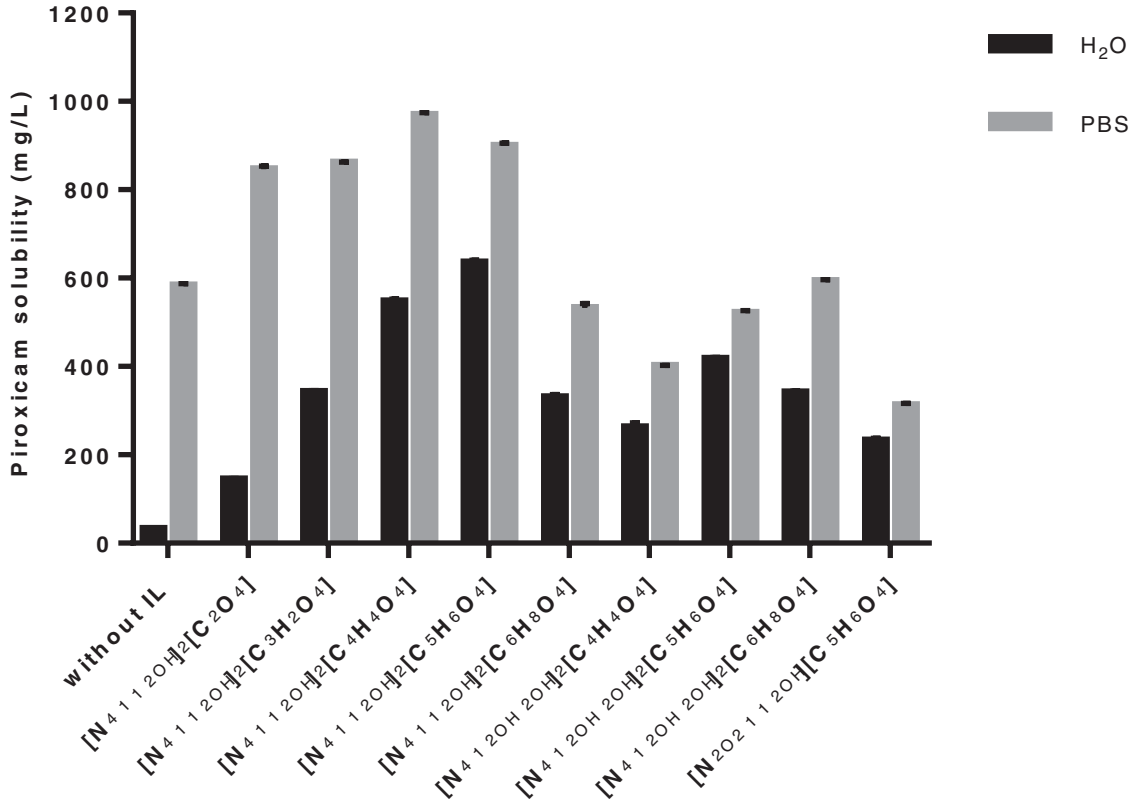

Fig. 7. Solubility of Piroxicam in aqueous and PBS solutions with 0.2 mol% of dianionic ionic liquids. The results were expressed as the mean ± SD of the three independent experiments. All results demonstrated statistical significant differences with $p<0.0001$.

the interaction between the two using a 2D ^{1}H-^{1}H NOESY experiment. The molar concentrations used mimic the conditions in the solubility assay. The 2D ^{1}H-^{1}H NOESY spectrum obtained is showed in Figure S15.

With a small 0.2 mol% of IL, there are no interactions between ibuprofen an IL, as can be seen in Figure S15.

Analogously, a ROESY experiment of a sample of piroxicam with 0.2 mol% of $[N_{4\,1\,1\,2OH}]_2[C_4H_4O_4]$ was performed in dmso-d_6. Also in this experiment, there are no interaction between piroxicam and IL, as can be observed in Figure 8.

3.5. Octanol-water partition coefficient and log P

Lipophilicity, commonly estimated by the partition coefficient (log P) can be a useful parameter to estimate the distribution profile of a drug within the body, which has a great influence in its ADME (absorption, distribution, metabolism and excretion) profile. In the case of an orally absorbed drug, it must permeate the lipid bilayers in the intestinal epithelium. Therefore, it is necessary a certain lipophilicity to partition into that bilayer but also require some hydrophilicity so that will not partition out again. According to literature these optimum log P values may vary between 0 and 3 (Pajouhesh and Lenz, 2005). According to literature, ibuprofen has a log P of 3.97 (Czyrski, 2019) and piroxicam has a log P of 3.06 (Shokri et al., 2012) meaning that are distributed to hydrophobic areas such as lipidic bilayers of cells.

The shake flask method was employed to determine the log P value of ibuprofen and piroxicam using $[N_{4\,1\,1\,2OH}]_2[C_4H_4O_4]$ as co-solvent, since it is the one that promoted the highest solubility.

Log P of ibuprofen and piroxicam in water were not determined by this method, since the separation of octanol-water phases was not possible, therefore the values reported in the literature were considered for comparison purposes, as already reported by some of us (Agostinho et al., 2021). It is important to highlight that in ionic liquid solutions we did not encounter the same problems described for the determination of log P of ibuprofen and piroxicam in water. The ability of both drugs to move to the aqueous phase seems to be therefore, highly affected by the presence of the excipient in solution. Log P for both drugs with only 0.2 mol% of $[N_{4\,1\,1\,2OH}]_2[C_4H_4O_4]$, can be seen in Table 2.

Table 2
Log P values for ibuprofen and piroxicam in aqueous and IL+water solutions.

System/Media	Log P
Ibuprofen/water	3.97 (Czyrski, 2019)
Piroxicam/water	3.06 (Shokri et al., 2012)
Ibuprofen/water + 0.2 mol% $[N_{4\,1\,1\,2OH}]_2[C_4H_4O_4]$	1.63 ± 0.14
Piroxicam/water + 0.2 mol% $[N_{4\,1\,1\,2OH}]_2[C_4H_4O_4]$	0.70 ± 0.06

In this study, the dianionic ionic liquids not only have an incredible increment in the solubility of both drugs in water but also meliorate its log P. In the presence of 0.2 mol% of IL both log P of ibuprofen and piroxicam have more hydrophilic values. As mentioned previously there must be a hydrophilicity/lipophilicity balance to achieve an ideal drug to be orally administered.

3.6. Permeability

The permeability in phosphate buffer solution PBS 0.01M pH = 7.4 of ibuprofen and piroxicam was studied along time in the presence and in the absence of IL. The concentration of the drug that permeated the membrane (mg/L) vs time is represented in Figure 9. Different parameters influence the diffusion, in particular, the affinity between membrane and the solute (He et al., 2004). In the presence of 0.2 mol% $[N_{4\,1\,1\,2OH}]_2[C_4H_4O_4]$, ibuprofen and piroxicam almost do not diffuse

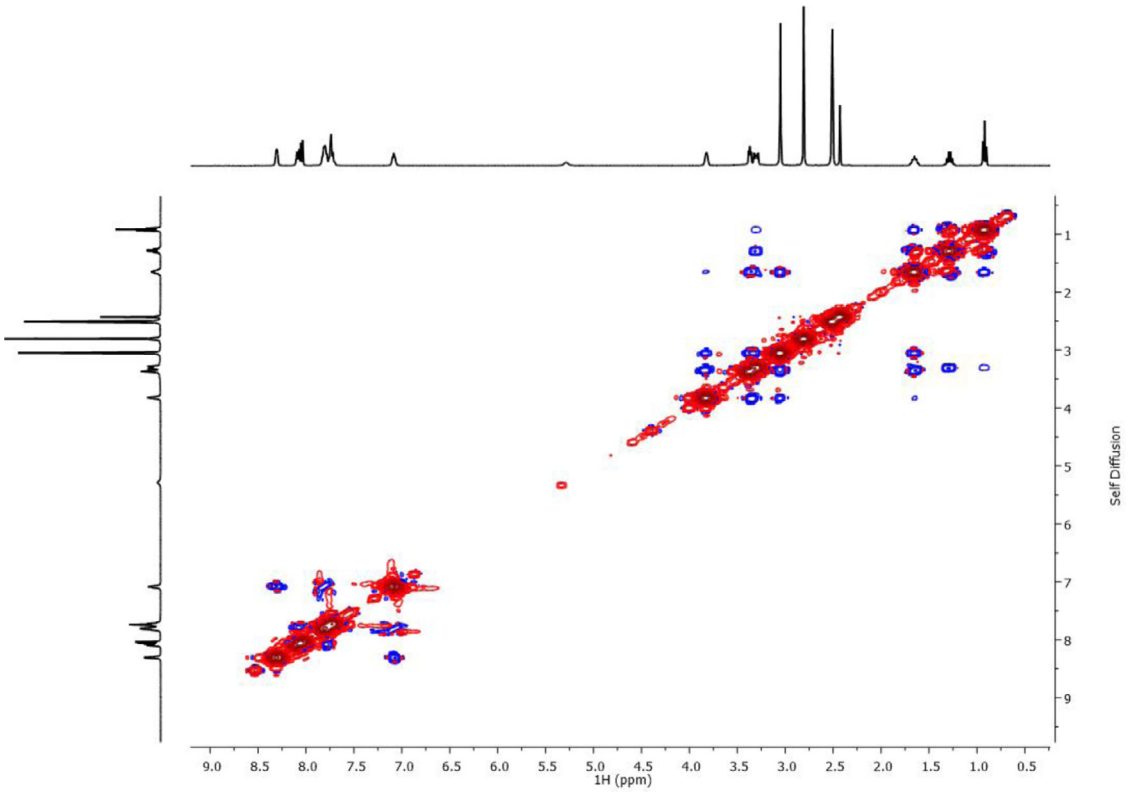

Fig. 8. 2D ^{1}H-^{1}H ROESY spectrum of piroxicam with 0.2 mol% of $[N_{4\,1\,1\,2OH}]_2[C_4H_4O_4]$ in dmso-d_6.

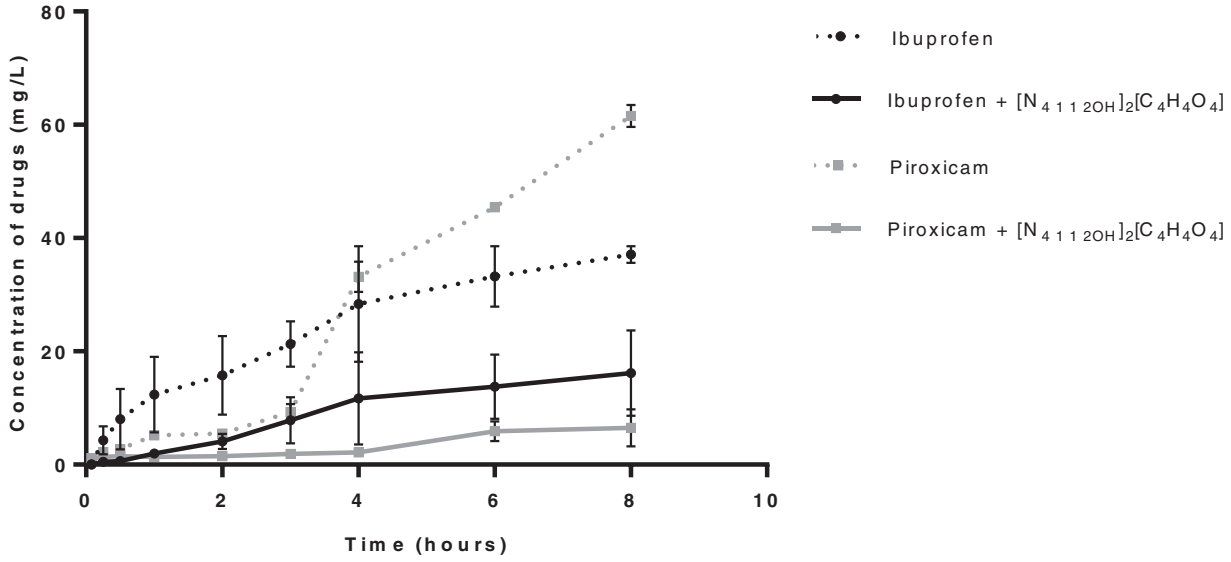

Fig. 9. Concentration of Ibuprofen and Piroxicam in PBS 0.01 M solution, in the presence or absence of $[N_{4\,1\,1\,2OH}]_2[C_4H_4O_4]$ along time, through PES-U membrane.

through the membrane polyethersulfone (PES-U). This effect is more pronounced for piroxicam.

The permeability of the oral drugs ibuprofen and piroxicam was determined in the presence or absence of IL in a concentration 0.2 mol% in PBS. The values of permeability, diffusion coefficient and partition coefficient of drugs are presented in Table 3.

The values of permeability and diffusion coefficient for ibuprofen in the presence of the IL decreased in comparison with absence of IL. In the case of piroxicam, in the presence of IL, the values were not calculated, because the quantity of drug that permeated through the membrane within the 8 h of assay, was negligible. Piroxicam is an API with a molecular structure bigger than ibuprofen and when interacts with $[N_{4\,1\,1\,2OH}]_2[C_4H_4O_4]$, becomes difficult to diffuse through PES-U. In the future, other membranes should be tested.

3.7. Cytotoxicity evaluation

For the cytotoxicity study, we have selected the six dianionic ILs, $[N_{4\,1\,1\,2OH}]_2[C_3H_2O_4]$, $[N_{4\,1\,1\,2OH}]_2[C_4H_4O_4]$, $[N_{4\,1\,1\,2OH}]_2[C_5H_6O_4]$, $[N_{4\,1\,1\,2OH}]_2[C_6H_8O_4]$, $[N_{4\,1\,2OH\,2OH}]_2[C_4H_4O_4]$ and $[N_{2O2\,1\,1\,2OH}]_2[C_5H_6O_4]$.

Table 3
Permeability, diffusion and partition coefficients obtained for oral drugs with 0.05% ILs.

	Permeability, P (10^{-5} cm/s)	Diffusion coefficient, D (10^{-6} cm^2/s)	Partition coefficient, Kd (10^{-1})
Ibuprofen	1.21 ± 0.30	1.78 ± 0.58	1.01 ± 0.11
Ibuprofen + $[N_{4\,1\,1\,2OH}]_2[C_4H_4O_4]$	0.21 ± 0.05	1.44 ± 0.11	0.21 ± 0.06
Piroxicam	1.56 ± 0.25	0.30 ± 0.02	7.91 ± 0.65
Piroxicam + $[N_{4\,1\,1\,2OH}]_2[C_4H_4O_4]$	-	-	-

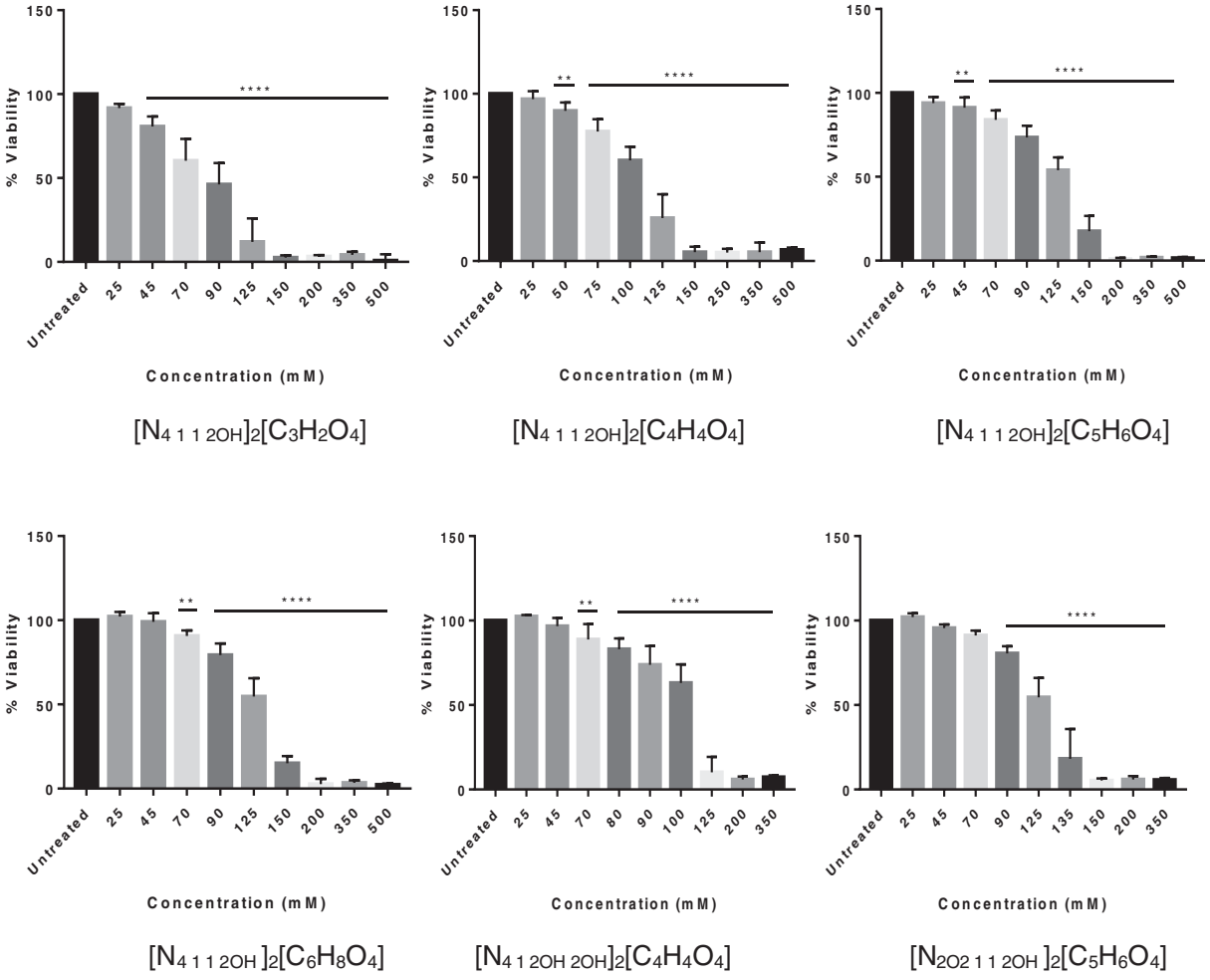

Fig. 10. Cytotoxicity of the ionic liquids in fibroblasts L929 cells to different concentrations of the respective ILs. The results are expressed as the average ± SD from three independent biological assays. The symbols ** and **** indicates that the viabilities are statistically significant with p = 0.0076 and p = 0.0001, respectively, when compared to the control.

Table 4
IC_{50} determination of dianionic ionic liquids.

Ionic Liquid	IC_{50} (mM)
$[N_{4\,1\,1\,2OH}]_2[C_3H_2O_4]$	78.1 ± 6.6
$[N_{4\,1\,1\,2OH}]_2[C_4H_4O_4]$	104.5 ± 5.8
$[N_{4\,1\,1\,2OH}]_2[C_5H_6O_4]$	116.0 ± 7.1
$[N_{4\,1\,1\,2OH}]_2[C_6H_8O_4]$	120.7 ± 5.2
$[N_{4\,1\,2OH\,2OH}]_2[C_4H_4O_4]$	102.8 ± 3.8
$[N_{2O2\,1\,1\,2OH}]_2[C_5H_6O_4]$	117.1 ± 11.4

The results of the IL cytotoxicity performed on the L929 cell line are presented at Figure 10 and Table 4. The results showed that in ionic liquids with the same cation $[N_{4\,1\,1\,2OH}]^+$, cytotoxicity decreases as the alkyl chain between the anion's carboxylic groups increases. On the other hand, in ILs with the same anion ($[C_4H_4O_4]^{2-}$ or $[C_5H_6O_4]^{2-}$) changes in the chemical structure of the cation do not influence the cytotoxicity of ILs. Therefore, it is the chemical structure of the anion that has been shown to influence the cytotoxicity of ILs. With exception of $[N_{4\,1\,1\,2OH}]_2[C_3H_2O_4]$, all dianionic ionic liquids have an IC_{50} higher than 100 mM.

4. Conclusion

New dianionic ionic liquids were synthesized and characterized in this work. They showed the ability to improve the solubility of two model poorly soluble oral drugs, ibuprofen and piroxicam in both aqueous and phosphate buffer solutions 0.01 M. Except for IL $[N_{4\,1\,1\,2OH}]_2[C_3H_2O_4]$, all new ILs have an IC_{50} higher than 100 mM (for 0.2 mol% IL) for fibroblasts L929 cells. For $[N_{4\,1\,1\,OH}]_2[C_4H_4O_4]$ in ibuprofen and for $[N_{4\,1\,1\,OH}]_2[C_4H_4O_4]$ and $[N_{4\,1\,1\,OH}]_2[C_5H_6O_4]$ in piroxicam, with a concentration of 0.2 mol% IL, it is possible to achieve an increase of 40-fold and 20-fold, respectively, in aqueous solutions.

In PBS, at the same IL concentration, it is possible an increase of 2-fold and 1.5-fold for ibuprofen and piroxicam.

In studies at 37 °C, 5 min dissolution time and 0.02 mol% of $[N_{4\,1\,1\,2OH}]_2[C_4H_4O_4]$, a solubility of ≈600 mg/L was obtained, which corresponds to one dose for an adult.

With 0.2 mol% of $[N_{4\,1\,1\,OH}]_2[C_4H_4O_4]$ it was possible to decrease the lipophilicity, log P, of both drugs which is important to enhance the pharmacodynamic and toxicological profile of both drugs.

Curiously, the dianionic ionic liquid $[N_{4\,1\,1\,2OH}]_2[C_6H_8O_4]$, is the less toxic IL having an IC_{50} of 121 mM. In these new dianionic ILs with biocompatible dicarboxylic acids, the increment in alkyl chain do not increase the toxic, as normally happens in monoanionic ionic liquids (Jesus et al., 2019). However, with bigger alkyl chains in anion, the solubility of both oral drugs decreases.

Thus, these new biocompatible ionic liquids are promising vehicles for aqueous administration of poorly water-soluble drugs.

Declaration of Competing Interest

None

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jil.2021.100003.

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