Journal of Pharmaceutical Sciences 110 (2021) 2489-2500

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Pharmaceutics, Drug Delivery and Pharmaceutical Technology

Improvement of New Dianionic Ionic Liquids *vs* Monoanionic in Solubility of Poorly Water-Soluble Drugs

Daniela A.S. Agostinho, Ana R. Jesus, Ana B.P. Silva, José M.S.S. Esperança, Alexandre Paiva, Ana R.C. Duarte**, Patrícia M. Reis*

LAQV, Requimte, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Caparica, 2829-516, Portugal

ARTICLE INFO

Article history:
Received 3 December 2020
Accepted 12 January 2021
Available online 21 January 2021

Keywords:
Ionic liquids
Dianionic ionic liquids
Cytotoxicity
Drug delivery
Bioavailability

ABSTRACT

New ionic liquids (ILs) based on dianionic phosphonate anions and ammonium cations were prepared and characterized. They were used as excipients to increase the water solubility of two oral drugs, piroxicam and ibuprofen, that are slightly soluble in water. An increment in solubility of 300-fold was achieved for ibuprofen when compared with pure water, with only 0.25 mol% of IL in water. Interestingly, this was achieved with the less toxic dianionic ionic liquid $[N_{4\ 1\ 2OH\ 2OH}]_2\ [C_2H_5PO_3]$, which presents an IC_{50} of 120 mM (≈0.25 mol%). On the other hand, piroxicam showed an increase of 480-fold for the same dianionic ionic liquid, with the same ionic liquid percentage. In contrast, for monoanionic ionic liquids, the effect was not so pronounced, and only a 10-fold was obtained, in the presence of 0.3 mol% of IL. The lipophilicity (logP) of drugs decreased in the presence of these ILs. Cytotoxicity profile of these ILs was determined and they did not show a significant impact towards healthy fibroblasts. The cytotoxicity of ibuprofen and piroxicam was also determined, and cellular viability almost did not change when ionic liquid was in the presence of 1 mM of oral drug.

Introduction

Ease of administration, cost-effectiveness, and flexibility in design of the dosage form are some reasons why oral drug delivery is the most advantageous and used form for drug delivery.[1] The oral drug bioavailability depends on several aspects, such as aqueous solubility and drug permeability. Oral drugs that are poorly water soluble (less than 100 mg/L[2]), show a limited dissolution rate and often low bioavailability, thus high doses are necessary to achieve therapeutic plasma concentrations after oral administration, but on its turn, it can lead to toxicity in the gastrointestinal mucosal.[3]

The low bioavailability and high lipophilicity of oral drugs in combination with high-dose requirements is a problem that pharmacy industry still has to solve.[4]

In the last few years, ionic liquids (ILs) have increasingly being exploited as solvents, co-solvents and active pharmaceutical ingredients because of their unique and tunable physicochemical properties.[5] The use of ILs can markedly improve the pharmacokinetic and pharmacodynamics properties of drugs. A number of strategies to improve the oral delivery of many drugs include prodrugs, salt formation, crystal engineering, solid dispersions, micellar systems and the use of excipients.[3] The common organic solvent excipients that are used to enhance the poor solubility of drugs are pyridine, *N*,*N*-dimethylformamide (DMF) and dimethyl sulfoxide (DMSO). These solvents are volatile, flammable and toxic, so ionic liquids are potential green alternatives for this purpose.[6] ILs are organic salts that are typically liquid below 100 °C. Some ILs are classified as room temperature ionic liquids (RTILs) that exist in liquid state at ambient temperature.[7] ILs possess numerous unique and useful physico-chemical properties including low vapor pressure, good dissolution capabilities, wide liquid range and good thermal stability.[8] As designer solvents, ILs properties could be manageable, simply by selecting the appropriate cation/anion combinations, by changing the side chain length of ions or by incorporating distinct functionalities in the cation.[9] The assessment of cytotoxicity is a mandatory preliminary test before application of ILs in either medical or pharmaceutical applications.[5,10] Cholinium-based ILs are some of the most biodegradable, less toxic, easily accessible and cost-effective ILs, when compared with other ILs such as pyridinium and imidazolium-based ILs.[11–13]

* Corresponding author.
** Corresponding author.
E-mail addresses: ard08968@fct.unl.pt (A.R.C. Duarte), preis@fct.unl.pt (P.M. Reis).

https://doi.org/10.1016/j.xphs.2021.01.014
0022-3549/

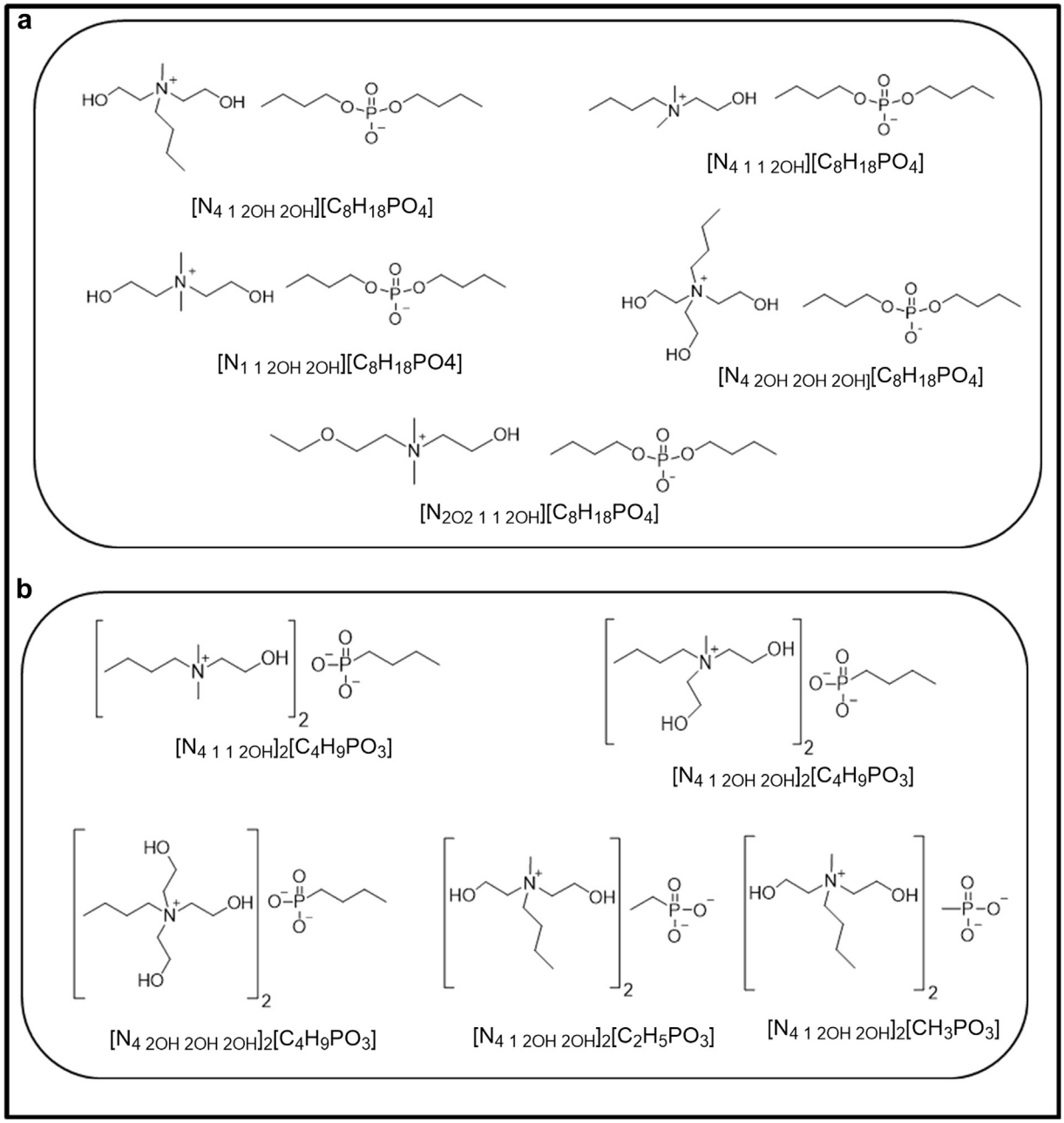


Fig. 1. Chemical structure of ILs used: a – monoanionic and b – dianionic ionic liquids.

We have previously shown that *N*-acetyl amino acid *N*-alkyl cholinium-based ILs were able to significantly increase the water solubility of two commercial drugs, paracetamol and sodium diclofenac, with no significant effect on normal human cells viability.[14] Our group also synthesized new biocompatible *N*-alkyl cholinium *N*-alkyl sulfonate ionic liquids, that were used to enhance the aqueous solubility of the same drugs.[15] In addition, four new dianionic ionic liquids, containing a succinyl-DL-alaninate anion and *N*-alkyl cholinium cation, were also prepared.

The aim of this work is to test the use of dianionic ILs as excipients, for the dissolution of low water soluble drugs, such as piroxicam and ibuprofen. Excipients are very important because of their ability to transport the active drug to the site in the body where the drug is intended to exert its action.[16,17] They also keep the drug from being released too early in the assimilation process. Therefore, we have prepared 10 new biocompatible ionic liquids, 5 monoanionic and 5 dianionic ionic liquids. The cation is based in the well-known ammonium cation, functionalized with different number of hydroxyethyl groups, and ether moietie and/or different alkyl chains, and the anion is based on phosphate and phosphonate groups, as presented in Fig. 1.

These new ILs were tested as excipients for two oral drugs, ibuprofen and piroxicam, which structure is presented in Fig. 2.

The application of this new ammonium ionic liquids as green excipients was evaluated, and their influence on the solubility of two poorly water soluble nonsteroidal anti-inflammatory drugs (NSAID), ibuprofen and piroxicam is reported herein. These drugs have analgesics and antipyretics properties, due to the inhibition of the cyclooxygenase enzyme, and are usually used in the treatment of headache, dysmenorrhea, dental pain, post-operative, rheumatoid arthritis, spondylitis, gouty arthritis and osteoarthritis.[18–22]

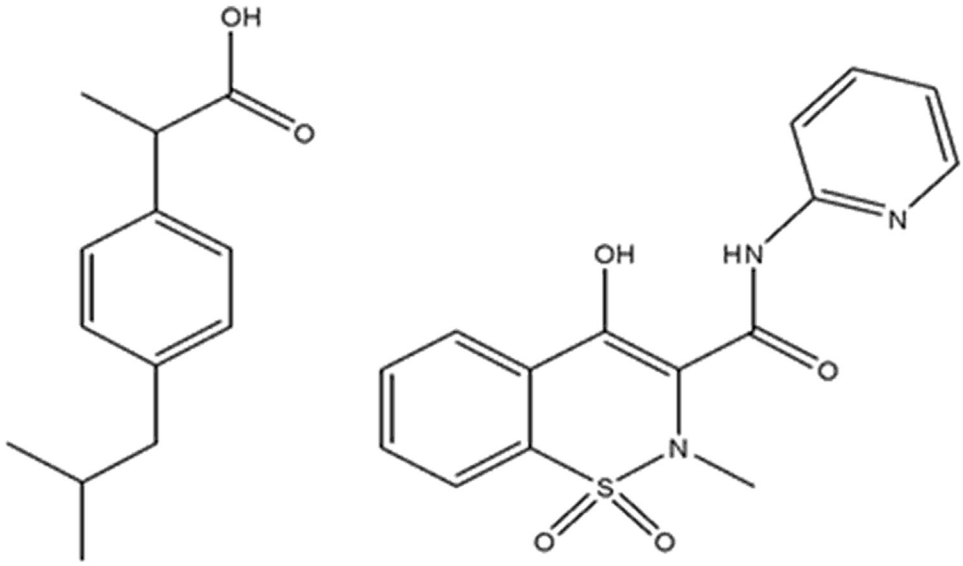


Fig. 2. Chemical structure of Ibuprofen (left) and Piroxicam (right).

Materials and Methods

Materials

N-Methyldiethanolamine (≥98.0%), 1-butylphosphonic acid (98.0%), ethylphosphonic acid (≥98.0%), methylphosphonic acid (98.0%), 1-octanol (99.0%), Amberlite™ IRN-78, 4-isobutyl-α-methylphenylacetic acid (Ibuprofen) (99.0%), piroxicam (97.0% min.) and D-lactose (98.0% min.) were purchased from Alfa Aesar. 1-chlorobutane (≥99.0%), 2-chloroethanol (99.0%), *N*-butyldiethanolamine (≥98.6%), dibutyl phosphate (≥97.0%), 1-bromobutane (≥98.0%), 2-dimethylaminoethanol (≥99.5%), 2-bromoethyl ethyl ether (90.0%) and sodium bicarbonate (≥99.7%) were acquired from Sigma-Aldrich. Diethyl ether (≥99.8%) and acetone (≥99.5%) were obtained from Honeywell – Riedel-de-Haën. Ethanol absolute anhydrous, deuterium oxide (99.9%) and hexane were purchased from Carlo Erba Reagents, Eurisotop and Valente e Ribeiro Lda., respectively.

General Procedure for the Synthesis of [$N_{4\ 1\ 2OH\ 2OH}$]Cl and [$N_{4\ 2OH\ 2OH\ 2OH}$]Cl

Methyldiethanolamine or butyldiethanolamine (100 mmol) was mixed with the alkylating agent, chlorobutane or 2-chloroethanol respectively (4.0 equiv.), and *n*-hexane in a 100 mL pressure reaction vessel. The mixture was kept at 80–90 °C for 4 days. The chloride ionic liquids were thoroughly washed with diethyl ether to remove the unreacted alkylating agent. Ionic liquids were then dried in the vacuum for 1–2 days, and their purity confirmed by ^{1}H NMR. These ILs were obtained in 95–99% yield.

General Procedure for the Synthesis of [$N_{4\ 1\ 1\ 2OH}$]Br, [$N_{2O2\ 1\ 1\ 2OH}$]Br and [$N_{1\ 1\ 2OH\ 2OH}$]Cl

2-Dimethylaminoethanol (100 mmol) was mixed with the alkylating agent, 1-bromobutane, 2-bromo ethyl ether or 2-chloroethanol respectively (1.1 equiv.), and *n*-hexane in a 100 mL pressure reaction vessel. The mixture was kept at 80–90 °C for 24 h. The ionic liquids were thoroughly washed with diethyl ether to remove the unreacted alkylating agent. Ionic liquids were then dried in the vacuum for 1–2 days, and their purity confirmed by ^{1}H NMR. These ILs were obtained in 95–99% yield.

General Procedure for the Metathesis Reaction

An aqueous solution of *N*-alkyl derivative cholinium bromide or chloride (20 mmol) was slowly passed through an anion exchange column Amberlite™ IRN-78. Then, the corresponding hydroxide solution was slowly added to a solution of dibutyl phosphate (1.0 equiv.) diluted in acetone or the corresponding aqueous alkylphosphonate 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 diethyl ether, obtained in quantitative yield, and dried in high vacuum for 2 days at 50–60 °C to guarantee minimum water content. Coulometric Karl-Fischer titrations yielded final water contents below 1000–1500 ppm depending on the IL. Moreover, $AgNO_3$ test was used to confirm the absence of halogen presence in the final IL.

Solubility Assays

A calibration curve was prepared for each drug, in water, 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 S1. Absorbance values were kept below 1. The statistical analysis was performed by Graphpad Prism 7.

General Procedure for the Determination of Solubility Limit of Ibuprofen and Piroxicam

Aqueous solutions (2 mL) of ionic liquids were prepared. Ibuprofen or piroxicam was added to each sample guaranteeing that we are above the solubility limit. In the case of ibuprofen, we used an aqueous solution of IL at the same concentration as our blank sample, because these ILs absorb in the region 190–230 nm. All solutions were stirred for 24 h at 37 °C until equilibrium was reached. Keeping the solutions in a 37 °C thermostatized water bath, the non-dissolved drugs were filtered off through a 0.22 μm syringe filter. Samples of each filtered solution were diluted in MilliQ® water, until the absorbance was smaller than 1. The drug

concentration was analysed in a UV−Vis VWR® spectrophotometer, model UV-6300PC. Triplicates were prepared for each sample and maximum absorbance was measured at 222 nm and 353 nm to ibuprofen and piroxicam, respectively. Solubility of drugs in the presence of each ionic liquid was calculated based on the calibration curve. The pH was checked during assay and no variation was observed.

General Procedure for the 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 literature[23] and also in our previous work.[14] Briefly, an octanol-saturated aqueous solution was used to prepare the ionic liquid solutions at 0.05 mol%. Known amounts of drug were dissolved in 1 g of IL solution. Afterwards, we mixed it with 1 g of a water-saturated octanol solution. The solutions were stirred vigorously for 18−24 h and then sat for some hours until full separation of organic and 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 S2.

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.

K_{ow} and log P of the three samples were determined using Equations (1) and (2), and the mean value was taken.

$$K_{ow} = [drug]_{oct} / [drug]_{aq} \quad (1)$$

$$\log P = \log K_{ow} \quad (2)$$

Permeability

Permeability measurements 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.[24,25]

The membrane used was a polyethersulphone (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 MilliQ® water and donor compartment (2 mL) loaded with a IL solution (0.05 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 MilliQ® water 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.

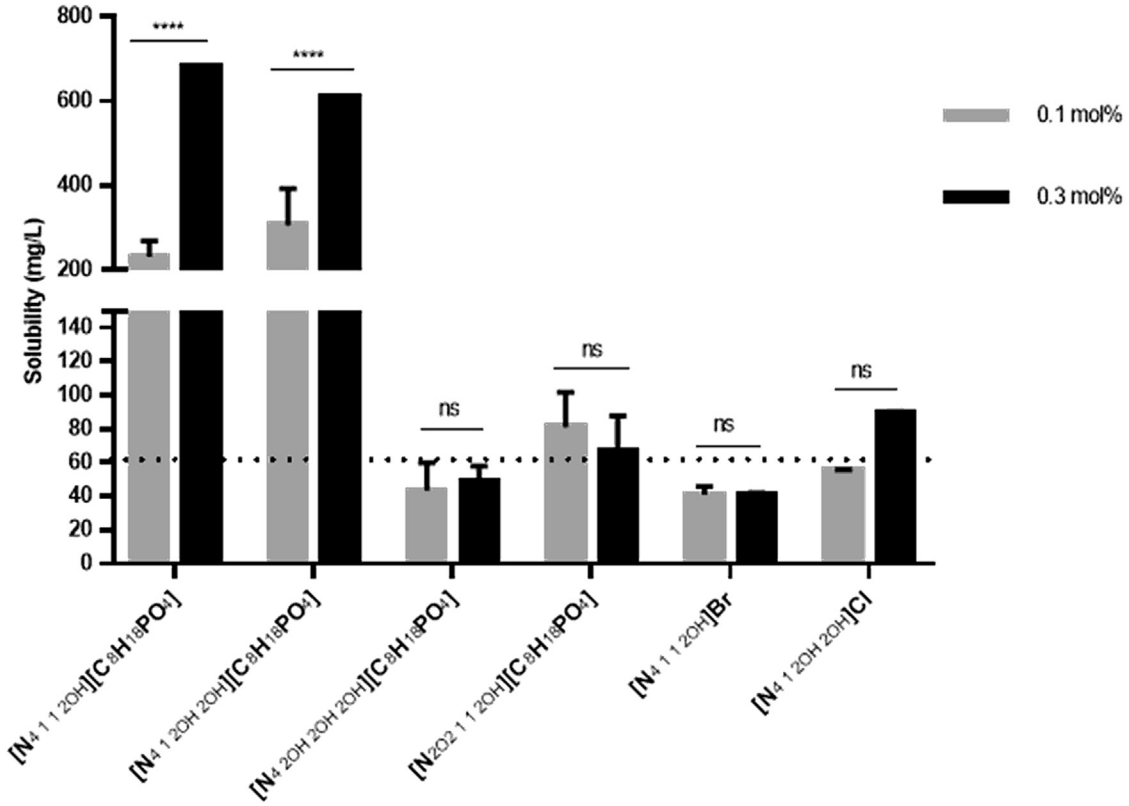


Fig. 3. Solubility of Ibuprofen in aqueous solutions with different mol percentages of monoanionic ionic liquids. The dashed line represents the solubility limit of ibuprofen in water. The results were expressed as the mean ± SD of the three independent experiments. Significantly statistical differences are represented in asterisks: ****$p < 0.0001$ and ns - non-significant (as compared to values of the same IL with different percentages).

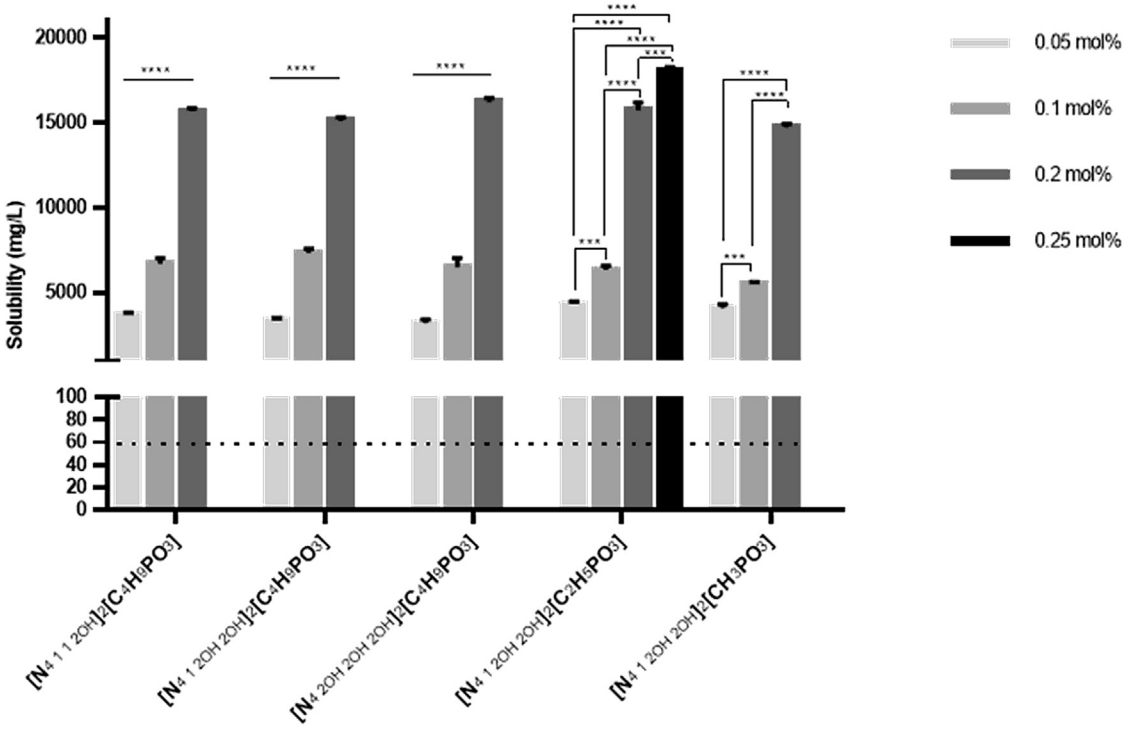


Fig. 4. Solubility of Ibuprofen in aqueous solutions with different mol percentages of dianionic ionic liquids. The dashed line indicates ibuprofen solubility in water and is use for comparison purposes. The results were expressed as the mean ± SD of the three independent experiments. Statistical significant differences are represented in asterisks: ****$p < 0.0001$, ***$p = 0.0001$ or $p = 0.0002$ ($[N_{4\ 1\ 2OH\ 2OH}]_2$ $[C_2H_5PO_3]$ and $[N_{4\ 1\ 2OH\ 2OH}]_2$ $[CH_3PO_3]$, respectively).

The permeability, P (cm s^{-1}) was calculated according to Equation (3):

$$-\ln(1-2\frac{Ct}{C0}) = \frac{2A}{V} \times P \times t \quad (3)$$

where C_t is the concentration in the receptor compartment at time t, C_0 is the initial concentration in the donor compartment, A is the effective mass transfer area, V is the total volume of solution in both compartments.

According to a derived solution of Fick's law of diffusion it is possible to determine the diffusion coefficient, D (cm^2 s^{-1}) of the ibuprofen and piroxicam in the presence of ILs, through the membrane, as can be seen in Equation (4):

$$D = \frac{V1 \times V2}{V1 + V2} \times \frac{h}{A} \times \frac{1}{t} ln\left(\frac{Cf - Ci}{Cf - Ct}\right) \quad (4)$$

where C_f and C_i are the final and initial concentration in the receptor compartment and C_t is the concentration in the receptor compartment at time t. V_1 and V_2 are the volume in the donor and in the receptor compartment respectively and h is the thickness of the membrane.

The partition coefficient, K_d is defined as measure of the solubility of the solute in the membrane, and is calculated as follow in Equation (5):

$$Kd = \frac{P \times h}{D} \quad (5)$$

where P is permeability, h is thickness of the membrane and D is the diffusion coefficient.

Cytotoxicity Evaluation

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

Viability Assays

Fibroblasts were incubated for 24 h 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 250 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. Graphpad Prism 7 was used for statistical analysis. Data is expressed as average ± standard deviation from at least three independent experiments.

Results and Discussion

Solubility Assays

In this work, the solubility of ibuprofen and piroxicam, two nonsteroidal anti-inflammatory drugs (NSAID) typically orally administered, and which present a low solubility in aqueous media,

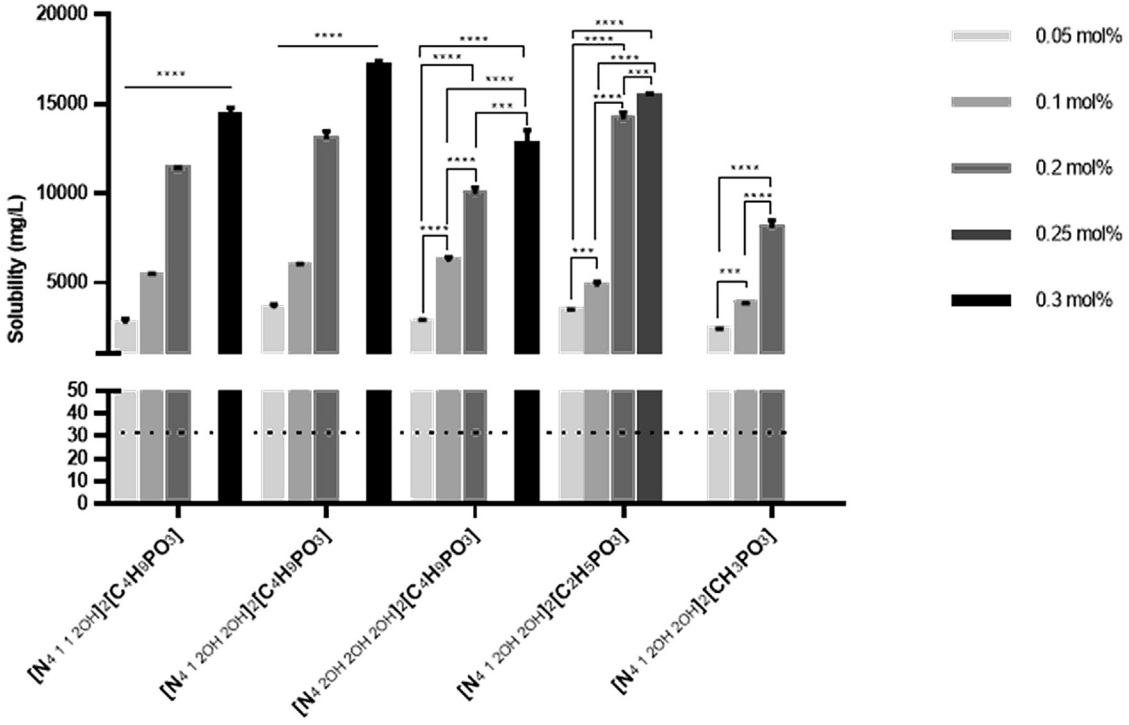


Fig. 5. Solubility of Piroxicam in aqueous solutions with different mol percentages of dianionic ionic liquids. The dashed line indicates buprofen solubility in water and is use for comparison purposes. The results were expressed as the mean ± SD of the three independent experiments. Statistical significant differences are represented in asterisks: ****$p < 0.0001$, ***$p = 0.0003$ ($[N_{4\ 2OH\ 2OH\ 2OH}]_2\ [C_4H_9PO_3]$), $p = 0.0008$ or $p = 0.0009$ ($[N_{4\ 1\ 2OH\ 2OH}]_2\ [C_2H_5PO_3]$ 0.05%–0.1% and 0.2%–0.25%, respectively) and $p = 0.0010$ $[N_{4\ 1\ 2OH\ 2OH}]_2\ [CH_3PO_3]$).

was determined. The assays were performed at 37 °C to mimic body temperature. At this temperature, their solubility was 60 mg/L and 32 mg/L, respectively, which is consistent with literature data.[26]

The effect of a small quantity of IL, as excipient, to promote the solubility of both drugs was evaluated. New monoanionic ionic liquids were tested as excipients, for ibuprofen, with promising results as presented in Fig. 3. Even at 0.3 mol% of $[N_{4\ 1\ 1\ 2OH}][C_8H_{18}PO_4]$ and $[N_{4\ 1\ 2OH\ 2OH}][C_8H_{18}PO_4]$, it was possible to achieve an increment of 10-fold in the solubility of ibuprofen when compared to its value in pure water.

In contrast, dianionic ionic liquids induced a greater increment in solubility compared to monoanionic ionic liquids. The solubility

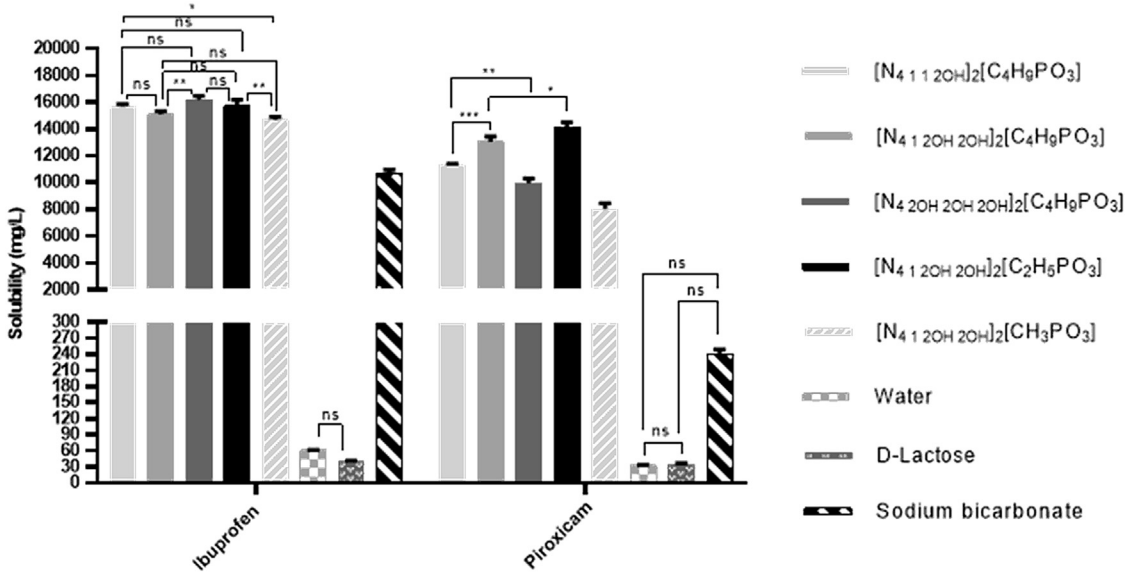


Figure-6. Solubility of Ibuprofen and Piroxicam with dianionic ILs and two commonly used excipients (0.2 mol%) compared to solubility of two drugs in water. The results were expressed as the mean ± SD of the three independent experiments. Statistical significant differences are represented in asterisks: ****$p < 0.0001$, Ibuprofen: **$p = 0.0013$ or $p = 0.0085$ ($[N_{4\ 1\ 2OH\ 2OH}]_2\ [C_4H_9PO_3]$ - $[N_{4\ 2OH\ 2OH\ 2OH}]_2\ [C_4H_9PO_3]$ or $[N_{4\ 1\ 2OH\ 2OH}]_2\ [C_2H_5PO_3]$ - $[N_{4\ 1\ 2OH\ 2OH}]_2\ [CH_3PO_3]$, respectively) and *$p = 0.0119$ ($[N_{4\ 1\ 1\ 2OH}]_2\ [C_4H_9PO_3]$ - $[N_{4\ 1\ 2OH\ 2OH}]_2\ [CH_3PO_3]$); Piroxicam: ** *$p = 0.0005$ ($[N_{4\ 1\ 1\ 2OH}]_2\ [C_4H_9PO_3]$ - $[N_{4\ 1\ 2OH\ 2OH}]_2\ [C_4H_9PO_3]$), **$p = 0.0032$ ($[N_{4\ 1\ 1\ 2OH}]_2\ [C_4H_9PO_3]$ - $[N_{4\ 2OH\ 2OH\ 2OH}]_2\ [C_4H_9PO_3]$) and *$p = 0.0102$ ($[N_{4\ 1\ 2OH\ 2OH}]_2\ [C_4H_9PO_3]$ - $[N_{4\ 1\ 2OH\ 2OH}]_2\ [C_2H_5PO_3]$, and ns - non-significant (as compared to values of the same IL with different percentages).

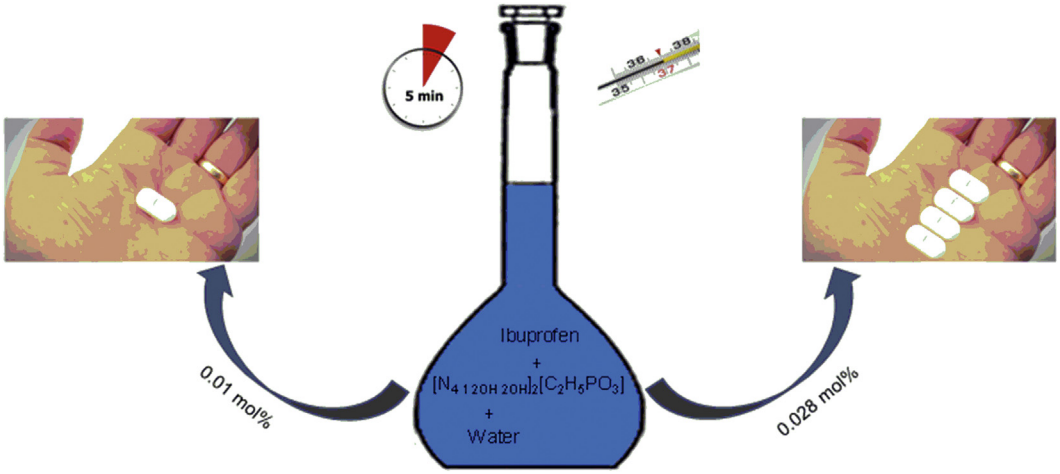
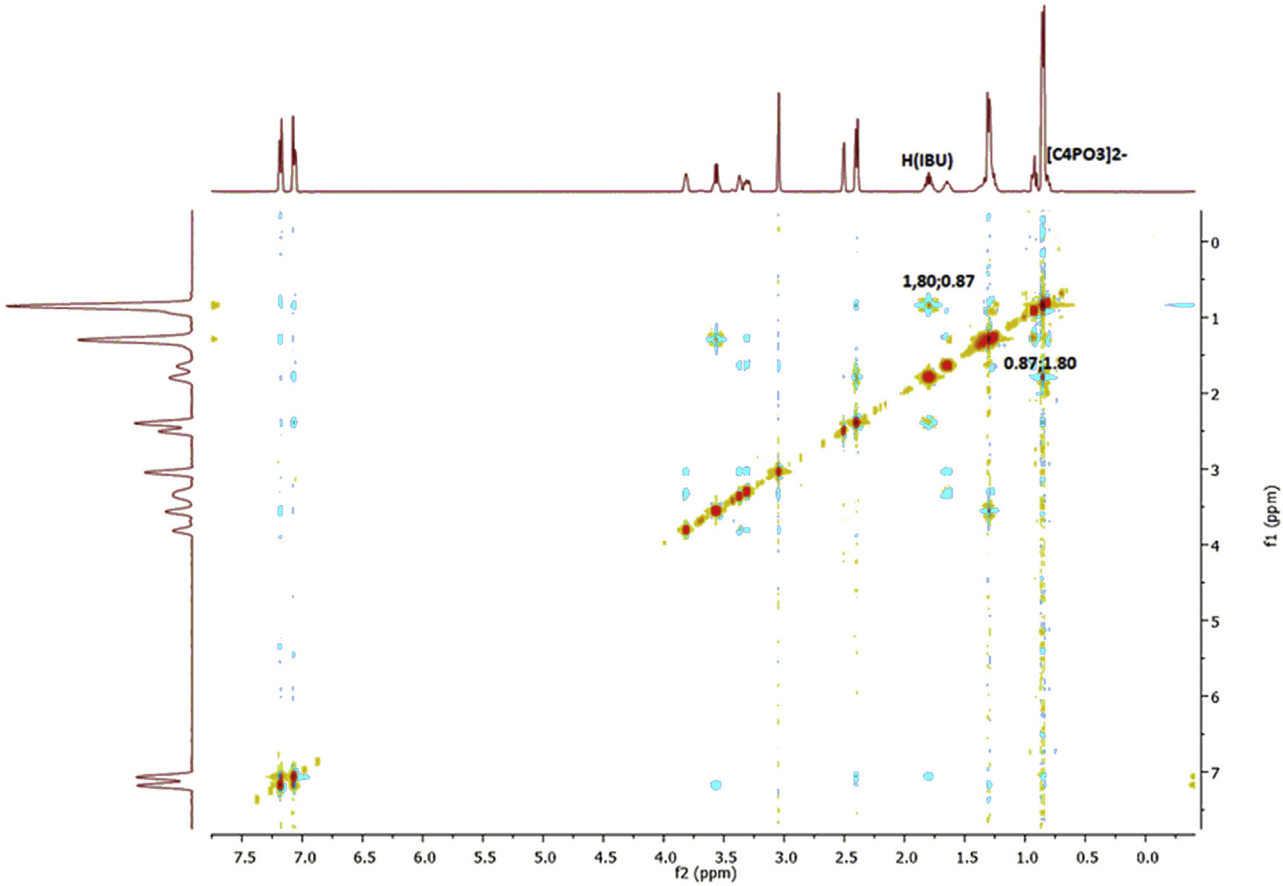


Fig. 7. Solubility of ibuprofen with ionic liquid $[N_{4\ 1\ 2OH\ 2OH}]_2\ [C_2H_5PO_3]$ at 0.01 and 0.028 mol% at 37 °C during 5 min.

results obtained for ibuprofen in aqueous solutions of dianionic ILs are presented in Fig. 4. Using ionic liquids with the same anion, $[C_4H_9PO_3]^{2-}$, it is possible to see that the insertion of additional hydroxyethyl groups (-CH_2CH_2OH) in the cation moiety, does not increase the solubility of ibuprofen. The same behaviour was observed when the alkyl chain of the anion was changed, from C_4 to C_1, using ILs with the same cation $[N_{4\ 1\ 2OH\ 2OH}]^+$.

The solubility of ibuprofen increases with increasing concentration of IL. For all ILs tested, there is an increase in solubility of more than 3 times when the amount of IL rise from 0.05 mol% to 0.2 mol%. The solubility enhancement in IL aqueous solutions (0.2 mol%) is over 250-fold when comparing with that in pure water.

With the monoanionic ionic liquids, the effect in the aqueous solubility of piroxicam is very small, see Figure S3. In contrast, major changes were observed, when using dianionic ionic liquids and the results are presented in Fig. 5. For this drug, the changes in the cation and/or anion had a distinct effect on the solubility. In the cation moiety, the insertion of a second hydroxyethyl group, lead to



Figs. 8. 2D $^1H-^1H$ NOESY spectrum of ibuprofen with 0.2 mol% of $[N_{4\ 1\ 1\ 2OH}]_2\ [C_4H_9PO_3]$ in DMSO-d_6 and representation of the interactions between ibuprofen and IL.

Table 1
- Log P values for Ibuprofen and Piroxicam.

System/Media	Log P
Ibuprofen/water	3.97[32]
Piroxicam/water	3.06[33]
Ibuprofen/water +0.05 mol% $[N_{4\ 1\ 2OH\ 2OH}]_2$ $[C_2H_5PO_3]$	0.60 ± 0.05
Ibuprofen/water + 0.05 mol% $[N_{4\ 1\ 1\ 2OH}]_2$ $[C_4H_9PO_3]$	0.57 ± 0.01
Piroxicam/water + 0.05 mol% $[N_{4\ 1\ 2OH\ 2OH}]_2$ $[C_2H_5PO_3]$	0.33 ± 0.06
Piroxicam/water + 0.05 mol% $[N_{4\ 1\ 1\ 2OH}]_2$ $[C_4H_9PO_3]$	0.29 ± 0.02

an increase in the solubility, but the addition of a third hydroxyethyl group lead to a decrease. These effect could be explained by our previous work,[27] which shows that addition of hydroxyl groups in the ammonium cation, enhances the hydrogen bonding ability of ILs with water. When the third hydroxyethyl group is added, the quantity of hydrogen bonds formed between IL and water is harmful, for interaction of piroxicam with ionic liquid. Comparing the results for ILs with the same cation ($[N_{4\ 1\ 2OH\ 2OH}]^+$) we can explore the effect of changes in the anion. In this case, the effect of increasing the alkyl chain from methyl to ethyl had a positive effect in solubility of piroxicam. However, increasing further the alkyl chain length in the anion, from an ethyl group to a butyl one, did not show the trend previously described and the solubility of piroxicam presents similar values in both IL/aqueous solutions. For the dianionic IL $[N_{4\ 1\ 2OH\ 2OH}]_2$ $[C_2H_5PO_3]$, with 0.25 mol% it was possible to have a 480-fold improvement on the drug solubility in comparison to pure water, and with $[N_{4\ 1\ 2OH\ 2OH}]_2$ $[C_4H_9PO_3]$ 0.3 mol%, an enhance of 530-fold.

The behaviour of some selected excipients that are used in the market to enhance the solubility of ibuprofen and piroxicam (sodium bicarbonate and lactose) were herein studied and the results are compared with the values obtained with the new dianionic ionic liquids herein designed, with an amount of 0.2 mol%. The values are presented for both drugs in Fig. 6. The lactose does not have any effect in solubility of both drugs. With sodium bicarbonate, the solubility of piroxicam has a 7-fold increment when comparing with that in pure water and with ibuprofen the increment in solubility was very high, but lower than the new dianionic ionic liquids, which demonstrated the advantage in the use of these new ILs.

Both ibuprofen and piroxicam are taken at 6–8 h intervals, hence we have studied their solubility in selected ionic liquids aqueous solutions after 6 h. After that period, it was observed that the solubility was practically the same than after 24 h, see Figure S4. The mean onset of action per 400 mg dose of ibuprofen is 45 min, which can be critical in an acute pain situation where a fast relief is critical.[28] So, we decide to decrease the time in assays to 5 min and the quantity of ionic liquid used, until 0.01 mol%.

As can be seen in Fig. 7, with only 0.01 mol% and 0.028 mol% of ionic liquid $[N_{4\ 1\ 2OH\ 2OH}]_2$ $[C_2H_5PO_3]$ at 5 min is possible to achieve a solubility of ≈600 mg/L and ≈2400 mg/L corresponding to one dose and maximum take daily, that an adult can take per day, however the normal dose should be 1200 mg/L.

Two Dimensional $^1H-^1H$ NOESY

A sample of ibuprofen with 0.2 mol% of $[N_{4\ 1\ 1\ 2OH}]_2$ $[C_4H_9PO_3]$ was prepared in DMSO-d_6 to observe the interaction between the two using a 2D $^1H-^1H$ NOESY experiment. The molar concentrations used mimic the conditions in the solubility assay. The 2D $^1H-^1H$ NOESY spectrum obtained is showed in Fig. 8.

The OH at δ 12.24 ppm from ibuprofen disappeared, which clearly demonstrate the intermolecular interaction between the oral drug and the ionic liquid, see Figure S15. It is also clear that exists a molecular interaction between the proton of ibuprofen at δ 1.80 ppm and methyl group of phosphonate dianion at

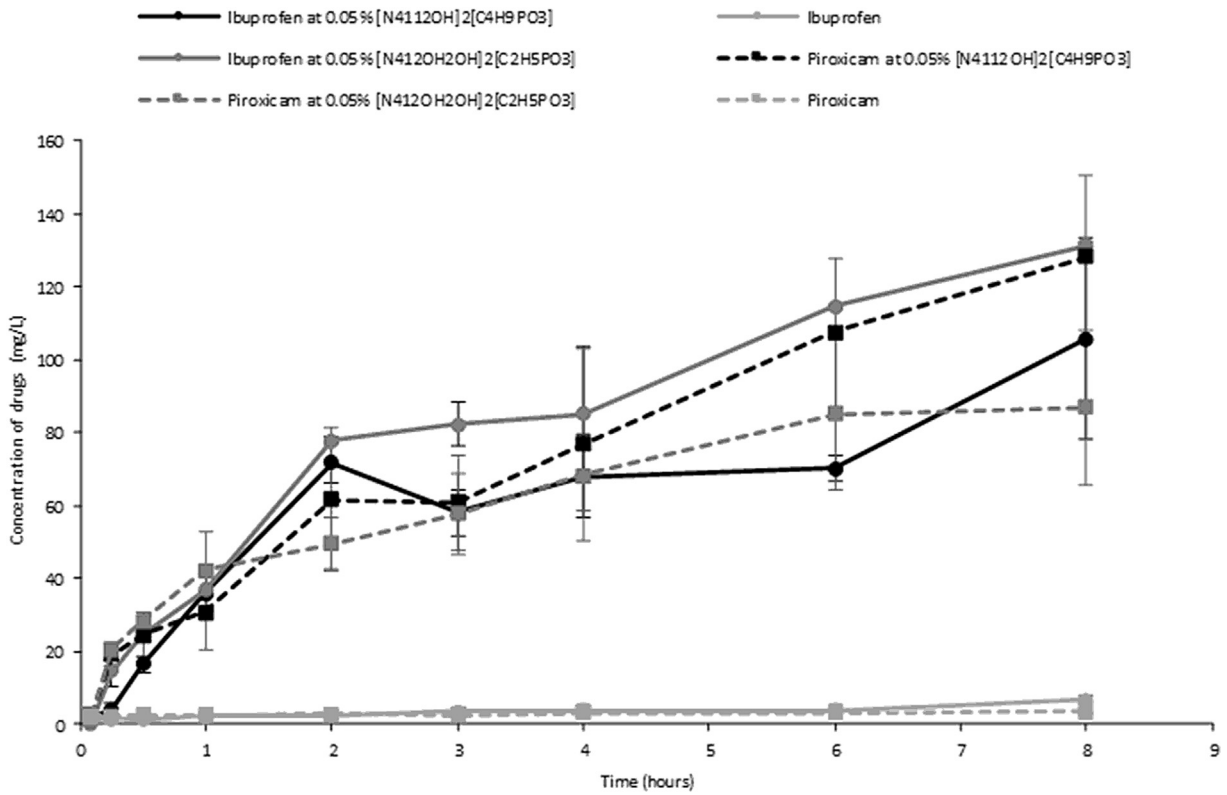


Fig. 9. Concentration of Ibuprofen and Piroxicam in solution, in the presence or absence of IL along time, through PES-U membrane.

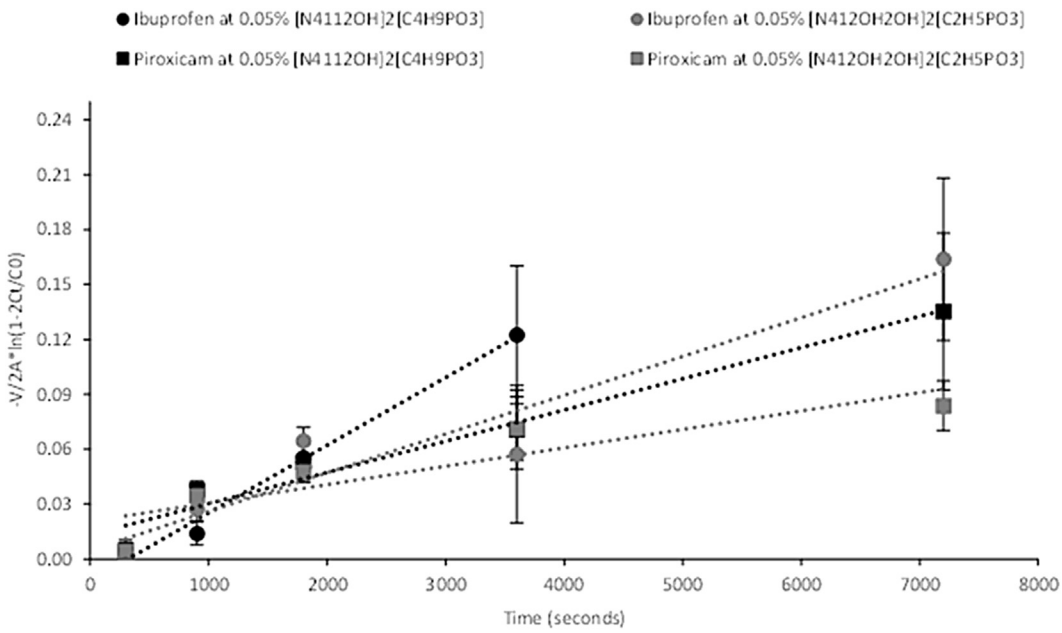


Fig. 10. Linearization of the permeation curves of Ibuprofen and Piroxicam with 0.05 mol% ILs.

δ 0.87 ppm. These interactions may justify the improvement on the solubility of this drug in the aqueous solution of this IL.

Analogously, a NOESY experiment of a sample of piroxicam with 0.2 mol% of the dianionic IL $[N_{4\ 1\ 1\ 2OH}]_2$ $[C_4H_9PO_3]$ was performed in CD_3CN. The OH at δ 13.52 ppm from piroxicam disappeared, suggesting the intermolecular interaction between the oral drug and the ionic liquid, see Figure S16. There is also a molecular interaction between the NH of piroxicam at δ 11.40 ppm and the methylene group connected to *N* atom from the cation (NCH_2CH_2OH) at δ 3.34 ppm, which may justify the improvement on the solubility of this drug in the aqueous solution of this IL, see Figure S17.

Octanol-Water Partition Coefficient and Log P

In the context of pharmacokinetics, 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. It also helps scientists to determine the most likely way of administration. It is relevant to notice that both drugs studied in this work belong to class II according to the Biopharmaceutics Classification System (BCS),[29,30] low solubility and high permeability. In the case of an orally absorbed drug, it has to 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.[31] According to literature, ibuprofen has a log P of 3.97[32] and piroxicam has a log P of 3.06[33] meaning that are distributed to hydrophobic areas such as lipidic bilayers of cells.

We have determined the log P value of these drugs using ionic liquids as co-solvents using the shake flask method. For each drug we have selected the ionic liquids that promoted the highest solubility. Namely, we have selected $[N_{4\ 1\ 2OH\ 2OH}]_2$ $[C_2H_5PO_3]$ and $[N_{4\ 1\ 1\ 2OH}]_2$ $[C_4H_9PO_3]$ dianionic ionic liquids to calculate log P.

Log P of ibuprofen and piroxicam in water were not determined by this method, due to the fact that the separation of octanol-water phases was not possible, therefore the values reported in the literature were considered for comparison purposes. 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 dianionic ionic liquids in solution. Log P for both drugs with only 0.05 mol% was very similar for both dianionic ionic liquids, as can be seen in Table 1.

In this study, the dianionic ionic liquids not only have an incredible increment in the solubility of both drugs but also meliorate its log P. In the presence of 0.05 mol% of ionic liquid both log P of ibuprofen and piroxicam have values more hydrophilic. As mentioned previously there must be a hydrophilicity/lipophilicity balance to achieve an ideal drug to be orally administered.

Table 2
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, K_d (10^{-1})
Ibuprofen	–	–	–
Ibuprofen + $[N_{4\ 1\ 1\ 2OH}]_2$ $[C_4H_9PO_3]$	3.70 ± 0.99	3.04 ± 1.05	1.83 ± 0.23
Ibuprofen + $[N_{4\ 1\ 2OH\ 2OH}]_2$ $[C_2H_5PO_3]$	2.12 ± 0.59	3.08 ± 0.20	1.03 ± 0.27
Piroxicam	–	–	–
Piroxicam + $[N_{4\ 1\ 1\ 2OH}]_2$ $[C_4H_9PO_3]$	1.71 ± 0.68	2.12 ± 0.98	1.21 ± 0.22
Piroxicam + $[N_{4\ 1\ 2OH\ 2OH}]_2$ $[C_2H_5PO_3]$	1.01 ± 0.22	2.60 ± 0.70	0.58 ± 0.17

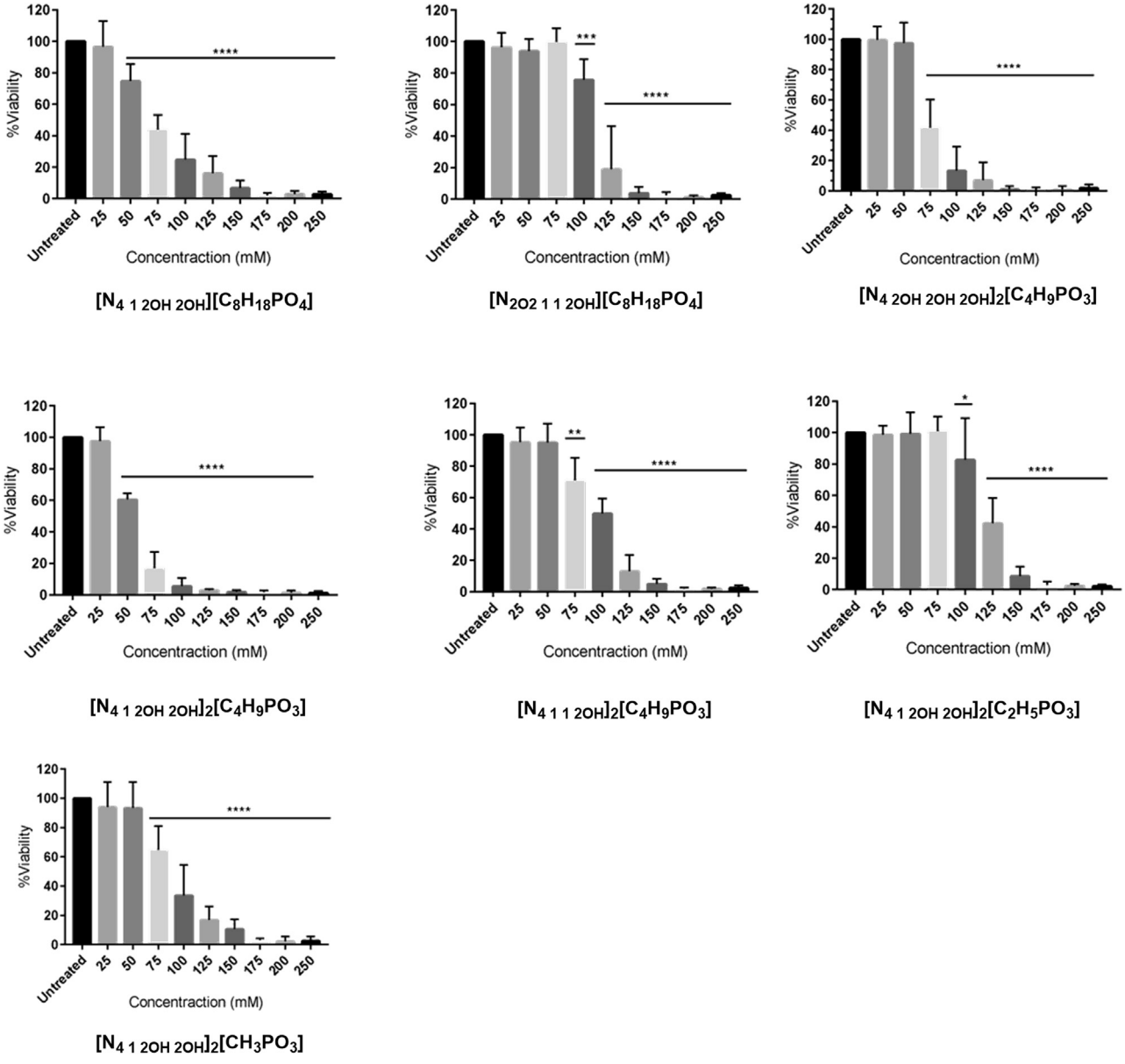


Fig. 11. Cytotoxicity of the ionic liquids in fibroblasts L929 cells to different concentrations of the respective ILs. The results are expressed as the average ± SD from three independent biological assays. The symbols *, **, *** and **** indicates that the viabilities are statistically significant with $p = 0.0152$, $p = 0.0018$, $p = 0.0002$ and $p = 0.0001$, respectively, when compared to the control.

Permeability

The permeability of ibuprofen and piroxicam was studied with the presence and in absence of IL along time. The concentration of the drug that permeate the membrane (mg/L) vs time is represented in Fig. 9. It is evident the effect that these dianionic ionic liquids have in the diffusion of ibuprofen and piroxicam over time through the membrane polyethersulphone (PES-U). Different parameters influence the diffusion. In particular, the amount of the solute permeated through a membrane depends on solute size, membrane pores size, pH, temperature and the affinity between membrane and the solute.[34] A temperature of 37 °C and a pH value of 6 were maintained constant during the whole assay.

The permeability of the oral drugs ibuprofen and piroxicam was determined with the presence of dianionic ionic liquids [$N_{4\ 1\ 2OH\ 2OH}$]$_2$ [$C_2H_5PO_3$] and [$N_{4\ 1\ 1\ 2OH}$]$_2$ [$C_4H_9PO_3$], in a concentration 0.05 mol% in aqueous solution. In absence of ILs it was not possible to determine the permeability, diffusion coefficient and partition

Table 3
- IC_{50} Determination of Mono and Dianionic Ionic Liquids and Drugs (Ibuprofen and Piroxicam).

Ionic Liquid	IC_{50}
[$N_{4\ 1\ 2OH\ 2OH}$][$C_8H_{18}PO_4$]	70.2 ± 5.6
[$N_{2O2\ 1\ 1\ 2OH}$][$C_8H_{18}PO_4$]	111.6 ± 3.7
[$N_{4\ 2OH\ 2OH\ 2OH}$]$_2$ [$C_4H_9PO_3$]	72.6 ± 3.4
[$N_{4\ 1\ 2OH\ 2OH}$]$_2$ [$C_4H_9PO_3$]	54.5 ± 2.3
[$N_{4\ 1\ 1\ 2OH}$]$_2$ [$C_4H_9PO_3$]	93.6 ± 5.5
[$N_{4\ 1\ 2OH\ 2OH}$]$_2$ [$C_2H_5PO_3$]	119.9 ± 5.2
[$N_{4\ 1\ 2OH\ 2OH}$]$_2$ [CH_3PO_3]	86.3 ± 5.2
Ibuprofen	2.5 ± 0.3
Piroxicam	2.3 ± 0.3

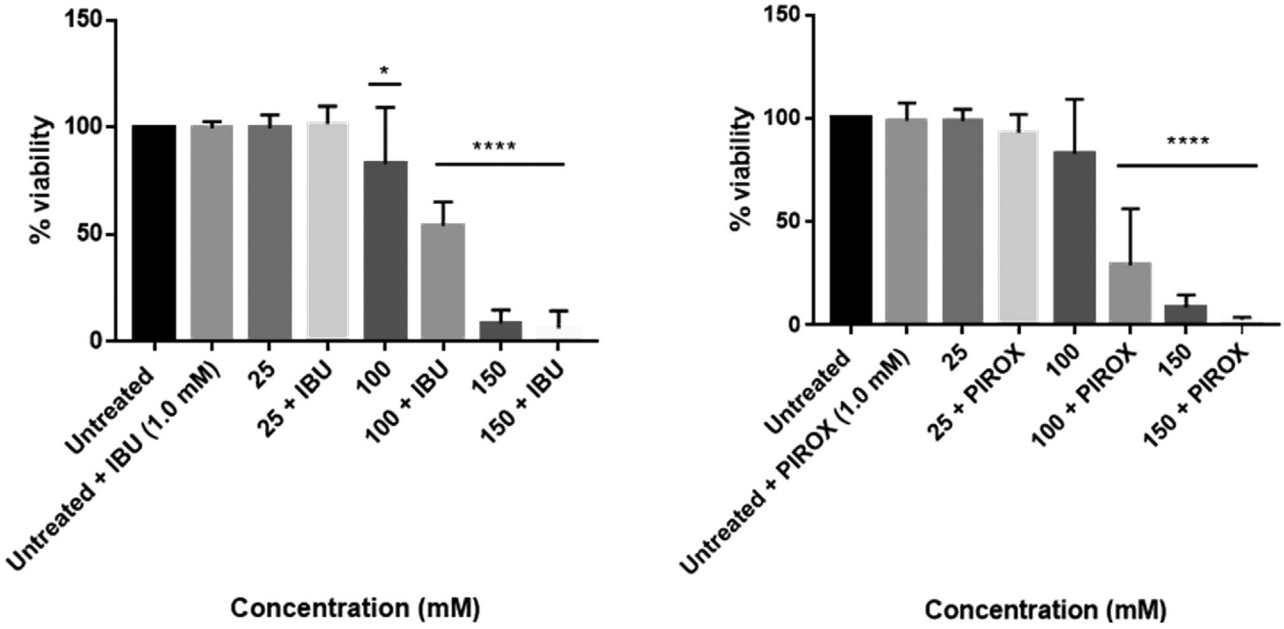


Fig. 12. Cytotoxicity of ibuprofen and piroxicam at 1 mM with some concentrations of [N4 1 2OH 2OH]2 [C2H5PO3] in fibroblasts L929 cells. The results are expressed as the average ± SD from three independent biological assays. The symbols * and **** indicates that the viabilities are statistically significant with $p = 0.0124$ and $p = 0.0001$, respectively, when compared to the control.

coefficient of drugs, since they not diffuse through membrane PES-U, as can be seen in Fig. 9.

The permeability coefficient was determined in the linear part of the permeation curve, up to 60% of the total drug permeated and as can be seen in Fig. 10 for ibuprofen and piroxicam.

A. R. Duarte et al.[24] determined the permeability and diffusion coefficient of ibuprofen in PBS solution, $4.6 \pm 0.14 \times 10^{-5}$ cm/s and $2.39 \pm 0.43 \times 10^{-6}$ cm^2/s, respectively. The permeability obtained for ibuprofen with both dianionic ILs were lower, as can be seen in Table 2, but diffusion coefficient was similar. Ibuprofen and piroxicam are drugs that belong to BCS class II, being high permeable. It would then be expected a different result in the permeability of these drugs. However, permeability depends on factors such as the medium and membrane, which may lead to completely different results. The difference in values may be due to small changes in the experimental procedure, such as the use of PBS in permeability studies, which is not the case in this study.

Cytotoxicity Evaluation

For the cytotoxicity study, we have selected the five dianionic ILs, $[N_{4\ 1\ 1\ 2OH}]_2$ $[C_4H_9PO_3]$, $[N_{4\ 1\ 2OH\ 2OH}]_2$ $[C_4H_9PO_3]$, $[N_{4\ 2OH\ 2OH\ 2OH}]_2$ $[C_4H_9PO_3]$, $[N_{4\ 1\ 2OH\ 2OH}]_2$ $[C_2H_5PO_3]$ and $[N_{4\ 1\ 2OH\ 2OH}]_2$ $[CH_3PO_3]$, which are those that showed the best results in terms of solubility for both drugs ibuprofen and piroxicam. For comparison purposes we have also chosen two monoanionic ILs, $[N_{4\ 1\ 2OH\ 2OH}]$ $[C_8H_{18}PO_4]$ and $[N_{2O2\ 1\ 1\ 2OH}][C_8H_{18}PO_4]$.

According to results showed in Fig. 11 and in Table 3, mono and dianionic ionic liquids presented low toxicity IC_{50} values ranging from 54.5 mM up to 119.9 mM.

Comparing $[N_{4\ 1\ 2OH\ 2OH}][C_8H_{18}PO_4]$ and $[N_{2O2\ 1\ 1\ 2OH}][C_8H_{18}PO_4]$ (both monoanionic with the same anion) it was possible to observe that the introduction of an ether group at the alkyl side chain is able to reduce significantly the cytotoxicity of these types of ILs. Moreover, the introduction of an *N*-hydroxyethyl group can lead to a decrease of IC_{50} values, fact that can be observed in ILs $[N_{4\ 1\ 2OH\ 2OH}]_2$ $[C_4H_9PO_3]$ and $[N_{4\ 1\ 1\ 2OH}]_2$ $[C_4H_9PO_3]$. For the ILs $[N_{4\ 1\ 2OH\ 2OH}]_2$ $[C_2H_5PO_3]$ and $[N_{4\ 1\ 2OH\ 2OH}]_2$ $[CH_3PO_3]$ which have the same cation with different anions we observed a decrease in IC_{50} with the decrease of the alkyl side chain of the anion, from ethyl to methyl. However, comparing ILs $[N_{4\ 1\ 2OH\ 2OH}]_2$ $[C_4H_9PO_3]$ and $[N_{4\ 1\ 2OH\ 2OH}]_2$ $[C_2H_5PO_3]$, there is an increment in IC_{50} when the anion changes from butyl to ethyl.

Interestingly, in this study the trend observed in other studies[14] regarding the increasing of IL's lipophilicity with the increase of toxicity, was not observed. In fact, in some cases we could not observe a direct correlation between the side chain length and the cytotoxicity. This may somehow suggest that dianionic ILs behave in different way than the monoanionic ones.

The cytotoxicity of both drugs was also tested, in fibroblasts L929 and the IC_{50} of ibuprofen is 2.5 mM and of piroxicam 2.3 mM, see Table 3 and Figure S18.

It is not straightforward though that if the IL and the API are non-toxic that the combination of the two renders a non-toxic formulation. Therefore, the cytotoxicity of ibuprofen and piroxicam at 1 mM (a non-toxic concentration) with different concentrations (25, 100 and 150 mM) of dianionic ionic liquid $[N_{4\ 1\ 2OH\ 2OH}]_2$ $[C_2H_5PO_3]$ was tested. The percentage of cell viability with 25 mM of IL is identical to that obtained for the same concentration of IL with 1 mM ibuprofen or piroxicam (Fig. 12). At 100 mM of IL with 1 mM of ibuprofen or piroxicam, the viability of cells is close 50% or below (29%) in the case of piroxicam. At 150 mM of IL + drug (1 mM), the cytotoxicity was highly visible.

Conclusions

The new dianionic ionic liquids synthesized and characterized in this work, have an excellent ability to improve the solubility of two poorly soluble oral drugs, ibuprofen and piroxicam, that belong to class II of the BCS (poor aqueous solubility and high permeability). Even at very small IL concentration (0.05−0.3 mol%) it was possible to convert these drugs into a class I (high permeability and high solubility). Also, at 0.05 mol% of IL 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. In studies at 37 °C, 5 min dissolution time and 0.01 mol% of $[N_{4\ 1\ 2OH\ 2OH}]_2$ $[C_2H_5PO_3]$, it was achieved a solubility of 600 mg/L, which corresponds to one dose for an adult. The dianionic ionic liquid $[N_{4\ 1\ 2OH\ 2OH}]_2$ $[C_2H_5PO_3]$ is the less toxic IL, having an IC_{50} of 120 mM ($\approx$0.25 mol%). Furthermore, the new dianionic ILs synthesized are not toxic to fibroblasts L929 cells. Thus, these new biocompatible ionic liquids are promising vehicles for aqueous administration of poorly water-soluble drugs.

Declarations of Interest

None.

Acknowledgements

The authors thank Fundação para a Ciência e Tecnologia, FCT/MCTES (Portugal) for financial support through an Investigator contract (IF/00621/2015 — P.M. Reis) and (IF/01146/2015 - A. Paiva), and through projects IF/00621/2015 and CryoDES (PTDC/EQU-EQU/29851/2017). This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme, under grant agreement No ERC-2016-CoG 725034. This work was also partially supported by the Associate Laboratory for Green Chemistry - LAQV which is financed by national funds from FCT/MCTES (UIDB/50006/2020 and UIDP/50006/2020) The NMR spectrometers are part of The National NMR Facility, supported by FCT/MCTES (RECI/BBB-BQB/0230/2012).

Appendix A. Supplementary Data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.xphs.2021.01.014.

References

1. Krishnaiah YSR. Pharmaceutical technologies for enhancing oral bioavailability of poorly soluble drugs. *J Bioequiv Availab*. 2010;2:28-36.
2. Horter D, Dressman JB. Influence of physicochemical properties on dissolution of drugs in the gastrointestinal tract. *Adv Drug Deliv Rev*. 2001;46:75-87.
3. Savjani KT, Gajjar AK, Savjani JK. Drug solubility: importance and enhancement techniques. *ISRN Pharm*. 2012;2012:195727.
4. Boyd BJ, Bergström CAS, Vinarov Z, et al. Successful oral delivery of poorly water-soluble drugs both depends on the intraluminal behavior of drugs and of appropriate advanced drug delivery systems. *Eur J Pharm Sci*. 2019;137:104967.
5. Adawiyah N, Moniruzzaman M, Hawatulaila S, Goto M. Ionic liquids as a potential tool for drug delivery systems. *Med Chem Commun*. 2016;7:1881-1897.
6. Moniruzzaman M, Goto M. Ionic liquids: future solvents and reagents for pharmaceuticals. *J Chem Eng Jpn*. 2011;44:370-381.
7. Buzzeo MC, Evans RG, Compton RG. Non-haloaluminate room-temperature ionic liquids in electrochemistry–a review. *Chem Phys Chem*. 2004;5:1106-1120.
8. Chiappe C, Pieraccini D. Ionic liquids: solvent properties and organic reactivity. *J Phys Org Chem*. 2005;18:275-297.
9. Kurnia KA, V Quental M, Santos LMNBF, Freire MG, Coutinho JAP. Mutual solubilities between water and non-aromatic sulfonium-{,} ammonium- and phosphonium-hydrophobic ionic liquids. *Phys Chem Chem Phys*. 2015;17:4569-4577.
10. Gomes JM, Silva SS, Reis RL. Biocompatible ionic liquids: fundamental behaviours and applications. *Chem Soc Rev*. 2019;48:4317-4335.
11. Gouveia W, Jorge TF, Martins S, et al. Toxicity of ionic liquids prepared from biomaterials. *Chemosphere*. 2014;104:51-56.
12. Jordan A, Gathergood N. Biodegradation of ionic liquids — a critical review. *Chem Soc Rev*. 2015;44:8200-8237.
13. Ventura SPM, e Silva FA, Gonçalves AMM, Pereira JL, Gonçalves F, Coutinho JAP. Ecotoxicity analysis of cholinium-based ionic liquids to Vibrio fischeri marine bacteria. *Ecotoxicol Environ Saf*. 2014;102:48-54.
14. Jesus AR, Soromenho MRC, Raposo LR, et al. Enhancement of water solubility of poorly water-soluble drugs by new biocompatible N-acetyl amino acid N-alkyl cholinium-based ionic liquids. *Eur J Pharm Biopharm*. 2019;137:227-232.
15. Jesus AR, Raposo LR, Soromenho MRC, et al. *N-alkyl Cholinium Based Ionic Liquids as Enhancers of Water Solubility for Poorly Water-Soluble Drugs, Unpubl. Results*. 2020.
16. Caparica R, Júlio A, Baby AR, et al. Choline-amino acid ionic liquids as green functional excipients to enhance drug solubility. *Pharmaceutics*. 2018;10.
17. Júlio A, Antunes C, Mineiro R, et al. Influence of two choline-based ionic liquids on the solubility of caffeine. *Biomed Biopharm Res*. 2018;15:96-102.
18. Garzón LC, F. Martínez Fleming. Temperature dependence of solubility for ibuprofen in some organic and aqueous solvents. *J Solut Chem*. 2004;33:1379.
19. Potthast H, Dressman JB, Junginger HE, et al. Biowaiver monographs for immediate release solid oral dosage forms: Ibuprofen. *J Pharm Sci*. 2005;94:2121-2131.
20. Escandar G, Bystol A, Campiglia A. Spectrofluorimetric method for the determination of piroxicam and pyridoxine. *Anal Chim Acta*. 2002;466:275-283.
21. Shohin IE, Kulinich JI, Ramenskaya GV, et al. Biowaiver monographs for immediate release solid oral dosage forms: piroxicam. *J Pharm Sci*. 2014;103:367-377.
22. Mahamat Nor SB, Woi PM, Ng SH. Characterisation of ionic liquids nanoemulsion loaded with piroxicam for drug delivery system. *J Mol Liq*. 2017;234:30-39.
23. Baka E, Comer JEA, Takács-Novák K. Study of equilibrium solubility measurement by saturation shake-flask method using hydrochlorothiazide as model compound. *J Pharm Biomed Anal*. 2008;46:335-341.
24. Duarte ARC, Ferreira ASD, Barreiros S, Cabrita E, Reis RL, Paiva A. A comparison between pure active pharmaceutical ingredients and therapeutic deep eutectic solvents: solubility and permeability studies. *Eur J Pharm Biopharm*. 2017;114:296-304.
25. Silva JM, Duarte ARC, Caridade SG, Picart C, Reis RL, Mano JF. Tailored free-standing multilayered membranes based on chitosan and alginate. *Biomacromolecules*. 2014;15:3817-3826.
26. Sintra TE, Shimizu K, Ventura SPM, Shimizu S, Canongia Lopes JN, Coutinho JAP. Enhanced dissolution of ibuprofen using ionic liquids as catanionic hydrotropes. *Phys Chem Chem Phys*. 2018;20:2094-2103.
27. de Ferro AM, Reis PM, Soromenho MRC, et al. Designing the ammonium cation to achieve a higher hydrophilicity of bistriflimide-based ionic liquids. *Phys Chem Chem Phys*. 2018;20:19307-19313.
28. Santos MM, Raposo LR, Carrera GVSM, et al. Ionic liquids and salts from ibuprofen as promising innovative formulations of an old drug. *Chem Med Chem*. 2019;14:907-911.
29. García-Arieta A, Gordon J, Potthast H. On the Biopharmaceutics classification system biowaiver of ibuprofen. *J Pharm Sci*. 2015;104:2429-2432.
30. Mirza S, Miroshnyk I, Habib MJ, Brausch JF, Hussain MD. Enhanced dissolution and oral bioavailability of piroxicam formulations: modulating effect of phospholipids. *Pharm Times*. 2010;2.
31. Pajouhesh H, Lenz GR. Medicinal chemical properties of successful central nervous system drugs. *NeuroRx*. 2005;2:541-553.
32. Czyrski A. Determination of the lipophilicity of ibuprofen, naproxen, ketoprofen, and flurbiprofen with thin-layer chromatography. *J Chemother*. 2019;2019:3407091.
33. Shokri J, Azarmi S, Fasihi Z, Hallaj-Nezhadi S, Nokhodchi A, Javadzadeh Y. Effects of various penetration enhancers on percutaneous absorption of piroxicam from emulgels. *Res Pharm Sci*. 2012;7:225-234.
34. He H, Cao X, Lee LJ. Design of a novel hydrogel-based intelligent system for controlled drug release. *J Control Release*. 2004;95:391-402.