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Evaluation of Solubility, Physicochemical Properties, and Cytotoxicity of Naproxen-Based Ionic Liquids

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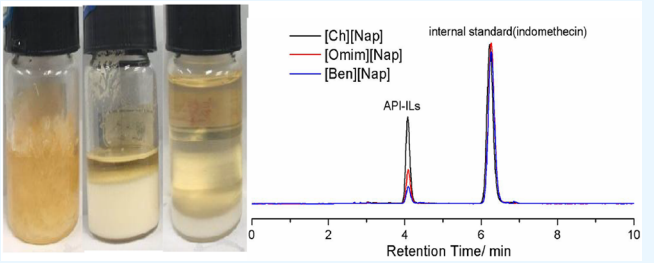
ABSTRACT: To solve the problems associated with poorly water-soluble nonsteroidal anti-inflammatory drugs (NSAIDs), naproxen-based ionic liquids (ILs) containing naproxen as an active pharmaceutical ingredient (API) anion were prepared with benzalkonium (tetradecyldimethylbenzyl ammonium), choline, and 1-octyl-3-methylimidazole as the cation. The structures and thermal properties were analyzed. Through the conductivity method, the solubility at 25 and 37 °C and the critical micelle concentration (CMC) at 25 °C were determined in water and ethanol. The octanol−water partition coefficients (K_{ow}) at 25 °C were measured with the shake-flask method. The cytotoxicity was evaluated with the MTT method. The results showed that the conversion of naproxen into the API-ILs increased the API's solubility in water by more than 850 times compared with the original API, and the thermostability was satisfactory with a lower glass transition temperature (t_g). Moreover, the variation trends of solubility, hydrophilicity, and K_{ow} were consistent with the different structures of naproxen-based ILs, except for benzalkonium naproxen. The CMC (10^{-5}−10^{-6} M) in water and ethanol demonstrated that the naproxen-based ILs were surface activite ILs. The IC_{50} values exhibited the low cytotoxicity of the naproxen-based ILs, which was better than 100 μM. The results provide essential information and a research basis for future topical and transdermal administration and oral administration of naproxen-based ILs.



INTRODUCTION

Active pharmaceutical ingredients (APIs) are mainly solid organic drugs, in particular, crystalline drugs because of their high purity, good thermal stability, and other advantages in the separation, storage, and evaluation processes. However, in the preclinical phase, some drugs with poor aqueous solubility have caused delivery concerns, erratic absorption, and low bioavailability.[1,2] Polymorphic conversion for some drugs affected the pharmaceutical action under the original dose.[3−7] Although scholars have attempted to address these problems with strategies or other treatments,[8−15] they can also lead to new problems such as drug dilution, uncertainty of the dosage, and stability.

To solve the aforementioned problems, changing the chemical structures of drugs is an effective way. Ionic liquids (ILs) can solve more pharmaceutical problems, i.e., solubility and polymorphism, and further improve the bioavailability and clinical effects. ILs are organic salts at room temperature, composed of organic cations and inorganic or organic anions. They have unique physicochemical properties.[16] Active pharmaceutical ionic liquids (API-ILs), belonging to the third-generation ILs,[17] can offer good physicochemical properties[17−23] and special biological activity.[17,24−27]

API-IL, ranitidine docusate ([Ran][Doc]), was first reported by Rogers and co-workers[17] in 2007. Since then, more and more reports on API-ILs have appeared in recent years.[18,28,29] Compared to the original and noncharged APIs, API-ILs have demonstrated enhanced aqueous solubility with single or dual therapeutic action.[17,30] Furthermore, they exhibit greater biological membrane permeability.[31] Most of the published work has focused on improving the solubility of APIs in aqueous media.[28]

Nonsteroidal anti-inflammatory drugs (NSAIDs) are one of the most widely prescribed classes of medications and are very effective in treating chronic and acute pain and inflammation. Among other serious side effects, long-term use of NSAIDs may increase the risk of gastrointestinal incidents, such as gastroduodenal ulcers, gastrointestinal bleeding, perforation, and dyspepsia.[32] One of the most recent studies on API-ILs involves the development of novel naproxen-based ILs in an attempt to optimize their solubility and other parameters that may influence gastrointestinal side effects.

Swiatek et al.[33] reported the conversion of naproxen into naproxen-based IL, L-proline alkyl ester naproxen ([ProOR]-[Nap]), suggesting the improved solubility and higher absorption of drug molecules by biological membranes.

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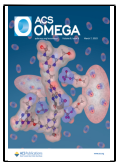

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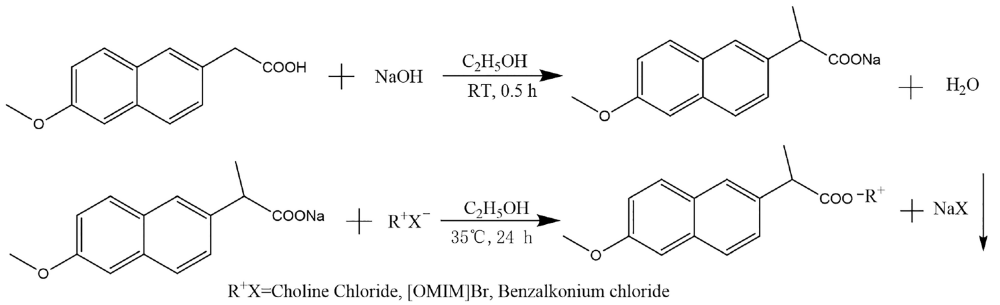
Scheme 1. Synthetic Procedure for Naproxen ILs



R^+X=Choline Chloride, [OMIM]Br, Benzalkonium chloride

Meanwhile, the use of L-proline isopropyl ester naproxen increased the permeation of naproxen through the skin by almost four times, and the increase in permeability is not associated with additional risk: all compounds had a similar effect on cell viability as the parent naproxen. Diphenlhydramine naproxen ([Dph][Nap]) has better clinical efficacy as an analgesic and sleep aid, with high solubility and good wettability. Meanwhile, when loaded into a mesoporous carrier, the [Dip][Nap] carrier composites exhibited improved dissolution rates, as well as excellent flow properties and ease of handling. Oral capsules of [Dip][Nap] were developed using such composites. The capsule product demonstrated acceptable drug release and bioavailability through a predictive artificial stomach−duodenum dissolution test.[34]

Choline naproxen ([Ch][Nap]) showed an aqueous solubility (at pH 7.4)[31,35] up to 100 times higher than the solubility of the naproxen precursor.[31] The addition of [Ch][Nap] into bacterial cellulose (BC) formed the transparent and homogeneous membranes, which further improved the absorption ability of phosphate-buffered saline (PBS) solution compared with the BC-naproxen.[31] Release tests showed faster and complete release of the [Ch][Nap] compared with the starting naproxen. The [Ch][Nap]-incorporated BC membranes have cytotoxicity and anti-inflammatory properties similar to those of naproxen.[31] The [Ch][Nap] not only showed the ability to interact with biological membranes but also improved the solubility, which positively impacted naproxen bioavailability and ultimately efficacy. The observed toxic alterations confirmed that choline influenced the toxicity of [Ch][Nap].[35] An automated system was investigated to study the interaction of [Ch][Nap] with human serum albumin (HSA).[36]

Balk et al.[30] reported tetrabutyl phosphonium naproxen ([Tbp][Nap]), which had a lower melting point and glass transition temperature. Its dissolution rates were improved by up to 1000 times compared with the original naproxen. Ossowicz et al.[37] reported an amino acid alkyl ester naproxen ([Aae][Nap]) that was not toxic to the murine macrophage cell line (RAW 264.7). The binding affinity of L-valine isopropyl ester naproxen for bovine serum albumin (BSA) was about 1 order of magnitude lower than that of parent naproxen and another [Aae][Nap], suggesting a faster diffusion rate in the circulatory system and faster time to reach the target system.

In this context, we synthesized a series of salts containing naproxen anion, choline naproxen ([Ch][Nap]), tetradecyldimethylbenzyl ammonium naproxen ([Ben][Nap]), and 1-octyl-3-methylimidazole naproxen ([Omim][Nap]), which can improve the solubility of the corresponding API naproxen in aqueous media. Meanwhile, their thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), solubility (quantification) in water or ethanol at 25 and 37 °C, critical micelle concentration (CMC) at 25 °C, octanol−water partition coefficients (K_{ow}), and cytotoxicity were investigated.

MATERIALS AND METHODS

Materials. Naproxen ($w \geq 99\%$) was purchased from Bide Pharmatech Ltd. Tetradecyldimethylbenzyl ammonium chloride ($w \geq 98\%$) and choline chloride ($w \geq 98\%$) were supplied by Aladdin. 1-Octyl-3-methyl imidazole bromide ([Omim]Br) was prepared in the lab. Ethanol ($w \geq 98\%$), *n*-octanol ($w \geq 98\%$), and ethyl acetate ($w \geq 99\%$) were supplied by Sinopharm Chemical Reagent Co., Ltd. Sodium hydroxide ($w \geq 98\%$) was supplied by Kemio Chemical Reagent Co., Ltd. Naproxen standard substance ($w \geq 99\%$, ID: Y15D8C50569) was supplied by Shanghai yuanye Bio-Technology Co., Ltd. Methyl thiazolyl tetrazolium (MTT) was supplied by Sigma. Trypsin was supplied by Shanghai Blue Base Biology Co., Ltd. Phosphate buffer (PBS) and trypsin cell digestive juice with 0.02% EDTA were supplied by Beijing Solaibao Technology Co., Ltd. High sugar Dulbecco's modified Eagle's medium (DMEM) was supplied by Gibco. Fetal calf serum (FCS) was supplied by Zhejiang Tianhang Biotechnology Co., Ltd. All chemicals were of analytical grade and used without further purification.

Synthesis of API-ILs. A mixture of sodium hydroxide (0.78 g, 19.5 mmol), naproxen (4.60 g, 20.0 mmol), and ethanol (40 mL) was placed in the flask and shaken vigorously for 0.5 h, and the ethanol was distilled off. The solid cake was treated with ethyl acetate. The solid white powder of naproxen sodium was obtained with a constant weight under vacuum at 45 °C. Naproxen sodium with the equimolecular [Omim]Br, benzalkonium chloride, or choline chloride was dissolved in ethanol. The mixture solution was stirred under the protection of nitrogen at 35 °C for 24 h and then naturally cooled to room temperature and filtered with a sand core funnel (bore diameter 3−4 μm). After removing the solvent by reduced pressure distillation, the oily or waxy substances were obtained and dried under reduced pressure at 45 °C to a constant weight (about 60 h). The faint yellow viscous liquids ([Ben][Nap] or [Omim][Nap]) or white waxy ([Ch][Nap]) substances, naproxen-based ILs, were obtained (Scheme 1), and the yield was above 90%.

All naproxen-based ILs were completely characterized by ^{1}H NMR (Bruker AVANCE 600 MHz, DE) to confirm the expected cation/anion ratios. A sand core funnel can filter the precipitates (NaCl or NaBr) better. High-performance liquid chromatography (HPLC) (Agilent 1260, USA) was used to check the structure and final purity of the obtained naproxen-

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based ILs. The purity was 99.6% (wt %) with the naproxen reference substance (ID: Y15D8C505 69).

TGA and DSC. Thermogravimetric measurements were conducted in a nitrogen atmosphere (flow rate of 50 mL·min^{-1}) on Q1000DSC + LNCS + FACEQ600 SDT (FA, USA) in a temperature range of 25−800 °C and a heating rate of 10 °C·min^{-1}. The DSC measurements were carried out in a nitrogen atmosphere (flow rate of 50 mL·min^{-1}) on Q1000DSC in a temperature range of −80 (5 min) to 100 °C and a heating rate of 10 °C·min^{-1}.

Solubility. The pure solvent (10 g, double-distilled water or ethanol) was placed in a beaker. The naproxen-based ILs were successively increased by a stepwise addition to pure solvent being initially placed in a cell for conductivity measurements. After each addition, the solution was stirred to ensure the homogeneous mixing, and then the electrical conductivity was recorded with a DDS-307 electrical conductivity meter (DJS-1C platinum black electrode) at 25 and 37 °C. The increased rate of the electrical conductivity slowly followed the concentration increase of naproxen-based ILs until the maximum values appeared.[38]

Critical Micelle Concentration. For the title naproxen-based ILs, the concentrations of standard solutions were prepared in turn with double-distilled water as the solvent (4×10^{-4}, 8×10^{-4}, 1.6×10^{-3}, 2.4×10^{-3}, 3.2×10^{-3}, 4.0×10^{-3}, and 1×10^{-2} mol·L^{-1}) and with ethanol as the solvent (1.0×10^{-2}, 1.0×10^{-3}, and 1.0×10^{-4} mol·L^{-1}).

First, the pure solvent (50 mL, double-distilled water or ethanol) was placed in a beaker. During different determination phases, the standard solution with different concentrations was chosen. After each addition of standard solution (1.00 mL), the solution was stirred to ensure homogeneous mixing, and the electrical conductivities and concentrations of the beaker were recorded with a DDS-307 electrical conductivity meter (DJS-1C platinum black electrode) at 25 °C. The DDS-307 electrical conductivity meter operated at an alternating current of 50 ± 0.5 Hz. The conductivity value of the naproxen-based ILs was determined after subtracting the electrical conductivity of solvents (water, 2.02 μS·cm^{-1}; ethanol, 0.35 μS·cm^{-1}) from the measured values.

Octanol−Water Partition Coefficients. The octanol−water partition coefficients of naproxen, naproxen sodium, and naproxen-based ILs were determined by the shake-flask method described by the OECD (Organizations for Economic Cooperation and Development) guidelines combined with UV−vis.

First, water (pH 7.4) and *n*-octanol had to saturate each other. Second, the standard solutions of different known concentrations of the naproxen salts (naproxen sodium and naproxen-based ILs) in water saturated with *n*-octanol and naproxen in *n*-octanol saturated with water were prepared sequentially, and the maximum absorption wavelength was measured at 230 nm using a Cary60 UV−vis spectrometer (Agilent, USA). The calibration curves were obtained. Third, a solution was prepared by completely dissolving the solute, naproxen salts (naproxen sodium and naproxen-based IL), in a 100 mL volumetric flask containing water saturated with *n*-octanol. Approximately 5 mL of this solution was added to a vial, and the same volume of *n*-octanol saturated with water was added. With naproxen as the solute, naproxen was completely dissolved in a 100 mL volumetric flask containing *n*-octanol saturated with water. Approximately 5 mL of this solution was added to a vial, and the same volume of water saturated with *n*-octanol was added.

For naproxen salts or naproxen, the solutions were prepared in quintuplicate. The vials were placed in an oscillator at a constant temperature of 25 °C for different periods. Subsequently, the samples were centrifuged for 15 min at 5000 rpm to ensure complete phase separation. Both phases were sampled by careful use of syringes. The syringe used to collect the water-rich phase was filled with air, which was slowly expelled while the syringe passed through the octanol phase. After the absorbance value became constant, the equilibrium time was obtained.

With the above-mentioned process and the equilibrium time, the concentrations in the water phase or *n*-octanol phase were determined using a UV spectrophotometer at the characteristic wavelength of naproxen (230 nm). By combining the calibration curves and the initial concentration of naproxen sodium, naproxen-based ILs, and naproxen, the equilibrium concentration of naproxen sodium, naproxen-based ILs, and naproxen in two phases was obtained. In the K_{ow} experiments, the measurement is always repeated three times.

Cytotoxicity. The cytotoxicity of the naproxen-based ILs of L929 cells in vitro was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were seeded at a density of 1×10^4 cells·mL^{-1} cells/well (200 μL) in DMEM containing 10% fetal bovine serum and 1% antibiotic solution in 96-well plates for 24−48 h at 37 °C. In serum-free DMEM, wells were rinsed with sterile PBS and treated with various concentrations of the API-IL samples. Each sample was reproduced three times, and the cells were cultured for 24 h at 37 °C. Twenty microliters of MTT (5 mg·mL^{-1}) was added to each well, and the cells were incubated for 4 h until purple precipitates were visible under a microscope. Finally, the medium was gently aspirated from the wells and rinsed with 1× PBS (200 μL). Then we added dimethylsulfoxide (DMSO) (100 μL) to dissolve the formazan crystals and shook the plate for 5 min. Using a microplate reader (Thermo Fisher Scientific, USA), the absorbance value of each well was measured at 490 nm, and the cell viability and IC_{50} value were calculated using GraphPad Prism 5.0 (USA).

RESULTS AND DISCUSSION

^{1}H NMR Analysis. [Ch][Nap]: ^{1}H NMR (ppm, $CDCl_3$, δ) 1.094 (s, 1H, OH), 1.390 (d, 3H, CH_3), 2.744 (s, 9H, CH_3), 3.090 (t, 2H, CH_2), 3.537−3.561 (q, 1H, CH), 3.663 (t, 2H, CH_2), 3.780−3.793 (s, 3H, CH_3), and 6.973−7.590 (s, 6H on benzene ring).

[Ben][Nap]: ^{1}H NMR (ppm, $CDCl_3$, δ) 0.806 (t, 3H, CH_3), 1.121−1.133 (m, 20H, intermediate hydrogen on the alkyl chain), 2.768−2.789 (m, 2H, N−CH_2), 2.911−2.939 (t, 2H, N−CH_2), 3.660 (s, 6H, CH_3), 3.588−3.600 (q, 1H, O=CCH), 3.787 (s, 3H, −OCH_3), 4.396 (s, 2H, N−CH_2), and 6.906−7.470 (s, 11H on benzene ring).

[Omim][Nap]: ^{1}H NMR (ppm, $DMSO_3$, δ) 0.853−0877 (t, 3H, CH_3), 1.240−1.261 (m, 12H, intermediate hydrogen on the octyl chain), 1.773 (d, 3H, O=CCH_3), 3.375−3.397 (t, 2H, N−CH_2), 3.848−3.867 (4H, O=CCH_3), 4.169 (s, 3H, N−CH_3), 7.064−7.475 (6H on naphthalene ring), 7.747 (s, 1H, N−CH), 7.819 (s, 1H, N−CH), and 9.322 (s, 1H, N−CH).

The results of ^{1}H NMR and HPLC showed that the structure of the cation for the title substance was naproxen-based API-ILs, and the title substance was extremely dry and

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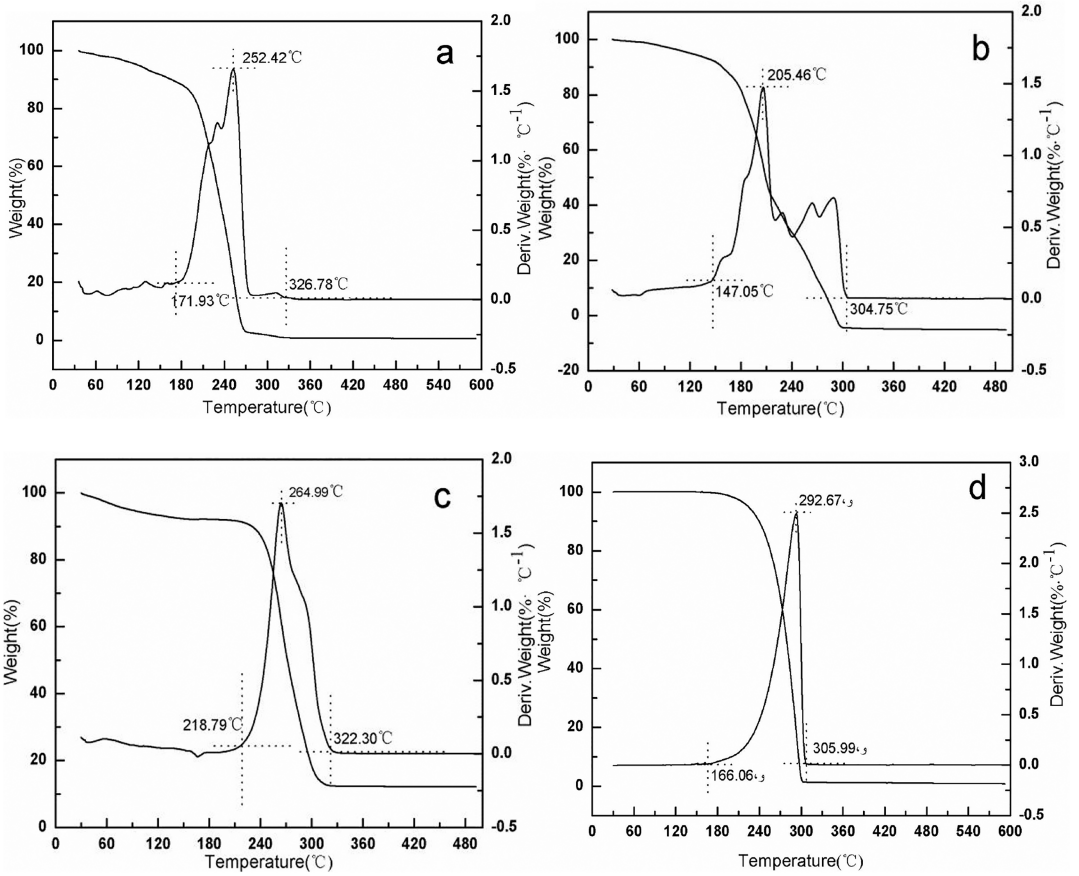

Figure 1. TG-DTG and DSC curves of naproxen-based ILs: (a) [Ch][Nap], (b) [Ben][Nap], (c) [Omim][Nap], (d) naproxen.

Table 1. Solubility of Naproxen and Naproxen Salts (Mean and Standard Deviation) in Water or Ethanol at 25 and 37 °C

naproxen and naproxen salts	solubility (mol·L^{-1}, 25 °C)	solubility (mol·L^{-1}, 37 °C)
naproxen	$1.15 \times 10^{-3} \pm 0.00012$[33,41]	$1.19 \times 10^{-3} \pm 0.00008$
Na[Nap]	0.7298 ± 0.0015[42,43]	1.1811 ± 0.0011[42a]
[Ch][Nap]	3.37 ± 0.04	4.32 ± 0.07
[Ben][Nap]	1.10 ± 0.03	1.18 ± 0.04
[Omim][Nap]	2.65 ± 0.08	3.03 ± 0.06
Na[Nap] (ethanol)	$5.092 \times 10^{-2} \pm 0.001$[42]	$7.54 \times 10^{-2} \pm 0.001$[42]
[Ch][Nap] (ethanol)	2.11 ± 0.05	2.32 ± 0.08
[Ben][Nap] (ethanol)	1.74 ± 0.06	1.77 ± 0.07
[Omin][Nap] (ethanol)	1.93 ± 0.08	1.98 ± 0.03

[a]Values are solubility of Na[Nap] at 35 °C.

free of water. ^{1}H NMR results also indicated that naproxen with [Omim]Br, benzalkonium chloride, or choline chloride formed naproxen-based ILs corresponding to 1 mol of [Nap]$^-$ to 1 mol of [Omim]$^+$, [Ben]$^+$, or [Ch]$^+$.

Thermal Properties. Naproxen-based ILs are hydrophilic ILs, and small amounts of water or solvents can greatly affect their physicochemical properties. To verify the moisture content and solvent residues in naproxen-based ILs (dried for 24 h), the thermal properties (TGA) of the ILs were studied, as shown in Figure 1. The weight loss from 25 to 120 °C is approximately 3−5%. This finding demonstrated that water and solvent residues in naproxen-based ILs were fewer.[39,40] The naproxen-based ILs still need to be dried under vacuum conditions at 45 °C for 36 h before the structural characterization and physicochemical properties analysis.

Glass transition (t_g) values of [Ch][Nap], [Ben][Nap], and [Omim][Nap] are −56.53, −39.83, and −69.03 °C, respectively. The t_g is related to the molecular structure and molecular movement. If the molecular motion is smoother, t_g is lower. When an anion is close to a cation and the interaction between them is bigger,[40] the resistance for the molecular chain movement is larger; therefore, t_g is higher.[40] Figure 1 shows that the beginning weight loss temperatures of [Ch][Nap], [Ben][Nap], [Omim][Nap], and naproxen were 171.93, 147.05, 218.79, and 166.05 °C, respectively, which indicates that naproxen-based ILs and naproxen have high heat stability. [Ch][Nap] in the derivative thermogravimetric (DTG) curve has two peak values (Figure 1a); its first maximum can be from the decomposition of the choline cation. The weight loss rate of [Ben][Nap] in all of the naproxen-based ILs is the largest. Four maximum values can

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observed in the DTG curve (Figure 1b), which originates from the long-chain structure of the cation and corresponds to the step-by-step cracking of the chain segment. When the temperature increased above 300 °C, naproxen-based ILs and naproxen decomposed completely.

Water Solubility and Octanol–Water Partition Coefficient. The solubility of the drugs affects their absorption, bioavailability, or efficacy, and poor solubility also produces toxicity and side effects in the application. Therefore, it is of great significance to study drug solubility in the process of drug research and development. The solubility of the naproxen-based ILs as well as the naproxen and naproxen sodium salt in water or ethanol at 25 and 37 °C and literature values are shown in Table 1 and Figure 2. The correlation of conductivity and solvent for naproxen-based ILs in water or ethanol at 25 °C is shown in Figure 3.

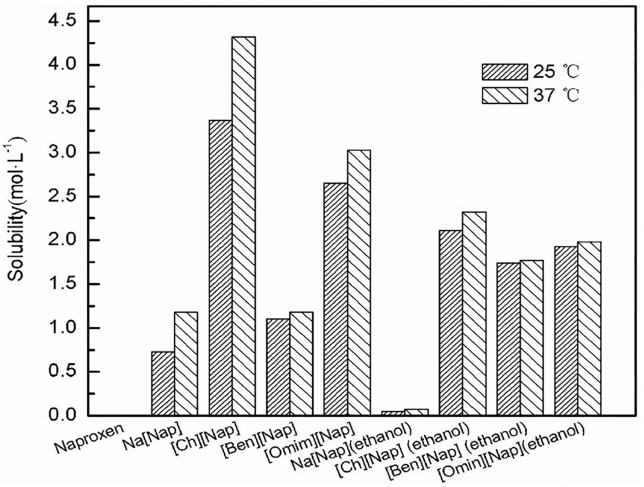

Figure 2. Solubility of naproxen, naproxen sodium salt, and naproxen-based ILs in water or ethanol at 25 and 37 °C.

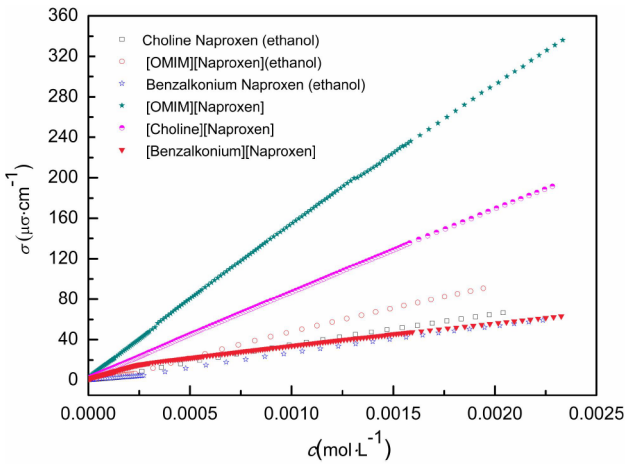

Figure 3. Influence of solvents and concentrations on electrical conductivities for naproxen-based ILs at 25 °C.

Solubility is affected by many factors, either extrinsic or intrinsic to APIs. Among the extrinsic factors, the effect of temperature was evaluated in this work. The solubility test was performed at 25 and 37 °C (body temperature). The influence of temperature on the solubility assays is shown in Figure 2. For naproxen, naproxen sodium salt, and naproxen-based ILs, the solubility always increases with temperature (Table 1 and Figure 2). The solubility trends at 37 and 25 °C are similar. As shown in Table 1, the difference in the water solubility of API-ILs can be observed (from 1.10 to 3.37 mol·L^{-1} at 25 °C), and the solubility of naproxen[33,41] in water is lower than the solubility of naproxen-based ILs. Compared with the water solubility of both naproxen sodium salt and naproxen-based ILs (0.730 mol·L^{-1}[42] and 0.919 mol·L^{-1}[43] at 25 °C, respectively), the solubility of [Ch][Nap] is the highest in this work. The results showed that the counterions can better tailor the aqueous solubility of the naproxen.

The solubility of the naproxen-based ILs in water or ethanol at 25 and 37 °C (body temperature) is $S_{[Ch][Nap]} > S_{[Omin][Nap]} > S_{[Ben][Nap]}$ (Figure 2 and Table 1). The dielectric constant of solvent (polarity) is an important property that characterizes the solvent's solvability to dissolve the solute. The higher the dielectric constant of the solvent, the stronger the ability to dissolve polar solutes.[16] From Figure 2, naproxen-based salts are easily soluble in water (ε_{water} = 80 F·m^{-1}) but insoluble in ethanol ($\varepsilon_{ethanol}$ = 24.5 F·m^{-1}). Under the identical anion, the mass of solute per 100 g of solvent increases in a polar solvent and decreases in a nonpolar solvent. For example, [Ch][Nap] (124.51 g·100 g^{-1}) > [Ben][Nap] (61.6251 g·100 g^{-1}) in water, [Ch][Nap] (70.23 g·100 g^{-1}) < [Ben][Nap] (97.61 g·100 g^{-1}) in ethanol at 25 °C. [Omim][Nap] (112.56 g·100 g^{-1} in water, 82.00 g·100 g^{-1} in ethanol) is between [Ch][Nap] and [Ben][Nap] at 25 °C. In nonpolar solvents, the superficial area of the cation increases, and the dispersion forces of the nonpolar solvents on naproxen-based increase, so the solubility in nonpolar solvents slightly increases.

Figure 3 shows that as the concentration for naproxen-based ILs increases in a certain concentration range, their electrical conductivity in water or ethanol tends to increase ($\sigma_{water} > \sigma_{ethanol}$). This is due to solvation, including the dielectric constant and polarity of the solvent, as well as the properties (diameter and mass) of the ions.[44]

The solvation of naproxen ILs in water is similar to that in ethanol. As the volumes of water and ethanol molecules increase, the size of the solvated molecules also increase after cation solvation. Under the action of the same external electric field, the larger the diameter and mass of the ion, the greater the movement resistance, and the slower the migration rate. Therefore, the conductivity of naproxen-based ILs is $\sigma_{water} > \sigma_{ethanol}$ (Figure 3). The polarity of the solvent also has some influence on the degree of ionization of API-ILs. The greater the polarity of the solvent, the more conducive the dissociation of electrolyte, and the greater the influence on the electrical conductivity. Water has a polarity higher than that of ethanol (ε_{water} (80 F·m^{-1}) > $\varepsilon_{ethanol}$ (24.5 F·m^{-1})),[44] so the conductivity of naproxen-based ILs varies with the degree of dissociation in the two solvents.

Drug metabolism is related to the transport of APIs in the distribution and their affinity to cell membranes, which are the key factors affecting drug bioavailability. However, the distribution coefficients of drugs in vivo is difficult to determine. Researchers used the octanol–water partition coefficient as a simplified model to mimic blood/lipidic membrane partition. It has become an essential tool to understand the tendency of a chemical to cross biological membranes. The equilibrium times of naproxen, naproxen-based ILs, and naproxen sodium salt are listed in Table 2. K_{ow} is defined as the ratio of the equilibrium concentrations of a

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Table 2. Octanol−Water Partition Coefficients of Naproxen, Naproxen Sodium Salt, and Naproxen-Based ILs (Mean and Standard Deviation) at 25 °C, Determined by the Shake-Flask Method

API/API-ILs	equilibrium time (h)	K_{ow}
naproxen	18	15.27 ± 0.02[33,46]
Na[Nap]	22	7.53 ± 0.02[35]
[Ch][Nap]	20	1.46 ± 0.02[35]
[Ben][Nap]	22	160.74 ± 0.08
[Omim][Nap]	22	2.73 ± 0.01

dissolved substance in a two-phase octanol and water system and is expressed using eq 1:[45]

$$K_{ow} = \frac{[\text{solute}]_{\text{octanol}}}{[\text{solute}]_{\text{water}}} \quad (1)$$

The octanol−water partition coefficients of the naproxen-based ILs, as well as the starting naproxen and naproxen sodium salt, were determined by the shake-flask method[46] as previously described, and the results are shown in Table 2 and Figure 4.

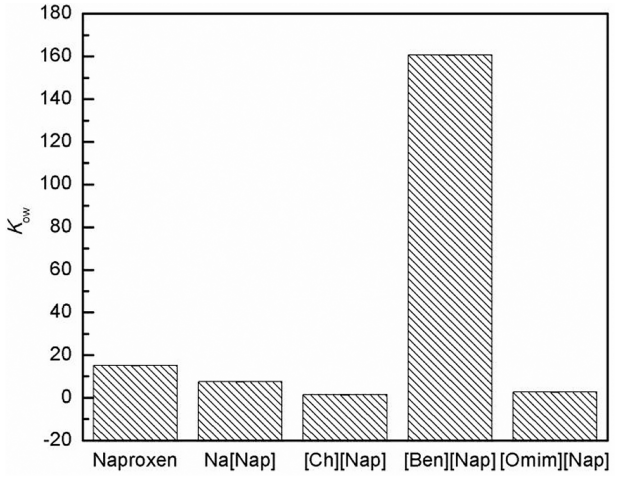

Figure 4. Octanol−water partition coefficients of naproxen, naproxen sodium salt, and naproxen-based ILs.

Two literature values were obtained for the octanol−water partition coefficient of naproxen.[33,35,47] The difference between the results in the assay (Table 2) and those in previous studies[33,35,47] is likely due to the different experimental methods.

As shown in Figure 4 and Table 2, all the naproxen-based ILs have a value of K_{ow} lower than that of naproxen except for [Ben][Nap]. As expected, more hydrophilic ILs showed lower K_{ow} values than more hydrophobic ones. However, not only the water solubility of [Ben][Nap] increased but also the K_{ow} value increased. This result indicated that both the water solubility and the "*n*-octanol solubility" increased, which illustrated the affinity of [Ben][Nap] for the organic phase was also increased. The variation trend of solubility in water (Figure 2) is similar to that of K_{ow} except for [Ben][Nap]-(Figure 4), which provided a better choice for pharmaceuticals and therapeutics. The aqueous solubility and octanol−water partition coefficient for both naproxen sodium salt and naproxen-based ILs (Figure 2 and Figure 4) revealed that the pathways of API-ILs are much more diverse than those of "traditional salt", naproxen sodium salt. Therefore, the enhancement of both the aqueous solubility and the octanol−water partition coefficient can be satisfied.

The K_{ow} value (160.74 ± 0.08) of [Ben][Nap] is extremely large, which can derive from an intermolecular interaction. The density functional theory (DFT) calculation can explain the intermolecular interaction. The calculations were carried out with Gaussian 16 software. The DFT-D3 with Becke−Johnson damping was applied to correct the weak interaction to improve the calculation accuracy. The calculation results indicate that the interactions between naproxen salts and water mostly depend on $[Nap]^-$. The interactions between naproxen salts and *n*-octanol mostly depend on the cation. So the K_{ow} values are determined mainly by the cation. The interaction between $[Ben]^+$ and *n*-octanol is the largest, which can cause the largest K_{ow} value.

Critical Micelle Concentration. The CMC is a ruler of surface activity for surfactants. The evaluation of micelle formation in drugs is essential for the assessment of their properties and their influence on biological processes. The smaller the CMC of the surfactant, the lower the concentration for micelle formation and surface saturation adsorption. The

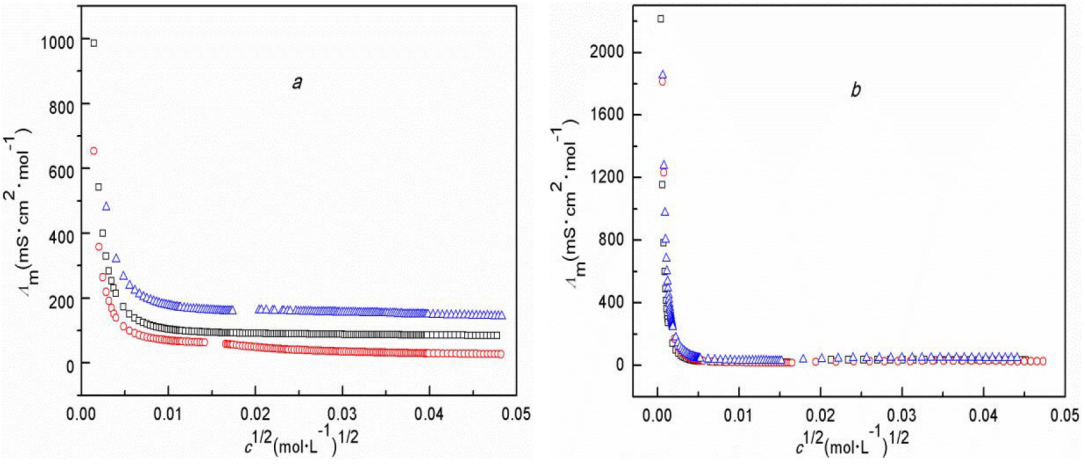

Figure 5. Curves of molar conductance vs square root of concentration for naproxen-based ILs in different solvents (a, water; b, ethanol). Experimental point: □, [Ch][Nap]; ○, [Ben][Nap]; △, [Omim][Nap].

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surfactant can obtain better wetting, emulsification, solubilization, and foaming effects. CMC is also a watershed, where the properties of surfactant solutions will change dramatically. It can diagnose and forecast the solubility to enhance the bioavailability of the drug candidates in biologically related media. It can also be used to express the intrinsic self-aggregate ability of compounds and predict the solubilization of drugs in the intestinal juice.

The conductivity of naproxen-based ILs at different concentrations was measured at 25 °C and converted to molar conductivity Λ_m. According to eq 2,[44] Λ_m of the naproxen-based ILs can be obtained.

$$\Lambda_m = \frac{\sigma}{c} \tag{2}$$

σ is the conductivity of naproxen-based ILs and c is the concentration of naproxen-based ILs. Figure 5a,b plots Λ_m versus te square root of the molarity (c) of naproxen-based ILs in water and ethanol solution at 25 °C.

Figure 5 shows that Λ_m of naproxen-based ILs decreases with the increase of concentrations in water or ethanol. Meanwhile, a breakpoint, the CMC of naproxen-based ILs, can be also observed at a certain concentration (Table 3).

Table 3. Critical Micelle Concentrations of the Surfactant Naproxen-Based ILs Calculated from Conductivity Measurements in Water or Ethanol at 25 °C

API-ILs	water (mol·L^{-1})	ethanol (mol·L^{-1})
[Ch][Nap]	3.75×10^{-5}	3.48×10^{-6}
[Ben][Nap]	3.05×10^{-5}	6.25×10^{-6}
[Omim][Nap]	5.68×10^{-5}	4.89×10^{-6}

Table 3 shows that the CMC values of the naproxen-based ILs solution range from 10^{-5} to 10^{-6} mol·L^{-1}, which are lower than those of typical surfactants.[48] This result indicated that the surface activity of the naproxen-based ILs in ethanol solution is better than that in water, and that a small number of naproxen-based ILs can achieve better wetting, emulsification, solubilization, and foaming effects.

Cytotoxicity. L929 cells were grown for 48 h at various concentrations of naproxen-based ILs, and the vitality of the cells was determined using the MTT assay with GraphPad Prism 5.0 (Figure 6). Treatment of L929 cells with naproxen-based ILs decreased cell proliferation in a dose-dependent manner (Figure 6). The best-fit IC_{50} values of [Ch][Nap], [Ben][Nap], and [Omim][Nap] were 783.3 (470.0),[35] 111.0, and 173.5 μM, respectively. Naproxen-based ILs are not toxic to L929 cells at concentrations up to 100 μM. The MTT assay demonstrated that the naproxen-based ILs were not cytotoxic.

CONCLUSION

This research focuses on evaluating the relevant pharmacological properties of novel naproxen-based API-ILs, such as water solubility, octanol−water partition coefficient (K_{ow}), critical micelle concentration (CMC), and cytotoxicity. The results clearly demonstrated the great potential of the API-IL approach due to its enhanced solubility properties in aqueous solutions. In addition, more sufficient properties, such as thermal properties (melting point and thermal stability), membrane permeation and membrane affinity (K_{ow}), biological processes (CMC), and cytotoxicity, were reported. The results showed that different counterions can fine-tune the physicochemical properties, and [Ch][Nap] is the most interesting among the prepared API-ILs due to its low melting point, enhanced aqueous solubility, low toxicity, and surfactant properties. Pharmacokinetic experiments in mice showed that the T_{max} of [Ch][Nap] and naproxen was 1.5 and 2.0 h, respectively, and the c_{max} of [Ch][Nap] was 23.051 mg·L^{-1} higher than that of naproxen (to be published in a different paper). Therefore, [Ch][Nap] may be a more interesting naproxen-based IL in further medicinal studies.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c07044.

^{1}H NMR curves of the naproxen-based ILs are appended in supplementary data Figures S1−S3 (PDF)

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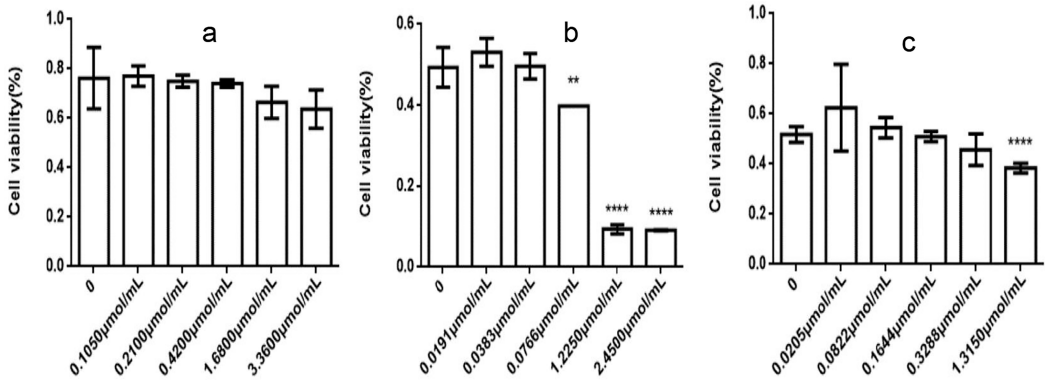

Figure 6. Cytotoxicities of naproxen-based ILs toward L929 cell lines (n = 3, mean ± SD for nonsignificant, ** for P < 0.01, *** for P < 0.001, **** for P < 0.0001): (a) [Ch][Nap], (b) [Ben][Nap]; (c) [Omim][Nap].

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Byrn, S. R.; Pfeiffer, R. R.; Stephenson, G.; Grant, W. J. D.; Gleason, W. B. *Solid-State Chemistry of Drugs*; SSCI: West Lafayette, IN, 1999.

(2) Schuster, D.; Laggner, C.; Langer, T. Why drugs fail-a study on side effects in new chemical entities. *Curr. Pharm. Des.* **2005**, *11*, 3545−3549.

(3) Censi, R.; Di Martino, P. Polymorph impact on the bioavailability and stability of poorly soluble drugs. *Molecules* **2015**, *20*, 18759−18776.

(4) Brittain, H. G.; Grant, D. J. R. Effects of polymorphism and solid-state solvation on solubility and dissolution rate. *Polymorphism in Pharmaceutical Solids*; Taylor and Francis: Abingdon, UK, 2009; pp 436−480.

(5) Bauer, J.; Spanton, S.; Henry, R.; Quick, J.; Dziki, W.; Porter, W.; Morris, J. Ritonavir: an extraordinary case of conformational polymorphism. *Pharm. Res.* **2001**, *18*, 859−866.

(6) Hulme, A. T.; Price, S. L.; Tocher, D. A. A new polymorph of 5-fluorouracil found following computational crystal structure predictions. *J. Am. Chem. Soc.* **2005**, *127*, 1116−1117.

(7) Aguiar, A. J.; Krc, J.; Kinkel, A. W.; Samyn, J. C. Effect of polymorphism on the absorption of chloramphenicol from chloramphenicol palmitate. *J. Pharm. Sci.* **1967**, *56*, 847−853.

(8) Li, Q.; Cao, J.; Wang, Q.; Zhang, J.; Zhu, S.; Guo, Z.; Zhu, W. H. Nanomized tumormicroenvironment-active NIR fluorescent prodrug for ensuring synchronous occurrences of drug release and fluorescence tracing. *J. Mater. Chem. B* **2019**, *7*, 1503−1509.

(9) El-Zhry El-Yafi, A. K.; El-Zein, H. Technical crystallization for application in pharmaceutical material engineering: review article. *Asian J. Pharm. Sci.* **2015**, *10*, 283−291.

(10) Madgulkar, A.; Bandivadekar, M.; Shid, T.; Rao, S. Sugars as solid dispersion carrierto improve solubility and dissolution of the BCS class II drug: clotrimazole. *Drug Dev. Ind. Pharm.* **2016**, *42*, 28−38.

(11) Okada, K.; Hirai, D.; Hayashi, Y.; Kumada, S.; Kosugi, A.; Onuki, Y. A novel approachto evaluate amorphous-to-crystalline transformation of active pharmaceuticalingredients in solid dispersion using time-domain NMR. *Chem. Pharm. Bull.* **2019**, *67*, 265−270.

(12) Poovi, G.; Damodharan, N. Lipid nanoparticles: A challenging approach for oraldelivery of BCS Class-II drugs. *Futur. J. Pharm. Sci.* **2018**, *4*, 191−205.

(13) Zhang, D.; Xu, Q.; Wang, N.; Yang, Y.; Liu, J.; Yu, G.; Yang, X.; Xu, H.; Wang, H. A complex micellar system co-delivering curcumin with doxorubicin againstcardiotoxicity and tumor growth. *Int. J. Nanomedicine* **2018**, *13*, 4549−4561.

(14) Park, S. H.; Choi, H. K. The effects of surfactants on the dissolution profiles of poorly water-soluble acidic drugs. *Int. J. Pharm.* **2006**, *321*, 35−41.

(15) Kumar, A.; Davern, P.; Hodnett, B. K.; Hudson, S. P. Carrier particle mediatedstabilization and isolation of valsartan nanoparticles. *Colloids Surf. B. Biointerfaces* **2019**, *175*, 554−563.

(16) Tang, Y. M.; Hu, X. L.; Guan, P.; Tian, T.; Wang, S. J. Investigation of surface properties and solubility of 1-vinyl-3-alkyl/esterimidazolium halide ionic liquids by density functional methods. *J. Chem. Eng. Data* **2014**, *59*, 2464−2471.

(17) Hough, W. L.; Smiglak, M.; Rodríguez, H.; Swatloski, R. P.; Spear, S. K.; Daly, D. T.; Pernak, J.; Grisel, J. E.; Carliss, R. D.; Soutullo, M. D.; Davis, J. H.; Rogers, R. D. The third evolution of ionic liquids: Active pharmaceutical ingredients. *New J. Chem.* **2007**, *31*, 1429−1436.

(18) Egorova, K. S.; Gordeev, E. G.; Ananikov, V. P. Biological activity of ionic liquids and their application in pharmaceutics and medicine. *Chem. Rev.* **2017**, *117*, 7132−7189.

(19) Araujo, J. M. M.; Florindo, C.; Pereiro, A. B.; Vieira, N. S. M.; Matias, A. A.; Duarte, C. M. M.; Rebelo, L. P. N.; Marrucho, I. M. Cholinium-based ionic liquids with pharmaceutically active anions. *RSC Adv.* **2014**, *4*, 28126−28132.

(20) Sahbaz, Y.; Williams, H. D.; Nguyen, T.; Saunders, J.; Ford, L.; Charman, S. A.; Scammells, P. J.; Porter, C. J. H. Transformation of Poorly Water-Soluble Drugs into Lipophilic Ionic Liquids Enhances Oral Drug Exposure from Lipid Based Formulations. *Mol. Pharmaceutics* **2015**, *12*, 1980−1991.

(21) Branco, L. C.; Jorda, N. Dipolar motions and ionic conduction in an ibuprofen derived ionic liquid. *Phys. Chem. Chem. Phys.* **2015**, *17*, 24108−24120.

(22) Ferraz, R.; Branco, L. C.; Marrucho, I. M.; Araujo, J. M. M.; Rebelo, L. P. N.; da Ponte, M. N.; Prudencio, C.; Noronha, J. P.; Petrovski, Z. Development of novel ionic liquids based on ampicillin. *Med. Chem. Commun.* **2012**, *3*, 494−497.

(23) Florindo, C.; Araujo, J. M.M.; Alves, F.; Matos, C.; Ferraz, R.; Prudencio, C.; Noronha, J. P.; Petrovski, Z.; Branco, L.; Rebelo, L. P. N.; Marrucho, I. M. Evaluation of solubility and partition properties of ampicillin-based ionic liquids. *Int. J. Pharm.* **2013**, *456*, 553−559.

(24) Berton, P.; Di Bona, K. R.; Yancey, D.; Rizvi, S. A. A.; Gray, M.; Gurau, G.; Shamshina, J. L.; Rasco, J. F.; Rogers, R. D. Transdermal bioavailability in rats of lidocaine in the forms of ionic liquids, salts, and deep eutectic. *ACS Med. Chem. Lett.* **2017**, *8*, 498−503.

(25) Johansson, K. M.; Izgorodina, E. I.; Forsyth, M.; Macfarlane, D. R.; Seddon, K. R. Protic ionic liquids based on the dimeric and oligomeric anions: [(AcO)(x)H(x-)](−). *Phys. Chem. Chem. Phys.* **2008**, *10*, 2972−2978.

(26) Bica, K.; Rogers, R. D. Confused ionic liquid ions—a "liquification" and dosage strategy for pharmaceutically active salts. *Chem. Commun.* **2010**, *46*, 1215−1217.

(27) Stoimenovski, J.; Dean, P. M.; Izgorodina, E. I.; Macfarlane, D. R. Protic pharmaceutical ionic liquids and solids: aspects of protonics. *Faraday Discuss.* **2012**, *154*, 335−352.

(28) Pedro, S. N.; Freire, C. S. R.; Silvestre, A. J. D.; Freire, M. G. The Role of ionic liquids in the pharmaceutical field: an overview of relevant applications. *Int. J. Mol. Sci.* **2020**, *21*, 8298−8348.

(29) Wu, X. Y.; Zhu, Q. G.; Chen, Z. J.; Wu, W.; Lu, Y.; Qi, J. P. Ionic liquids as a useful tool for tailoring active pharmaceutical ingredients. *J. Controlled Release* **2021**, *338*, 268−283.

(30) Balk, A.; Wiest, J.; Widmer, T.; Galli, B.; Holzgrabe, U.; Meinel, L. Transformation of acidic poorly water soluble drugs into ionic liquids. *Eur. J. Pharm. Biopharm.* **2015**, *94*, 73−82.

(31) Chantereau, G.; Sharma, M.; Abednejad, A.; Neves, B. M.; Sebe, G.; Coma, V.; Freire, M. G.; Freire, C. S. R.; Silvestre, A. J. D. Design of nonsteroidal anti-inflammatory drug-based ionic liquids with improved water solubility and drug delivery. *ACS Sustain. Chem. Eng.* **2019**, *7*, 14126−14134.
(32) Wongrakpanich, S.; Wongrakpanich, A.; Melhado, K.; Rangaswami, J. A comprehensive re-view of non-steroidal anti-inflammatory drug use in the elderly. *Aging Dis.* **2018**, *9*, 143−150.
(33) Swiatek, E.; Ossowicz-Rupniewska, P.; Janus, E.; Nowak, A.; Sobolewski, P.; Duchnik, W.; Kucharski, L.; Klimowicz, A. Novel naproxen salts with increased skin permeability. *Pharmaceutics* **2021**, *13*, 2110.
(34) Wang, C. G.; Chopade, S. A.; Guo, Y. W.; Early, J. T.; Tang, B. X.; Wang, E.; Hillmyer, M. A.; Lodge, T. P.; Sun, C. C. Preparation, Characterization, and formulation development of drug-drug protic ionic liquids of diphenhydramine with ibuprofen and naproxen. *Mol.Pharmaceut.* **2018**, *15*, 4190−4201.
(35) Azevedo, A. M.O.; Costa, S. P.F.; Dias, A. F.V.; Marques, A. H.O.; Pinto, P. C.A.G.; Bica, K.; Ressmann, A. K.; Passos, M. L.C.; Araujo, A. R.T.S.; Reis, S.; Saraiva, M. L. M.F.S. Anti-inflammatory choline based ionic liquids: Insights into their lipophilicity, solubility and toxicity parameters. *J. Mol. Liq.* **2017**, *232*, 20−26.
(36) Ribeiro, R.; Pinto, P. C. A. G.; Azevedo, A. M. O.; Bica, K.; Ressmann, A. K.; Reis, S.; Saraiva, M. L. M. F. S. Automated evaluation of protein binding affinity of anti-inflammatory choline based ionic liquids. *Talanta* **2016**, *150*, 20−26.
(37) Ossowicz, P.; Janus, E.; Klebeko, J.; Swiatek, E.; Kardaleva, P.; Taneva, S.; Krachmarova, E.; Rangelov, M.; Todorova, N.; Guncheva, M. Modulation of the binding affinity of naproxen to bovine serum albumin by conversion of the drug into amino acid ester salts. *J. Mol. Liq.* **2020**, *319*, 114283.
(38) Zhang, Q.-G.; Sun, S.-S.; Pitula, S.; Liu, Q.-S.; Welz-Biermann, U.; Zhang, J.-J. Electrical conductiviy of solution of ionic liquids with methanol, ethanol, acetonitrile, and propylene carbonate. *J. Chem. Eng. Data* **2011**, *56*, 4659−4664.
(39) Tang, Y. M.; Hu, X. L.; Guan, P.; Lin, X. P.; Li, X. Q. Physico-chemical characterization of paramagnetic ionic liquids 1-viny-3-alkylimidazolium tetrahalogenidoferrate (III) [VRIM] [$FeCl_mBr_{4-m}$]. *J. Phys. Org. Chem.* **2014**, *27*, 498−503.
(40) Tang, Y. M.; Qin, B.; Zhang, B. Correlation between Structure and thermal properties of *N*-vinyl-3-alkylimidazolium magnetic ionic liquids. *J. Wuhan Univ. Technol.* **2020**, *35*, 26−31.
(41) Kumar, L.; Suhas, B.; Pai, G. K.; Verma, R. Determination of saturated solubility of naproxen using UV visible spectrophotometer. *Res. J. Pharm. Technol.* **2015**, *8*, 825.
(42) Delgado, D. R.; Ruidiaz, M. A.; Gomez, S. M.; Gantiva, M.; Martinez, F. Thermodynamic study of the solubility of sodium naproxen in some ethanol plus water mixtures. *Quim. Nova* **2010**, *33*, 1923−1927.
(43) Jimenez, D. M.; Cardenas, Z. J.; Martinez, F. Solubility and apparent specific volume of some pharmaceutical salts in propylene glycol plus water mixtures at 298.15K. *Chem. Eng. Commun.* **2016**, *203*, 1013−1019.
(44) Tang, Y.; Hu, X.; Guan, P.; Li, X.; Tian, T. Influence of structural variations on the solubility and electrical conductivity of 1-vinyl-3-alkylimidazole halogen ionic liquids. *J. Wuhan Univ. Technol.* **2014**, *29*, 1090−1097.
(45) Sangster, J. Octanol−water partition-coefficients of simple organiccompounds. *J. Phys. Chem. Ref.* **1989**, *18*, 1111−1229.
(46) OECD Guideline for the testing of chemicals: 107 partition coefficient (*n*-octanol/water): shake flask method; OECD: Paris, 1993.
(47) Yang, D. G.; Liu, C.; Ding, D. W.; Quan, P.; Fang, L. The molecular design of drug-ionic liquids for transdermal drug delivery: Mechanistic study of counterions structure on complex formation and skin permeation. *Inter. J. Pharmaceut.* **2021**, *602*, 120560.
(48) Silva, A. T.; Teixeira, C.; Marques, E. F.; Prudencio, C.; Gomes, P.; Ferraz, R. Surfing the third wave of ionic liquids: a brief review on the role of surface-active ionic liquids in drug development and delivery. *Chem. Med. Chem.* **2021**, *16*, 2604−2611.

https://doi.org/10.1021/acsomega.2c07044
ACS Omega 2023, 8, 8332−8340