THE INFLUENCE OF IONIC LIQUIDS ON THE SOLUBILITY/PERMEATION OF POORLY SOLUBLE ACTIVE COMPOUNDS

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After all this time?
Always!
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Abstract

The pharmaceutical industry has developed alternative forms of drug delivery over the years and the delivery through the skin, topical or transdermal delivery, has been increasingly used. However, incorporating poorly soluble or partially insoluble drugs into the developed delivery systems has been difficult to solve.

Ionic liquids have specific properties, arising from their structure, which allows them to be used in aqueous, oily or hydroalcoholic solutions. Hence, ionic liquids can be used as excipients with the aim of increasing/improving the topical and transdermal delivery of drugs.

Herein, the efficacy of ionic liquids derived from amino acids, [Cho][Phe] and [Cho][Glu], as solubility and/or permeation promoters of poorly soluble actives were evaluated. The studied actives were ferulic acid and rutin, both natural antioxidant compounds, with low solubility, which restricts their use in topical and dermocosmetic products, since they require the use of high proportions of organic solvents.

The solubility studies carried out showed that the solubility of both active compounds is always increased in the presence of the prepared ionic liquids in all the studied conditions. Regarding the permeation studies, the ionic liquids did not shown a significant effect on the permeation of the studied actives.

The obtained results indicate that the ionic liquids studied can be efficient functional ingredients, since they allow an increase in the solubility of the studied actives, even at low concentrations of the studied ionic liquids.

Key Words: Ionic Liquids; Solubility; Rutin; Ferulic Acid; Permeation.
Resumo

A indústria farmacêutica ao longo dos anos tem vindo a desenvolver formas alternativas de veicular fármacos. A veiculação através da pele, veiculação tópica ou trandérmica, tem vindo a ser cada vez mais utilizada. No entanto, a dificuldade de incorporar fármacos pouco solúveis ou parcialmente insolúveis, nos sistemas de veiculação desenvolvidos, tem sido difícil de solucionar.

Os líquidos iónicos têm características específicas, provenientes da sua estrutura, que permite que estes possam ser utilizados em soluções aquosas, oleosas ou hidroalcoólicas. Desta forma, os líquidos iónicos podem ser utilizados como excipientes em formulações tópicas com o objetivo de aumentar/melhorar a veiculação tópica e trandérmica de fármacos.

No âmbito deste trabalho, foi avaliada a eficácia de líquidos iónicos derivados de aminoácidos, [Cho][Phe] e [Cho][Glu], como promotores da solubilidade e/ou da permeação de ativos pouco solúveis. Os ativos estudados foram o ácido ferúlico e a rutina, ambos compostos antioxidantes naturais, com baixa solubilidade, o que restringe a sua utilização em produtos tópicos e dermocosméticos, por exigirem a utilização de altas proporções de solventes orgânicos.

Os estudos de solubilidade realizados permitiram demonstrar que a solubilidade de ambos os ativos é sempre aumentada na presença dos líquidos iónicos preparados, em todas as condições analisadas. Relativamente aos estudos de permeação, os líquidos iónicos não demonstraram ter um efeito significativo na permeação dos ativos estudados.

Os resultados obtidos indicam que os líquidos iónicos estudados podem ser eficientes ingredientes funcionais, uma vez que permitem um aumento da solubilidade dos ativos estudados, mesmo a baixas concentrações de líquido iónico.

**Palavras Chave:** Líquidos Iónicos; Solubilidade; Rutina; Ácido Ferúlico; Permeação.
Abbreviations and Symbols

%  Percentage
% (m/m)  Percentage weight per weight
°C  Celsius degree
APIs  Active Pharmaceutical Ingredients
API-IL  Active Pharmaceutical Ingredients-Ionic Liquids
cm²  Square centimetres
CO₂  Carbon dioxide
D₂O  Deuterium oxide
g  Gram
h  Hour
IL  Ionic Liquid
ILs  Ionic Liquids
kg  Kilogram
M  Molar
m²  Square meter
mg  Milligram
mg/mL  Milligram per millilitre
MHz  Mega-hertz
mL  Millilitre
n  Number of samples
O/W  Oil/Water
PBS  Phosphate buffer Solution
PILs  Protic Ionic Liquids
SD  Stander Deviation
SPF  Sun Protection Factor
UV  Ultraviolet radiation
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Introduction
Over the years, the pharmaceutical industry has been encouraged to explore other alternative routes, besides oral and/or parenteral, which also allow the delivery of the drug efficiently and effectively to the target site (Singh Malik, Mital, & Kaur, 2016).

Drug delivery through the skin is one of the alternatives that has been increasingly explored and can be divided into two classes: the first class refers to the dermal/topical delivery systems and involves the topical application of drugs for dermatological treatment or cosmetic applications, that is, should only be used for pathologies where the target site is within the skin, so systemic absorption should be minimal (Brown, Martin, Jones, & Akomeah, 2006; Gabriel, 2016; Torin Huzil, Sivaloganathan, Kohandel, & Foldvari, 2011). On the other hand, the second class, the transdermal delivery systems, involve the application of drugs to the skin for systemic therapy (Brown et al., 2006; Gabriel, 2016; Torin Huzil et al., 2011).

The topical drug delivery comparatively with other routes, mainly with the oral route, avoids hepatic first pass metabolism and gastric pH variations (Brown et al., 2006; Singh Malik et al., 2016; Torin Huzil et al., 2011). The other advantages associated with the topical drug delivery include: improved patient compliance and acceptance, ease and convenience of application, painless and non-invasive techniques, improvement in drug bioavailability, direct access to target or diseased site, provides an alternative in circumstances where oral dosing is not possible and ease of dose termination in the event of any adverse reactions. As for transdermal drug delivery systems, besides the above mentioned characteristics, the advantages of these systems also include the reduction in side effects associated with systemic toxicity, better physiological and pharmacological response and minimal systemic toxicity and exposure of drug to non-infectious tissue and sustained and controlled delivery over a prolonged period of time (Brown et al., 2006; Singh Malik et al., 2016).

Furthermore, to develop formulations for topical application it is necessary to understand the characteristics and properties of the skin.

**Skin Properties**

The skin is one of the main and most important organs of the body, covering about 1.7 m² and representing approximately 10% of the human body mass in adults (Heylings, 2011). The primary function of the skin is to provide a barrier between the body and the external
environment by protecting it against external factors that can induce damages in human body, such as ultraviolet radiation, chemicals, allergens and microorganisms, and preventing the loss of moisture and body nutrients (Barry, 2004; Brown et al., 2006; Gabriel, 2016; Heylings, 2011; Hussain, Limthongkul, & Humphreys, 2013). Additionally, the skin is also an important sensory organ and contributes to the maintenance of homeostasis (Heylings, 2011). With respect to its constitution, the skin is composed of the appendages, hair follicles and eccrine and apocrine sweat glands, and by three different main layers, the stratum corneum, the viable epidermis, dermis, and subcutaneous tissue (Gabriel, 2016; Heylings, 2011; Torin Huzil et al., 2011). The stratum corneum is the primary barrier of the skin and has the ability to regulate water loss and to prevent the permeation of potentially dangerous substances/microorganisms (Heylings, 2011). This layer is composed of several layers of flattened, non-living corneocytes (Torin Huzil et al., 2011), which are composed of 70% – 80% keratin and 20% lipid within a cell envelope (Heylings, 2011). This cell envelope, located in the exterior of the corneocytes, consists of two parts: a protein envelope and a lipid envelope. The connection of this two envelopes, more concretely the anchoring between intercellular lipids and corneocyte protein envelope, provides the important barrier function of the stratum corneum and, consequently, of the skin (Heylings, 2011).

Thus, to develop formulations for drug delivery through the skin, it is necessary to careful select, not only the drug but also, the excipients used and understand their properties, since the barrier properties of the skin may limit the bioavailability of the drug.

Hence, for drugs to enter the skin they should have the ability to penetrate, consecutively, the hydrophilic and hydrophobic domains of the skin (Heylings, 2011). This ability will depend on factors such as drug solubility. The solubility is a property of a chemical substance, called solute, to dissolve in a certain solvent to form a homogeneous solution of the solute in the solvent. It is a dynamic equilibrium that establishes when two processes, dissolution and phase joining, reach a constant rate. The solubility of a substance depends on the solvent, temperature, pH and pressure. The solvents used are mostly in the liquid state and can be a pure substance or a mixture of substances. The solubility of a solute in a solvent can be measured and therefore the constant addition of a solute does not, constantly, increase its concentration in the solution. The extent of solubility varies greatly, from complete soluble to poorly soluble (Savjani, Gajjar, & Savjani, 2012). In this context, the poor solubility of some molecules, has been a growing problem in the discovery and development of new drugs. About
75% of the drugs candidates have low solubility (Di, Fish, & Mano, 2012) and belong to classes II and IV of the Biopharmaceutical Classification System (Pouton, 2006), which relate to molecules with low solubility, and more than 40% of these are practically insoluble in water (Savjani et al., 2012).

Rutin and ferulic acid, Figure 1, are examples of drugs with low solubility in aqueous medium. Thus, despite the potential of their pharmacological properties, the resulting difficulty in their delivery and bioavailability limits their use.

![Figure 1](image)

**Figure 1** – The chemical structure of **a)** ferulic (Mancuso & Santangelo, 2014) and **b)** rutin (Sharma et al., 2013).

**Active Compounds - Ferulic Acid and Rutin**

Ferulic acid, 4-hydroxy-3-methoxycinnamic acid (Ou & Kwok, 2004), is a phenolic acid (Ou & Kwok, 2004) and a constituent of the ubiquitous plant that results from the metabolism of phenylalanine and tyrosine (Srinivasan, Sudheer, & Menon, 2007). It is widely found in plants, such as rice, wheat, oats and pineapple, grasses, grains, vegetables, flowers, fruits, leaves, beans, seeds of coffee, artichoke, peanut and nuts. The amount of ferulic acid present in these products varies greatly, ranging from 0.19 mg/0.1 kg in pot grown lettuces to 800 mg/0.1 kg in sugar-beet pulp (Kumar & Pruthi, 2014).

In recent years, the interest in the possible applications of ferulic acid has increased (Srinivasan et al., 2007). This is due to its wide range of therapeutic effects including antioxidant and anticarcinogenic (Mancuso & Santangelo, 2014; Ou & Kwok, 2004; Srinivasan et al., 2007), antidiabetic and vasodilatory (Mancuso & Santangelo, 2014; Srinivasan et al.,...
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2007), hepatoprotective (Food, Rukkumani, Aruna, & Varma, 2004; Srinivasan et al., 2007), anti-inflammatory (Ou & Kwok, 2004; Srinivasan et al., 2007), antimicrobial (Ou & Kwok, 2004; Shushizadeh & Dalband, 2012), antithrombotic (Mancuso & Santangelo, 2014; Ou & Kwok, 2004) and antiviral effects (Kumar & Pruthi, 2014).

Rutin, 3’,4’,5,7-tetrahydroxy-flavone-3-rutinoside, (Chua, 2013) also known as quercetin-3-O-rutinoside (Chua, 2013), is a glycoside derivative of quercetin (Magalingam, Radhakrishnan, & Haleagrahara, 2013). It commonly occurs in fruits, especially in citrus fruits, such as orange and lemon, and plants such as buckwheat and asparagus (Magalingam et al., 2013; Sharma et al., 2013).

In the pharmaceutical industry, rutin is considered a relevant flavonoid. More than 130 therapeutic medicinal preparations contain rutin in their formulations. In addition, because of its pharmacological activity, its use as a phytochemical is increasingly attractive (Chua, 2013). As regards to its pharmacological properties rutin shows antioxidant, anti-inflammatory, antitumor and anticarcinogenic, antibacterial, antiviral, antilipidemic, antidiabetic and antihypertensive activity (Chua, 2013; Sharma et al., 2013).

Thus, it is clear that these two compounds, ferulic acid and rutin, have valuable properties whereby their incorporation into delivery systems may be a useful strategy in the pharmaceutical sciences. For example, recent studies for the development of sunscreens have shown that rutin-loaded gelatin nanoparticles in association with UV filters allowed to increase SPF as well as percentage of free radical scavenging (Oliveira et al., 2016). Furthermore, ferulic acid being similar to tyrosine, by competitive inhibition, leads to the inhibition of melanin formation and consequently to the protection of the skin against erythema induced by UV-B (Kumar & Pruthi, 2014).

Nonetheless, their low solubility in water represents a challenge to the pharmaceutical industry and thus it is fundamental to find alternatives that allow us to incorporate this drugs in delivery systems.

In this sense, ionic liquids, ILs, may be valuable as functional ingredients by enhancing drug solubility and/or drug permeation (Almeida, Júlio, Portugal, Mota, & Reis, 2017).
Ionic Liquids

Ionic liquids, are organic salts (Dobler, Schmidts, Klingenhoefer, & Runkel, 2013; Ferraz, Branco, Prudêncio, Noronha, & Petrovski, 2011), constituted by ions, an organic cation and an organic or inorganic anion, (Dobler et al., 2013; Gouveia et al., 2014) which are liquid at a temperature below 100 °C (Balk, Holzgrabe, & Meinel, 2015; Ferraz et al., 2011; Mitkare, Lakhane, & Kokulwar, 2013) or, in some cases, liquid at room temperature (Dobler et al., 2013; Gouveia et al., 2014). These compounds have various properties, such as negligible vapor pressure (Balk et al., 2015; Dobler et al., 2013; Moniruzzaman, Kamiya, & Goto, 2010), the ability to dissolve organic, inorganic and polymeric materials (Dobler et al., 2013; Moniruzzaman, Kamiya, et al., 2010), their viscosity (Gouveia et al., 2014), and they are also non-flammable (Balk et al., 2015; Ghandi, 2014), non-volatile (Ghandi, 2014; Gouveia et al., 2014), recyclable (Ghandi, 2014), and have high thermal and chemical stability and high ionic conductivity (Moniruzzaman, Kamiya, et al., 2010). But the high susceptibility to modifications is the most important property of these salts, since it allows flexibility and variability (Balk et al., 2015) enabling ILs to be synthesized with specific chemical and physical properties, towards a particular application (Dobler et al., 2013; Mitkare et al., 2013; Moniruzzaman, Kamiya, et al., 2010), by deliberately changing the anion/cation combinations, some represented in Figure 2 (Moniruzzaman, Tamura, Tahara, Kamiya, & Goto, 2010). The number of possible combinations is overwhelming, about \(10^{18}\), and shows that almost all desirable properties can be combined within an IL molecule (Egorova et al., 2017). So, ILs can be used as designer solvents, as some may represent a "green" alternative for toxic, flammable, hazardous and highly volatile organic solvents (Moniruzzaman, Kamiya, et al., 2010).

![Figure 2 - Examples of cations and anions commonly used in ionic liquids (Egorova et al., 2017).](image-url)
Regarding their synthesis and structure, ionic liquids may be considered as protic ILs, PILs, when they are formed through proton Brönsted acid to a Brönsted base, or aprotic ILs when different synthetic strategies, from the simple acid-base reactions, are required to obtain PILs. To facilitate their understanding, ionic liquids were divided into four main categories dialkylimidazolium cation, N-alkyl-pyridinium cation, phosphonium cation or a alkylammonium cation (Almeida et al., 2017).

The Imidazolium-based ILs are characterized by low viscosity, stability in oxidative and reductive reactions and easy synthesis, making them the most studied ILs. However, these ILs, in basic media, should be used with caution since side reactions have been reported (Almeida et al., 2017; Ghandi, 2014). For their applications, the imidazolium based liquids can be used as catalysts, solvents (Almeida et al., 2017) and solubility promoters (Dobler et al., 2013). However, given that ILs with the long alkyl chain attached to the imidazolium cation have greater toxicity, to their use while permeation promoters are limited, this being one of their main drawbacks (Almeida et al., 2017).

The pyridinium-based ILs are more recent than the previously reported ILs and therefore, studies on their stability, reactivity and catalytic role in organic synthesis are still in progress (Ghandi, 2014). But according to studies already conducted, the Pyridinium-based ILs show poor regioselectivity in palladium-catalyzed telomerization of butadiene with methanol (Almeida et al., 2017; Ghandi, 2014) and a negative effect on the rate of some Diels-Alder reactions (Khupse & Kumar, 2011). Despite this, the use of this type of ILs in Friedel-Crafts (Snelders & Dyson, 2011) and Grignard reactions (Almeida et al., 2017; Ghandi, 2014) has been successful. In addition, pyridinium-based ILs have shown good results as catalysts in the synthesis reactions of some pharmaceutical agents such as 1,4-dihydropyridine, dihydropyrimidinones and 3,5-bis(dodecyloxycarbonyl)-1,4-dihydropyridine derivatives (Almeida et al., 2017).

The quaternary ammonium-based ILs, when compared to imidazole-based and pyridinium-based ILs, have lower toxic effects towards the organism (Melo, Bogel-lukasik, Nunes, & Bogel-lukasik, 2013) and are thus described as less toxic (Almeida et al., 2017). Regarding their applications these ILs have a varied industrial use due to their bioactivity and antimicrobial activity (Melo et al., 2013).

These ILs can also be used as electrolytes, because their melting point and viscosity are low, and more recently have been used to enhance topical drug delivery and antibiotic activity (Almeida et al., 2017). In addition, Melo et al., in 2013, concluded that quaternary ammonium-
based ILs are appropriate solvents for drug manufacturing and such solvents can be suitable for pharmaceutical processing; in other words, these ILs may be used as an alternative to the commonly used solvents in pharmaceutical industry (Melo et al., 2013).

The Phosphonium-based ILs are more recent than imidazolium and pyridinium-based ILs (Ghandi, 2014) and thermally more stable than ammonium and imidazolium-based ILs thus making them suitable for reactions that are carried out at greater than 100 °C (Almeida et al., 2017; Ghandi, 2014) As for their applications, these ILs have been used as solvents and catalysts in several reactions and more recently for CO₂ capture (Ghandi, 2014).

However, ILs can also be classified in generations, **Figure 3**, more specifically, in three generations that differ according to their properties and chemical structures.

![Image of ILs generations](image)

**Figure 3** - Ionic liquids evolution.(Egorova et al., 2017)

The first generation, which attracted attention because of their physical properties, was sensitive to water and air and most of these ILs result from the combination of dialkylimidazolium and alkylpyridinium cations with metal halide anions. The second generation is stable to air and water being the dialkylimidazolium, alkylpyridinium, ammonium and phosphonium the most commonly used cations, whereas the halides, tetrafluoroborate and hexafluorophosphat are the most commonly used anions. Finally, the third generation of ILs is composed of biodegradable and natural ions, such as amino acids and choline, or ions with known biological activity. This generation has shown interest because of its chemical properties and possible applications in the field of ecology, biology (Egorova et al., 2017) and pharmaceutical (Almeida et al, 2017).

In general, ILs can be used as reaction media and catalysts (Hapiot & Lagrost, 2008; Miao & Tak, 2006; Sowmiah, Srinivasadesikan, Tseng, & Chu, 2009), for separations and
ex extractions (Armstrong & Welch, 2007; Moriel et al., 2010), as electrolytes (Hapiot & Lagrost, 2008), as lubricants and propellants (Balk et al., 2015) or in areas such as nanotechnology (Palacio & Bhushan, 2010) and biotechnology (Almeida et al., 2017; Balk et al., 2015). However, it is in the pharmaceutical and medical field that ILs have attracted special attention since they have several applications, namely in the synthesis of drugs, for instance as additives for enzymatic catalysis, as catalysts or as reaction media. ILs have also been used in drug delivery, as dispersing agents, as catalysts or reaction media, as solvents/antisolvents, cosolvents, copolymers or emulsifiers. More recently, APIs have been used in the form of ionic liquids API-ILs in order to decrease the polymorphism of the compounds (Egorova et al., 2017).

It becomes clear that ILs may be valuable in the pharmaceutical area and the fact that these salts may be included in aqueous, oily or hydroalcoholic solutions, represents an asset towards the development of pharmaceutical and cosmetic formulations (Santos de Almeida et al., 2017), namely to increase drug solubility and/or to enhance topical and transdermal administration. In fact, recently choline based ILs have been used as caffeine solubility enhancers and have efficiently been incorporated in O/W emulsions and gels (Santos de Almeida et al., 2017). Nonetheless, it is of the utmost importance to evaluate if these ILs have an impact on drug solubility of sparingly soluble actives. In this context, the aim of this study was to evaluate the influence of two choline-based ILs on the solubility/permeation of the poor water soluble ferulic acid and rutin.
Chapter I: Material and Reagents
1.1 Materials

Horizontal shaker Stuart®
Magnetic stirrer bars
Analytical balance Satorius® (± 0.001 g)
Thermostatic bath Memmert®
Glass Franz cells
Quartz Cells
Centrifuge Jouan BRA®
Freezer
Elastic bands
Spectrophotometer Evolution 300 UV-Vis — Thermo scientific®
Rotary evaporator with bath IKA Labortechnik HB4 basic® e Elevador IKA WERKE RV06-ML®
Desiccator
Heating and shaking incubator Heidolph UNIMAX 1010®
Current laboratory material
pH meter Metrohm 827 pH labor®, with calibration buffer at pH = 4 and pH = 7
Micropipettes P2, P10, P50, P200, P1000 and P5000
Scissors
Ultrasound Bandelin Sonorex SUPER AK 510H®
Vortex Stuart®

1.2 Reagents

Acetonitrile 99.5% (purity), VWR®
Hydrochloric Acid 1M
Ferulic Acid
Deionized water
Choline hydroxide 45% m/m in methanol, SIGMA-ALDRICH®
Sodium Hydroxide 1 M, 10 M
L-phenylalanine (Ph. Eur., USP) pure, pharma grade, PANREAC®
L-glutamine (Ph. Eur., USP) pure, pharma grade, PANREAC®
Methanol 99.9% (purity), VWR®
Rutin
Phosphate buffer pH 7.4 (USP 32)
Sodium Chloride 99.5% (purity), VWR®
Sodium Phosphate dihydrate 98.0% (purity), FLUKA®
Dihydrate dihydrate 99.9% (purity), VWR®
Chapter II: Methods
2.1 Ionic Liquid Synthesis

The two ILs derived from amino acids used, 2-hydroxyethyl-trimethylammonium-L-phenylalanine [Cho][Phe] and 2-hydroxyethyl-trimethylammonium-L-glutamate [Cho][Glu], were synthesized in the scope of this study, having been prepared according to literature (Gouveia et al., 2014) with slight modifications (Santos de Almeida et al., 2017). These ILs were characterized by $^1$H-NMR performed on a 400 MHz Brucker Avance 400® apparatus using D$_2$O.

2.2 Ionic Liquid Cytotoxicity

The cytotoxicity of the ILs studied in this work was previously evaluated (Santos de Almeida et al., 2017).

2.3 Solubility Studies

For the solubility studies, several saturated solutions were prepared with each of the active compounds, ferulic acid or rutin, in several water:IL mixtures or phosphate buffer pH 7.4:IL. For both systems were also evaluated the respective blank samples, active in deionized water or active in phosphate buffer, PBS, at pH 7.4. These solutions were then placed on a horizontal shaker at 25 ºC and 32 ºC for 72 h.

All solutions were prepared in triplicate and after filtration to remove the excess solute, the samples were analysed by UV-visible spectrophotometry at the maximum absorption wavelength of the active substance under study.

2.4 Permeation Studies

In the permeation studies (n=5) vertical diffusion glass cells (Franz cells) were used with a volume of approximately 4 mL in the receiver compartment and a diffusion area of 0.95 cm$^2$ (Santos de Almeida et al., 2017) using silicone membrane.
For this study, 500 μL of the saturated solution of the active ingredient was placed in the donor compartment and in the recipient compartment pH 7.4 phosphate buffer was placed as medium. Thereafter, the receiver compartment is placed in a thermostated bath at 37 °C to ensure that the silicone membrane is at 32 °C.

At pre-established time intervals, complete collection of the medium in the receiver compartment was performed and then a new pre-heated medium was replaced. By UV-visible spectrophotometry the quantitative analysis of the medium was performed. The determination of the steady state flow of the active is obtained by determining the cumulative amount of drug diffusing and measuring the slope of the graph.

2.5 pH Measurements

The pH of all prepared solutions was evaluated with a pH meter Metrohm 827 pH labor® and calibration buffer at pH= 4 and pH= 7.
Chapter III: Results and Discussion
In this work two different ionic liquids, derived from amino acids, [Cho][Phe] and [Cho][Glu], were prepared to evaluate their ability as solubility and permeation enhancers of the two actives studied, ferulic acid and rutin. The ionic liquids used were synthesized according to the literature (Almeida et al., 2017).

3.1 Solubility and Influence of pH

To understand the influence of the IL content on the solubility of each active, the solubility studies, were carried out in the presence of different water:IL mixtures and PBS pH 7.4:IL mixtures. Thus, several percentages of ILs, namely, 0.1; 0.2; 0.5 and 1% (m/m), were studied. Additionally, the pH of all the prepared mixtures was also evaluated.

3.1.1 Ferulic Acid

The obtained solubility of ferulic acid in water, at 25 °C, 0.64 mg/mL, is in agreement with the results published in the literature of 0.78 mg/mL (Mota, Queimada, & Pinho, 2008). Furthermore, when evaluating the influence of the ILs on the solubility of ferulic acid, at 25 °C and 32 °C, results show that the solubility in water:IL mixtures are always higher than its solubility in water, as shown in Table 1 and Figures 4 and 5. Additionally, between the two ILs studied, at both temperatures, [Cho][Phe] is shown to be the better solubility promoter, which may be indicative of a higher affinity between the active and this IL. Moreover, at both temperatures, it was observed that when the percentage of IL increases, the solubility of ferulic acid also increases.
Table 1 - Solubility of ferulic acid in deionized water and in different deionized water:IL mixtures.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Water:IL Ratio</th>
<th>25 °C Average</th>
<th>25 °C SD</th>
<th>32 °C Average</th>
<th>32 °C SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>100:0</td>
<td>0.64</td>
<td>0.02</td>
<td>0.73</td>
<td>0.06</td>
</tr>
<tr>
<td>Water:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Cho][Phe]</td>
<td>99.9:0.1</td>
<td>0.87</td>
<td>0.02</td>
<td>1.00</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>99.8:0.2</td>
<td>1.30</td>
<td>0.08</td>
<td>1.47</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>99.5:0.5</td>
<td>2.47</td>
<td>0.19</td>
<td>2.62</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>99:0:1</td>
<td>4.02</td>
<td>0.37</td>
<td>5.39</td>
<td>0.24</td>
</tr>
<tr>
<td>Water:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Cho][Glu]</td>
<td>99.9:0.1</td>
<td>0.76</td>
<td>0.03</td>
<td>1.25</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>99.8:0.2</td>
<td>0.78</td>
<td>0.01</td>
<td>1.43</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>99.5:0.5</td>
<td>1.42</td>
<td>0.06</td>
<td>1.68</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>99:0:1</td>
<td>1.97</td>
<td>0.1</td>
<td>2.90</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Figure 4 - Solubility of ferulic acid at 25 °C for water and water:IL mixtures with proportions ranging from 99.9:0.1 to 99:1 (n = 3) (where * p<0.05, ** p<0.01, *** p<0.001 (ANOVA, Tukey's test)).
Figure 5 - Solubility of ferulic acid at 32 °C for water and water:IL mixtures with proportions ranging from 99.9:0.1 to 99:1 (n = 3) (where * p<0.05, ** p<0.01, *** p<0.001 (ANOVA, Tukey’s test)).

Regarding the solubility of ferulic acid in phosphate buffer, results are similar at both temperatures and once again the solubility in the presence of ILs is always higher than in phosphate buffer pH 7.4, as shown in Table 2 and Figure 6 and 7. Additionally, results show once more that [Cho][Phe] is the best solubility promoter and that higher percentages of IL have a higher impact on the solubility of ferulic acid.

Table 2 - Solubility of ferulic acid in phosphate buffer pH 7.4 and in different phosphate buffer pH 7.4:IL mixtures.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Phosphate buffer:IL ratio</th>
<th>Solubility (mg/mL)</th>
<th>25 °C</th>
<th>SD</th>
<th>32 °C</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate buffer</td>
<td>100:0</td>
<td>3.62</td>
<td>0.23</td>
<td></td>
<td>6.61</td>
<td>0.20</td>
</tr>
<tr>
<td>Phosphate buffer:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Cho][Phe]</td>
<td>99.9:0.1</td>
<td>7.31</td>
<td>0.54</td>
<td></td>
<td>7.35</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>99.8:0.2</td>
<td>8.18</td>
<td>0.57</td>
<td></td>
<td>9.23</td>
<td>1.01</td>
</tr>
<tr>
<td></td>
<td>99.5:0.5</td>
<td>10.29</td>
<td>1.27</td>
<td></td>
<td>10.59</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>99.0:1</td>
<td>12.38</td>
<td>1.00</td>
<td></td>
<td>12.85</td>
<td>0.03</td>
</tr>
<tr>
<td>Phosphate buffer:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Cho][Glu]</td>
<td>99.9:0.1</td>
<td>7.46</td>
<td>0.65</td>
<td></td>
<td>7.18</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>99.8:0.2</td>
<td>7.88</td>
<td>0.60</td>
<td></td>
<td>8.72</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>99.5:0.5</td>
<td>9.87</td>
<td>1.82</td>
<td></td>
<td>9.89</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>99.0:1</td>
<td>10.23</td>
<td>1.57</td>
<td></td>
<td>11.56</td>
<td>0.60</td>
</tr>
</tbody>
</table>
Figure 6 - Solubility of ferulic acid at 25 °C phosphate buffer pH 7.4 and phosphate buffer pH 7.4:IL mixtures with proportions ranging from 99.9:0.1 to 99:1 (n=3) (in That * p<0.05, ** p<0.01, *** p<0.001 (ANOVA, Tukey's test)).

Figure 7 - Solubility of ferulic acid at 32 °C phosphate buffer pH 7.4 and phosphate buffer pH 7.4:IL mixtures with proportions ranging from 99.9:0.1 to 99:1 (n=3) (where * p<0.05, ** p<0.01, *** p<0.001 (ANOVA, Tukey's test)).
When comparing the solubility in water and PBS pH 7.4 and in the water:IL and PBS 7.4:IL mixtures, the analysis of the obtained results, presented in Table 3 and Figures 8-11, allows to conclude that the solubility of ferulic acid is always higher in phosphate buffer than in water, which would be expected since this compound is an acid and its solubility is expected to be lower in more acidic solutions. The observed enhancement in the solutions pH arises from the presence of the choline-based ILs, which have a high basic pH.

Table 3 - pH values of ferulic acid in water and phosphate buffer pH 7.4 and in different water:IL and phosphate buffer pH 7.4:IL mixtures.

<table>
<thead>
<tr>
<th>IL</th>
<th>Mixture Ratio</th>
<th>Water:IL</th>
<th>Phosphate buffer:IL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100:0</td>
<td>3.64</td>
<td>5.35</td>
</tr>
<tr>
<td>[Cho][Phe]</td>
<td>99.9:0.1</td>
<td>4.52</td>
<td>5.92</td>
</tr>
<tr>
<td></td>
<td>99.8:0.2</td>
<td>4.82</td>
<td>5.95</td>
</tr>
<tr>
<td></td>
<td>99.5:0.5</td>
<td>5.28</td>
<td>5.97</td>
</tr>
<tr>
<td></td>
<td>99.0:1</td>
<td>5.60</td>
<td>6.05</td>
</tr>
<tr>
<td>[Cho][Glu]</td>
<td>99.9:0.1</td>
<td>4.21</td>
<td>5.86</td>
</tr>
<tr>
<td></td>
<td>99.8:0.2</td>
<td>4.23</td>
<td>5.88</td>
</tr>
<tr>
<td></td>
<td>99.5:0.5</td>
<td>4.50</td>
<td>5.90</td>
</tr>
<tr>
<td></td>
<td>99.0:1</td>
<td>4.54</td>
<td>5.89</td>
</tr>
</tbody>
</table>

Figure 8 - Solubility of ferulic acid at 25 °C in water, in phosphate buffer pH 7.4 or in water:[Cho][Phe] or phosphate buffer pH 7.4:[Cho][Phe] mixtures with proportions ranging from 99.9:0.1 to 99:1 (n=3) (where * p<0.05, ** p<0.01, *** p<0.001 (ANOVA, Tukey’s test)).
Figure 9 - Solubility of ferulic acid at 25 °C in water, in phosphate buffer pH 7.4 or in water:[Cho][Glu] or phosphate buffer pH 7.4:[Cho][Glu] mixtures with proportions ranging from 99.9:0.1 to 99:1 (n=3) (where * p<0.05, ** p<0.01, *** p<0.001 (ANOVA, Tukey’s test)).

Figure 10 - Solubility of ferulic acid at 32 °C in water, in phosphate buffer pH 7.4 or in water:[Cho][Phe] or phosphate buffer pH 7.4:[Cho][Phe] mixtures with proportions ranging from 99.9:0.1 to 99:1 (n=3) (where * p<0.05, ** p<0.01, *** p<0.001 (ANOVA, Tukey’s test)).
Regarding the solubility of rutin in water, the obtained results at 25 °C, 0.20 mg/mL, are slightly higher than the results published in the literature, 0.125 mg/mL (Pedriali, Fernandes, Bernusso, & Polakiewicz, 2008). Additionally, results show that the solubility of the rutin in water:IL mixtures, is always higher when compared to the solubility in deionized water, as shown in Table 4 and Figures 12 and 13. Once again the [Cho][Phe] proves to be the better solubility promoter. Furthermore, higher percentages of IL enable a higher enhancement of the rutin solubility.
**Table 4** - Solubility of the rutin in deionized water and in different deionized water:IL mixtures.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Water:IL ratio</th>
<th>25 °C</th>
<th>Average</th>
<th>SD</th>
<th>32 °C</th>
<th>Average</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>100:0</td>
<td>0.20</td>
<td>0.01</td>
<td></td>
<td>0.20</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Water: [Cho][Phe]</td>
<td>99.9:0.1</td>
<td>0.62</td>
<td>0.04</td>
<td></td>
<td>0.63</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>99.8:0.2</td>
<td>1.15</td>
<td>0.88</td>
<td></td>
<td>1.62</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>99.5:0.5</td>
<td>4.45</td>
<td>0.15</td>
<td></td>
<td>4.64</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td>99:0.1</td>
<td>7.25</td>
<td>0.21</td>
<td></td>
<td>8.50</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>Water: [Cho][Glu]</td>
<td>99.9:0.1</td>
<td>0.36</td>
<td>0.02</td>
<td></td>
<td>0.43</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>99.8:0.2</td>
<td>0.68</td>
<td>0.03</td>
<td></td>
<td>0.86</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>99.5:0.5</td>
<td>1.05</td>
<td>0.04</td>
<td></td>
<td>1.14</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>99:0.1</td>
<td>2.12</td>
<td>0.28</td>
<td></td>
<td>2.12</td>
<td>0.06</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 12** - Solubility of rutin at 25 °C for water and water:IL mixtures with proportions ranging from 99.9:0.1 to 99:1 (n = 3) (where * p<0.05, ** p<0.01, *** p<0.001 (ANOVA, Tukey's test)).
In regards to the solubility of rutin in phosphate buffer, data presented in Table 5 and Figures 14 and 15, it was found that, as observed in water:IL mixtures, the solubility of the rutin in the phosphate buffer pH 7.4:IL mixtures is always higher than the solubility in phosphate buffer pH 7.4, except for the percentage of 0.1 of the [Cho][Glu] were no significant differences were observed; and that [Cho][Phe] appears to be, at both temperatures, a better promoter of solubility. In addition, it has been observed that, at both temperatures, as the percentage of IL increases the solubility of the rutin also increases.

\[ \text{Solubility (mg/mL)} \]

![Solubility of rutin at 32 °C for water and water:IL mixtures with proportions ranging from 99.9:0.1 to 99:1 (n = 3) (where * p<0.05, ** p<0.01, *** p<0.001 (ANOVA, Tukey's test)).}
Table 5 - Solubility of rutin in phosphate buffer pH 7.4 and in different phosphate buffer pH 7.4: IL mixtures.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>phosphate buffer:IL ratio</th>
<th>Solubility (mg/mL)</th>
<th>25 °C</th>
<th>32 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Average</td>
<td>SD</td>
<td>Average</td>
</tr>
<tr>
<td>Phosphate buffer</td>
<td>100:0</td>
<td>0.49</td>
<td>0.03</td>
<td>0.64</td>
</tr>
<tr>
<td>Phosphate buffer:</td>
<td>99.9:0.1</td>
<td>0.55</td>
<td>0.04</td>
<td>0.69</td>
</tr>
<tr>
<td>[Cho][Phe]</td>
<td>99.8:0.2</td>
<td>0.61</td>
<td>0.01</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>99.5:0.5</td>
<td>0.87</td>
<td>0.04</td>
<td>1.24</td>
</tr>
<tr>
<td></td>
<td>99.0:1</td>
<td>2.43</td>
<td>0.26</td>
<td>1.96</td>
</tr>
<tr>
<td>Phosphate buffer:</td>
<td>99.9:0.1</td>
<td>0.47</td>
<td>0.04</td>
<td>0.67</td>
</tr>
<tr>
<td>[Cho][Glu]</td>
<td>99.8:0.2</td>
<td>0.50</td>
<td>0.02</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td>99.5:0.5</td>
<td>0.65</td>
<td>0.01</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>99.0:1</td>
<td>0.68</td>
<td>0.04</td>
<td>0.94</td>
</tr>
</tbody>
</table>

Figure 14 - Solubility of rutin at 25 °C phosphate buffer pH 7.4 and phosphate buffer pH 7.4:IL mixtures with proportions ranging from 99.9:0.1 to 99.1 (n=3) (in That * p<0.05, ** p<0.01, *** p<0.001 (ANOVA. Tukey's test)).
Relative to the pH values, according to Table 6, it was found that the presence of ILs significantly alters the pH in water, whereas in phosphate buffer pH 7.4 the variations are less significant, as would be expected.

Comparing the solubility of rutin in water and in phosphate buffer pH 7.4, data shown in Figures 16-19, show that at both temperatures, the solubility is higher in water than in phosphate buffer.
Table 6 - pH values of rutin in water and phosphate buffer pH 7.4 and in different water:IL and phosphate buffer pH 7.4: IL mixtures.

<table>
<thead>
<tr>
<th>IL</th>
<th>Mixtures ratio</th>
<th>Water:IL</th>
<th>phosphate buffer:IL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100:0</td>
<td>6.49</td>
<td>7.26</td>
</tr>
<tr>
<td>[Cho][Phe]</td>
<td>99.9:0.1</td>
<td>7.49</td>
<td>7.41</td>
</tr>
<tr>
<td></td>
<td>99.8:0.2</td>
<td>8.02</td>
<td>7.52</td>
</tr>
<tr>
<td></td>
<td>99.5:0.5</td>
<td>8.62</td>
<td>7.72</td>
</tr>
<tr>
<td></td>
<td>99.0:1</td>
<td>8.87</td>
<td>8.31</td>
</tr>
<tr>
<td>[Cho][Glu]</td>
<td>99.9:0.1</td>
<td>6.44</td>
<td>7.28</td>
</tr>
<tr>
<td></td>
<td>99.8:0.2</td>
<td>6.55</td>
<td>7.30</td>
</tr>
<tr>
<td></td>
<td>99.5:0.5</td>
<td>7.17</td>
<td>7.36</td>
</tr>
<tr>
<td></td>
<td>99.0:1</td>
<td>7.56</td>
<td>7.38</td>
</tr>
</tbody>
</table>

Figure 16 - Solubility of rutin at 25 °C in water, in phosphate buffer pH 7.4 or in water:[Cho][Phe] or phosphate buffer pH 7.4:[Cho][Phe] mixtures with proportions ranging from 99.9:0.1 to 99:1 (n=3) (where * p<0.05, ** p<0.01, *** p<0.001 (ANOVA, Tukey's test)).
Figure 17 - Solubility of rutin at 25 °C in water, in phosphate buffer pH 7.4 or in water:[Cho][Glu] or phosphate buffer pH 7.4:[Cho][Glu] mixtures with proportions ranging from 99.9:0.1 to 99:1 (n=3) (where * p<0.05, ** p<0.01, *** p<0.001 (ANOVA, Tukey's test)).

Figure 18 - Solubility of rutin at 32 °C in water, in phosphate buffer pH 7.4 or in water:[Cho][Phe] or phosphate buffer pH 7.4:[Cho][Phe] mixtures with proportions ranging from 99.9:0.1 to 99:1 (n=3) (where * p<0.05, ** p<0.01, *** p<0.001 (ANOVA, Tukey's test)).

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Figure 1 - Solubility of rutin at 32 °C in water, in phosphate buffer pH 7.4 or in water:[Cho][Glu] or phosphate buffer pH 7.4:[Cho][Glu] mixtures with proportions ranging from 99.9:0.1 to 99:1 (n=3) (where * p<0.05, ** p<0.01, *** p<0.001 (ANOVA, Tukey's test)).

3.2 Permeation studies

To evaluate the influence of ILs on the permeation of ferulic acid and rutin, permeation tests were performed with saturated solutions of each active. These solutions were placed in the donor compartment to ensure that the chemical potential between the Franz cells was equal and consequently that the motive force was constant and the coefficient of variability decreased. Since the barrier properties of the membrane are not affected by the carrier and/or solvent that permeates, the permeation flow of the saturated solution is independent of these factors.

3.2.1 Ferulic Acid

Results show that the ILs did not influence the ferulic acid permeation, since there were no significant variations in the flux values obtained, as shown in Figure 20.
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Figure 20 - Results (mean ± standard deviation of 3 independent assays where * p<0.05, ** p<0.01, *** p<0.001 (ANOVA, Tukey's test)) of the steady state fluxes of the permeate assay of ferulic acid saturated solutions in phosphate buffer pH 7.4 and in the phosphate buffer pH 7.4: IL mixture (99.8:0.2).

3.2.2 Rutin

For rutin, results seem to indicate that the ILs may have a positive influence on the permeation of this active, as shown in Figure 21. However, the obtained values are below the detection capacity of the UV-Visible apparatus used and since they are not within the calibration curve, this test must be repeated to confirm these values.

Figure 21 - Results (mean ± standard deviation of 3 independent assays where * p<0.05, ** p<0.01, *** p<0.001 (ANOVA, Tukey's test)) of the steady state fluxes of the permeate assay of rutin saturated solutions in phosphate buffer pH 7.4 and in the phosphate buffer pH 7.4: IL mixture (99.8:0.2).
Conclusion
The aim initially established for this work was reached, since it was possible to evaluate the influence of two choline-based ILs, [Cho][Phe] and [Cho][Glu] on the solubility/permeation of the studied poor soluble actives. This study demonstrated that the solubility of ferulic acid and rutin increased in the presence of both ILs in both studied temperatures, 25 °C and 32 °C, and in both mixtures, water:IL and phosphate buffer pH 7.4: IL.

Moreover, it was observed that, at both temperatures, the solubility of ferulic acid in PBS pH 7.4 and in PBS pH 7.4:IL mixtures is superior to the solubility, of the same active, in water and water: IL mixtures. However, unlike ferulic acid, the rutin, at both temperatures, exhibits higher solubility in water:IL mixtures than in PBS pH 7.4:IL mixtures. Additionally, for both active and at both temperatures, [Cho][Phe] was shown to be a better promoter of solubility when compared to [Cho][Glu].

Regarding the permeation studies with saturated solutions of ferulic acid and rutin, in the presence and absence of the studied ILs, at a percentage of ILs were cell viability is maintained, 0.2% (m/m), allowed to conclude that the presence of the ILs did not influence the ferulic acid permeation. For rutin, although results may indicate a slight positive influence in the permeation of these active, these tests need to be repeated in the future to confirm the flux values obtained.

In conclusion, the ionic liquids studied had a positive impact on the solubility allowing the increase of the solubility of the active compounds studied. This is a very relevant result in view of the low solubility of these compounds in water. Moreover, the fact that the ILs studied do not significantly influence the permeation of the studied assets, may be useful to guarantee a low incidence of adverse effects in topical formulations where a low permeation of the incorporated asset is sought. Hence, the results obtained may be very promising towards the incorporation of higher amounts of poorly soluble drugs in drug delivery systems.
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