ANA LUÍSA GOMES JÚLIO

IONIC LIQUIDS AS FUNCTIONAL INGREDIENTS IN DRUG DELIVERY SYSTEMS: SOLUBILITY, PERMEATION AND CYTOTOXICITY STUDIES

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Universidade Lusófona de Humanidades e Tecnologias

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Lisboa

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Dissertação defendida em provas públicas na Universidade Lusófona de Humanidades e Tecnologias no dia 27 de Setembro de 2017, perante o júri, nomeado pelo Despacho de Nomeação n.º: 294/2017 de 20 de Setembro, com a seguinte composição:

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Escola de Ciências e Tecnologias da Saúde

Lisboa

2017

In the middle of difficulty lies opportunity.

Albert Einstein

Acknowledgments

After all the work, today is the day to thank all of those who have supported and helped me so much in this journey. It has been a period of intense learning for me, not only professionally, but also on a personal level.

I would like to thank the Research Center for Biosciences & Health Technologies, CBIOS, for providing me excellent laboratory facilities. I also would like to acknowledge the Centro de Química e Bioquímica, CQB, of Faculdade de Ciências da Universidade de Lisboa and São Paulo Research Foundation, FAPESP, for the collaboration and Suinimais, Lda. for donating the porcine ears for the skin permeation studies.

I would like to express my sincere acknowledgements to the following people. First, I would like to thank my supervisor Professor Tânia Almeida. You were the person who discovered me for research, being able to see who I was and what I liked, when not even I knew who I was, what I liked and what direction I would take in my professional life. More than a mentor, you have become a friend who I know supports me and who I can count on. You were always available to listen to me, help me and support me greatly. I also must thank you for all the advices, as well as for the opportunities you have given me, and for everything you have taught me, as for all the time you have spent with me in the laboratory. This work would never have been completed without your guidance.

Additionally, I would like to thank Professor Joana Portugal Mota for her valuable teaching. You gave me tools I needed to improve my skills. Without your passionate participation and input, my work in the development of drug delivery systems would not have been so successful.

I would also want to thank Professor Catarina Rosado, not only for her precious guidance during the permeation studies, but also for all her encouragement.

I would like to express my acknowledgements to Professor Ana Sofia Fernandes and Professor Nuno Saraiva, members of Pharmacology and Therapeutics Group, PT Group, of CBIOS, for doing the cytotoxicity assays. And I would also like to acknowledge Professor Nuno Saraiva for helping me with the microscopy analysis. Both of you were also very important in this research and a valuable support.

It is also important to acknowledge, Professor Maria Eduarda Araújo, member of CQB, that provide us the imidazole-based ionic liquids and allowed us to perform the Nuclear Magnetic Resonance Spectrum to confirm the structure of the synthesised choline-based ionic liquids.

Finally, I express my gratitude to my family and my little stars that wherever you are I know you are looking for me. Particularly, I would like to thank my parents for providing me with unfailing support and encouragement throughout my years of study and through this research path. This work and dissertation would not have been possible without you, because you are my safe harbour.

I also must mention my friends and colleagues, since they also were an important part of this work. You listened to my problems and my difficulties, you understood those days that I did not have time for you and you also worked with me in the days that I needed help.

Thank you very much!

Abstract

Poor drug solubility/loading and stability, represents a problem in the development of drug delivery systems.

Since ionic liquids can be placed in either lipophilic or hydrophilic solutions, they may be helpful to overcome these problems. Nonetheless, it is vital to determine their usefulness when used at concentrations were cell viability is maintained, which was considered herein.

Five different ILs were studied - three imidazole-based ILs: [C2mim][Br], [C4mim][Br] and [C6mim][Br]; and two choline based ILs: [Cho][Phe] and [Cho][Glu]. Their cytotoxicity in human keratinocytes, their influence in drug solubility and in permeation, and their influence in drug release from lipidic implants, was evaluated.

Caffeine and salicylic acid were used as model actives. Choline-based ILs proved to be more suitable as functional ingredients, since they showed higher impact on drug solubility and lower cytotoxicity. The major solubility enhancement was observed for caffeine and further solubility studies were carried out with this active in several concentrations of these ILs, 0.1; 0.2; 0.5; 1.0; 3.0 and 5.0%, w/w, at 25 °C and 32 °C. Solubility was greatly influenced by concentrations up to 0.5%. The choline-based ILs showed no significant impact on the skin permeation, for both actives. For the imidazole-based ILs the size of the alkyl chain enhances the caffeine solubility and permeation, but also the ILs cytotoxicity, limiting their use.

O/W emulsions and gels were prepared containing the less toxic choline-based ILs and caffeine. All prepared formulations were stable. Lipidic implants containing IL and salicylic acid were also successfully prepared.

Our results indicate that the choline-based ILs were effective functional ingredients, since, when used at non-toxic concentrations, they allowed a higher drug loading, in topical formulations, while maintaining their stability, and allowed a higher drug release from lipidic implants.

Keywords: Ionic Liquids; solubility enhancement; pig skin permeation; cytotoxicity; drug delivery systems

Resumo

A baixa solubilidade/incorporação e estabilidade de alguns fármacos, representa um problema no desenvolvimento de sistemas de veiculação de fármacos.

Como os líquidos iónicos, LI, podem ser colocados em soluções lipofílicas ou hidrófilas, podem ser úteis para superar estas dificuldades. No entanto, é crucial determinar a sua utilidade em quantidades onde se mantenha a viabilidade celular, facto que foi considerado neste estudo.

Cinco LI diferentes foram estudados - três LI derivados de imidazol: [C2mim][Br], [C4mim][Br] e [C6mim][Br]; e dois LI derivados da colina: [Cho][Phe] e [Cho][Glu]. Foi avaliada a sua citotoxicidade, a sua influência na solubilidade e na permeação cutânea dos fármacos estudados e a sua influência na libertação de fármacos a partir de implantes lipídicos.

A cafeína e o ácido salicílico foram utilizados como ativos modelo. Os LI derivados da colina revelaram-se mais adequados como ingredientes funcionais, dado apresentarem maior impacto na solubilidade do fármaco e uma menor citotoxicidade. O maior aumento de solubilidade foi observado para a cafeína, tendo sido realizados outros estudos de solubilidade com este ativo em várias concentrações desses LI, 0,1; 0,2; 0,5; 1,0; 3,0 e 5,0%, m/m, a 25 °C e 32 °C. A solubilidade foi fortemente influenciada nas concentrações até 0,5 %. Os LI derivados de colina não mostraram um impacto significativo na permeação de nenhum dos fármacos. Para os LI derivados de imidazol, o tamanho da cadeia de alquilo aumenta a solubilidade e permeação da cafeína, mas também a citotoxicidade do LI, limitando seu uso.

As emulsões A/O e geles foram preparados contendo os LI menos tóxicos, derivados de colina, e a cafeína. Todas as formulações preparadas foram estáveis. Os implantes lipídicos, contendo LI e ácido salicílico, também foram preparados com sucesso.

Os resultados indicam que os LI derivados de colina eram ingredientes funcionais efetivos, pois quando utilizados em concentrações não tóxicas, permitiram um aumento da concentração de fármaco, em formulações tópicas, mantendo sua estabilidade e permitiram uma maior liberação de fármaco a partir de implantes lipídicos.

Palavra-Chave: Líquidos Iónicos; promoção da solubilidade; permeação em pele de porco; sistemas de veiculação de fármacos.

List of abbreviations and acronyms

% V/V	Percentage volume by volume
% w/w	Percentage weight by weight
%	Percentage
[C2mim][Br]	1-ethyl-3-methylimidazolium bromide
[C4mim][Br]	1-butyl-3-methylimidazolium bromide
[C6mim][Br]	1-hexyl-3-methylimidazolium bromide
[Cho][Glu]	(2-hydroxyethyl)-trimethylammonium-L-glutamate
[Cho][OH]/MeOH	Cholinium hydroxide in methanol
[Cho][Phe]	(2-hydroxyethyl)-trimethylammonium-L-phenylananinate
±	More or Less
µg	Microgram
ºC	Degrees Celsius
ANOVA	One-way analysis of variance
APIs	Active Pharmaceutical Ingredients
BHT	Butylated hydroxytoluene
CBIOS	Research Center for Biosciences & Health Technologies
cm	Centimetre
cm^2	Square centimetre
cm^3	Cubic centimetre
CQB	Centro de Química e Bioquímica
Crodafos CES	Cetearyl alcohol and diacetyl phosphate and ceteth-10 phosphate
DDS	Drug delivery systems

Disodium EDTA	Ethylenediamine tetraacetic acid disodium
Dynasan® 118	Glycerin triestearate
FAPESP	São Paulo Research Foundation
FDA	Food and Drug Administration
g	Gram
g/mol	Gram by mole
h	Hours
IL	Ionic Liquid
M	Molar
mg	Milligram
mg/mL	Milligram by millilitres
mins	Minute
MIP	Isopropyl myristate
mL	Millilitres
mmol	Millimole
mPa.s	Millipascal-second
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide
n	Number of samples
nm	Nanometres
O/W	Oil in water
PBS	Phosphate-buffered saline
PEG 400	Polyethylene glycol 400
PT Group	Pharmacology and Therapeutics Group

q.s.	Quantity sufficient
RT	Room Temperature
SD	Standard deviation
USA	United States of America
USP 32	United States Pharmacopeia 32
UV	Ultraviolet radiation
x g	Times gravity
λ	Maximum absorption wavelength

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1. Introduction

The Pharmaceutical Industry spends a great amount of their financial resources in drug development, to improve the safety and efficacy of the developed drugs. Additionally, to overcome weaknesses such as poor drug solubility/loading and release, and reduced stability, amongst others, it is also crucial for the pharmaceutical field to develop new and more efficient Drug Delivery Systems, DDS (Almeida, Júlio, Mota, Rijo, & Reis, 2017; Tiwari et al., 2012).

DDS are formulations or devices that introduce the drug in the body, increase the efficacy and safety of the drug, and control the rate, time and place of its release. Hence, DDS are considered an interface between the drug and the body (Jain, 2008). These systems are also used to solve some non-ideal properties of drugs that interpose with their therapeutic implications, such as poor solubility and biodistribution, unfavorable pharmacokinetics, tissue damage on extravasation, rapid breakdown of the drug *in vivo* and low selectivity for target tissues (Allen, 2004). Additionally, some DDS have limited stability, which represents an additional challenge. Because of these difficulties, several strategies have been made to develop new and/or more efficient DDS, as well as to find new functional ingredients that may increase drug solubility, loading, permeation and stability.

In this sense, some ionic liquids, ILs, may be potential candidates as solvents, or even as vehicles, in DDS to solve or minimize these problems, since they are substances that may be introduced in water, oils or alcoholic solutions, due to their peculiar characteristics, arising from their structures. In our days, some studies have already shown the use of ILs to enhance drug solubility, permeation and stability (Almeida et al., 2015; Dobler, Schmidts, Klingenhoefer, & Runkel, 2012; Frade & Afonso, 2010; Frizzo et al., 2013; Hough et al., 2007; Mizuuchi, Jaitely, Murdan, & Florence, 2008; Stoimenovski, MacFarlane, Bica, & Rogers, 2010).

ILs are salts in which ions, an organic cation and an organic or inorganic anion, are poorly coordinated, resulting in solvents that are liquid below 100 °C or even at room temperature, RT. Furthermore, ILs have key properties, like high thermal and chemical stability, low vapour pressure, nonflammability, negligible volatile and re-usability (Almeida et al., 2015; Earle et al., 2006; Ghandi, 2014; Kubota, Shibata, & Yamaguchi, 2016). Depending on the type of ions used, the ILs can be miscible, immiscible or partially miscible with water, so by changing their chemical structure we can adjust the solvent characteristics of these salts (Czekanski, Santos de Almeida, Portugal Mota, Rijo, & Araújo, 2014; Gouveia et al., 2014).

Most importantly, the high suitability of these salts for alterations, allows the modification of their overall properties (Ghandi, 2014).

As a consequence of the above mentioned properties, these salts have been used in the Pharmaceutical Industry with different purposes, namely as extractants of pharmaceutical compounds from aqueous solutions (Álvarez, Esperança, Deive, Sanromán, & Rodríguez, 2015; Marrucho, Branco, & Rebelo, 2014; Mitkare, Lakhane, & Kokulwar, 2013), as solubility promotors (Marrucho et al., 2014) and as solvents and catalysts of active pharmaceutical ingredients, APIs (Marrucho et al., 2014; Shamshina, Barber, & Rogers, 2013). Also, some studies have used ILs as oil or water substitutes, as additives or as surfactants in emulsions and micro emulsions (Dobler et al., 2012; Lawrence & Rees, 2000; Qiu & Texter, 2008; Zech et al., 2009; Zhu et al., 2009). Hence, there are multiple and valuable uses for these salts.

ILs may be divided in four categories depending on the cation present in their composition: either a dialkylimidazolium cation, an N-alkylpyridinium cation, an alkylammonium cation or a phosphonium cation (Almeida et al., 2017). Nonetheless, since the imidazole-based ILs are amongst the most studied ILs and the choline-based ILs have been considered less toxic (Gouveia et al., 2014) this study focus on these two particular classes of ILs, shown in **Figure 1**, and in their applicability as functional ingredients in DDS.

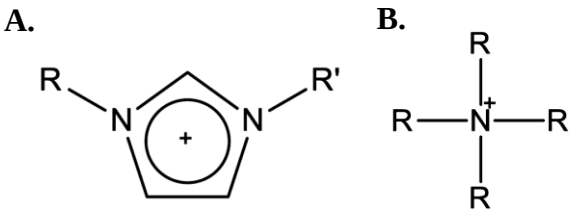

Figure 1: **A.** Dialkylimidazolium and **B.** Alkylammonium cations contained in the ILs used herein.

The reason why Imidazole-based ILs are amongst the most studied ILs, is due to their lower viscosity and to the stability of the imidazolium cation in oxidative and reductive conditions. Hence, they have been used for several applications, such as solvents and catalysts in synthesis and as solubility enhancers in DDS. In terms of their use as solubility promoters, their cytotoxicity may represent a drawback, particularly for those ILs with a long alkyl chain attached to the imidazolium cation and this should always be considered (Almeida et al., 2017).

On the other hand, some choline based ILs, derived from amino acids, have been described has less toxic (Gouveia et al., 2014) and consequently they may be more suited to incorporate in DDS. These ILs have choline as a cation and different amino acids as the anion. Choline is an essential micronutrient for cells and the amino acids are nontoxic, biocompatible and biodegradable compounds and that is why these ILs are less toxic and more biodegradable and biocompatibility than other ILs not derived from non-renewable sources. In terms of their applicability, they have also been used as solvents and catalysts in synthesis (De Santis et al., 2015; Gouveia et al., 2014). Nonetheless, in terms of their utility in DDS, their utility at different concentrations and their incorporation in formulations at non-toxic concentrations needs to be considered.

To evaluate the influence of the studied Ionic liquids on drug solubility, loading and realise, to active models, widely used in the pharmaceutical field, have been used in this study, caffeine as a hydrophilic model and salicylic acid as a more lipophilic model.

Caffeine, 1,3,7-trimethylpurine-2,6-dione (Pubchem: Open chemistry database, n.d.-a), which structure is shown in **Figure 2**, is a natural methylxanthine alkaloid that may act, as a mild stimulant of the central nervous system (Ferré, 2016), as an alertness and agitation promotor in systemic uses, as a cosmetologically active ingredient to prevent accumulation of fat in cells and as an antioxidant (Herman & Herman, 2012). Since caffeine, during lipolysis, stimulates the fats cells degradation by the inhibition of the phosphodiesterase activity, by the stimulation of β-adrenergic receptors and by the increase of cyclic adenosine monophosphate levels, it has the capacity to act as an anti-cellulite substance (Herman & Herman, 2012; Lupi, Semenovitch, Treu, Bottino, & Bouskela, 2007; Velasco et al., 2008). On the other hand, its antioxidant capacity is due to the ability to protect cells against the UV radiation, which slows the photoaging process of the skin (Herman & Herman, 2012). The antioxidant capacity of caffeine comes from its ability to improve the cellular redox status followed by increasing the levels of intracellular glutathione (Jung, Kim, Park, Jeong, & Ko, 2017; Vignoli, Bassoli, & Benassi, 2011).

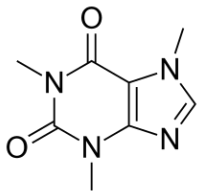

Figure 2: Caffeine Structure (Pubchem: Open chemistry database, n.d.-a).

Hence, because caffeine has several characteristics useful for both the pharmaceutical and the cosmetic fields and since it is a relatively accessible drug it has been widely used as active model and that is why it was chosen in this present study as a more hydrophilic drug model.

On the other, Salicylic acid, 2-hydroxybenzoic acid (Pubchem: Open chemistry database, n.d.-b), which structure is shown in **Figure 3**, will be used as a more lipophilic active model.

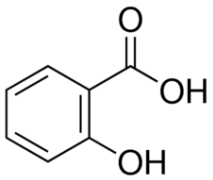

Figure 3: Salicylic Acid Structure (Pubchem: Open chemistry database, n.d.-b).

This drug is a natural β-hydroxy acid that has been used for the treatment of acne, psoriasis, callouses, corns, keratosis pilaris and warts, since it acts as bacteriostatic, keratolytic agent and chemical peeling agent (Arif, 2015). As a keratolytic agent in callouses and keratosis pilares, it may disrupt cellular junctions and break or lyse intercellular keratin filaments (Arif, 2015). In the chemical peeling, salicylic acid does a exfoliation of the superficial layers of the skin, which promotes the skin and dermal tissues regeneration (Arif, 2015). When salicylic acid acts as peeling agent in the acne and psoriasis, its application is improved by the combination of phototherapy or pulsed dye laser (Alba, Gerenutti, Yoshida, & Grotto, 2017; Arif, 2015; Lekakh et al., 2015). It has also been described has acting as an anti-inflammatory drug (Arif, 2015; Klessig, Tian, & Choi, 2016). Furthermore, this drug is the active metabolite of the acetyl salicylic acid, that is used for pain, fever and inflammation (Klessig, Tian, & Choi, 2016).

Hence, both chosen actives have many different applications in the pharmaceutical and cosmetic field that justify their choice as models for this study.

Therefore, herein five ILs, three imidazole-based ILs — [C2mim][Br], [C4mim][Br] and [C6mim][Br] — and two choline-based ILs —[Cho][Phe] and [Cho][Glu]. [Cho][Phe] and [Cho][Glu] were studied and synthetized as drug solubility and/or permeation enhancers (Almeida et al., 2015; Gouveia et al., 2014).

Finally, one of the focus of this study was to evaluate if the synthesized ILs could act as drug solubility, loading and/or release enhancers, at concentrations were cell viability is

maintained, since this is crucial to confidently assert that these salts may act as functional ingredients in Drug Delivery Systems. For this purpose, the cytotoxicity of the prepared ILs was performed in human keratinocytes, HaCaT cells. Then, considering the cytotoxicity results, different delivery systems were prepared containing nontoxic concentrations of the ILs and 2% w/w of the active. So, Oil-in-Water, O/W, emulsions and gels containing the active and the IL were prepared and the stability of the prepared formulations was evaluated.

Furthermore, to assess the influence of ILs on the drug release of a more lipophilic drug from subcutaneous systems, lipidic implants containing salicylic acid and an IL were also prepared. The influence of the IL on the drug content and release was then evaluated.

2. Materials and Reagents

2.1. Materials

- Analytical scale Sartorius BP221S®
- Centrifuge Jouan BR 4i®
- Common laboratory material
- Desiccator
- Freezer Omina®
- Glass Franz type diffusion cells
- Heating plate with stirring
- Horizontal shaker IKA-Vibrax-VWR®
- Incubator Heidolph 1000® with Stirring Heidolph Unimax 1010®
- Inverted microscope OLYMPUS CKX41® with Camera OLYMPUS SC20®
- Lyophilizer LABCONCO FreeZone 25®
- Magnetic stirrer
- Micropipette P100, P200, P1000 and P5000 VWR Ergonomic High-Performance®
- Minivortex MS2 IKA®
- Multi-point plate
- Oven Memmert U30®
- pH meter 827 pH lab Metrohm®
- Rotary Evaporator with bath IKA Labortechnik HB4 basic® and Elevator IKA WERKE RV06-ML® Thermostatic water bath Memmert®
- Ultrasounds Bandolin Sonorex Super RK 510 H®
- UV-visible spectrophotometer Evolution 300 UV-Vis—Thermo Scientific®
- Viscometer Brookfield®

2.2. Reagents

- Acetone
- Acetonitrile by Sigma-Aldrich, USA
- Butylated hydroxytoluene, BHT, by Mapric, Brazil
- Caffeine anhydrous by Fagron, Spain
- Carbopol® 940 by José Vaz Pereira, Portugal
- Cholinium hydroxide in methanol [Cho][OH]/MeOH 45 % by Sigma-Aldrich, USA
- Crodafos CES®, cetearyl alcohol and diacetyl phosphate and ceteth-10 phosphate, by Mapric, Brazil
- Deionized water
- Diethyl ether by Panreac, Spain
- Dynasan® 118, Glycerin triestearate, by Cremer Oleo GmbH, Germany
- Ethylenediaminetetraacetic acid disodium, Disodium EDTA, by Fagron, Spain
- Gelucire® 50/02, mixture of monoesters, di-esters and tri-esters of glycerol and mono- and di-esters of polyethylene glycol, by GatteFossé, France
- Isopropyl myristate, MIP, by Pharma Special, Brazil
- L-glutamine by Sigma-Aldrich, USA
- L-phenylalanine by Sigma-Aldrich, USA
- Methanol by Sigma-Aldrich, USA
- Methylene blue
- Methylparaben by Sigma-Aldrich, USA
- Nitric acid by VWR, USA
- Polyethylene glycol 400, PEG 400, by Panreac, Spain
- Propylene glycol by Fagron, Spain
- Propyl paraben by Sigma-Aldrich, USA
- Salicylic acid by Sigma-Aldrich, USA
- Silver Nitrate by Sigma-Aldrich, USA
- Sodium azide by Sigma-Aldrich, USA
- Sodium Chloride by Sigma-Aldrich, USA
- Sodium phosphate dibasic dihydrate by Sigma-Aldrich, USA
- Sodium phosphate monobasic dihydrate by Sigma-Aldrich, USA
- Sucrose

- Sudan III
- Triethanolamine by José Vaz Pereira, Portugal

3. Methods

3.1. Synthesis of Ionic Liquids

The imidazole-based ILs, 1-ethyl-3-methylimidazolium bromide [C2mim][Br], 1-butyl-3-methylimidazolium bromide [C4mim][Br] and 1-hexyl-3-methylimidazolium bromide [C6mim][Br], were kindly provided by a collaboration with the Centro de Química e Bioquímica, CQB, of Faculdade de Ciências da Universidade de Lisboa.

The two amino acid derivative ILs, (2-hydroxyethyl)-trimethylammonium-L-phenylalaninate [Cho][Phe] and (2-hydroxyethyl)-trimethylammonium-L-glutamate [Cho][Glu], were prepared according to the literature (Czekanski et al., 2014; Gouveia et al., 2014) with slight modifications. Briefly, 15.6 mL of cholinium hydroxide in methanol [Cho][OH]/MeOH 45 % was evaporated under vacuum, on the Rotary Evaporator with bath IKA Labortechnik HB4 basic® and Elevator IKA WERKE RV06-ML®, at 50 °C. Furthermore, it was added an aqueous solution of 57.79 mmol of amino acid, the solution was already prepared with the minimum amount of water necessary to completely solubilize the amino acid. The mixture was stirred in an ice bath for 17h and it was evaporated on the rotary evaporator at 60 °C. Next, it was added a mixture of acetonitrile:methanol, 9:1 with vigorously stirring for amino acid in excess on the solution to precipitate. To remove the solid, a gravity filtration was made simultaneously, followed by centrifugation at 10 080 x g, for 20 mins and by vacuum evaporation at 60 °C, to remove the solvent (Santos de Almeida et al., 2017). The prepared ILs were stored, and conserved without light, in a desiccator.

3.2. Solubility Studies

Solutions of caffeine and salicylic acid were saturated and they were prepared in triplicate in deionized water and several mixtures of deionized water:IL. After that, saturated solutions were placed in a horizontal shaker, at 0.5 x g, at 25 ± 2 °C, RT, and 32 ± 2 °C, skin surface temperature, for 72 h. After being filtered to gravity to remove the solute in excess, all samples were analyzed by UV-visible spectrophotometry, at wavelength of maximum absorption for caffeine, 273 nm, and salicylic acid, 278 nm, in water.

3.3. Permeation Studies

Permeation studies, n=5, were carried out in glass Franz type diffusion cells with a receiver volume of approximately 4 mL and a diffusion area of 0.95 cm^2, using pig ear skin. The porcine ears were gently provided from a local slaughterhouse, Suinimais Lda, Leiria, Portugal. This skin is previously prepared by shearing, carefully, not to damage the stratum corneum, followed by separation of the epidermis existing cartilage in the outer part of the ear, using a scalpel. Previously, to remove impurities the ear was washed with tap and deionized water. After these processes, the skin is kept in the freezer at -20 °C, until it was used (Santos de Almeida et al., 2017).

Furthermore, 500 µL of the active compound saturated solutions, in a water:IL mixture, with a ratio of 95:5, was placed in the donor compartment. This compartment was occluded by sealing it with microscope coverslips. The receptor compartment was filled with a medium, phosphate-buffered saline, PBS, pH 7.4, and was placed in a thermostatic bath at 37 °C, to ensure that it was at 32 °C.

The full content of the receptor compartment was collected and simultaneously replaced with fresh and preheated medium at pre-determined intervals. Quantitative analysis of the medium was done by using UV-visible spectrophotometry. The determination of the active's steady state flux was made by monitoring the cumulative amount of drug diffusing and measuring the slope of the graph once steady-state diffusion was reached.

3.4. Cytotoxicity of Ionic Liquids

The cytotoxicity of choline-based ILs was characterized in human keratinocytes, HaCaT cells line, by MTT assay, according to previously published literature (Fernandes et al., 2010; Wagemaker et al., 2015). Different concentrations of ILs were tested between 0.01 and 1.0 % V/V, with an incubation period of 24 h. This study was accomplished by the Pharmacology and Therapeutics Group, PT Group, from Research Center for Biosciences & Health Technologies, CBIOS.

3.5. Gravimetric studies: Percentage of imidazole-based ILs that permeated the skin

To quantify the amount of halogenated ILs that permeated the porcine ear, contained in the caffeine saturated solutions, gravimetric studies of the samples collected from the receptor compartment, were performed.

The assay was done in triplicate and a mass of 0.03 g of the sample was weighed. Then, 13.5 mL of deionized water and 0.075 mL of aqueous solution of nitric acid 6 M were added. This solution was stirred and 4.5 mL of an aqueous solution of silver nitrate 0.125 M was the added. Afterwards, the solution was gently warmed and then cooled to RT, followed by gravity filtration, with a previously weighed fluted paper filter. The paper filter was washed with two portions of 0.75 mL of deionized water and three portions of 0.75 mL of acetone. Then, the paper filter was placed to dry in the oven at 60 °C for one night and after that it was removed and placed in the desiccator. The quantification was made by weighing the solid mass which was retained on the paper filter, with successive daily weighing's until three consecutive equal weights were obtained (Nelson, 1985).

3.6. Preparation of Topical Formulations

3.6.1. *O/W Emulsions*

The composition of the emulsions is shown in **Tables 1** and **2**. In the preparation of the O/W emulsions, the raw materials of the aqueous and oily phases were weighed. Both phases were placed in a thermostat bath at 65 °C to melt the oily phase, while at the same time the temperature of the two phases was equalized. Then, the aqueous phase was added to the oil phase, still in the bath and always with vigorous stirring. Finally, the mixture was removed from the bath under continuous stirring until the temperature decreased to RT and the emulsion appeared homogeneous.

Table 1: Qualitative and quantitative composition, % w/w, for O/W emulsions with and without caffeine and/or [Cho][Phe].

Compound	O/W Emulsions		
	Without caffeine/IL	**With caffeine and without IL**	**With caffeine and IL**
Crodafos CES®	4	4	4
MIP	2	2	2
BHT	0.1	0.1	0.1
Disodium EDTA	0.1	0.1	0.1
Propylene Glycol	5	5	5
PEG 400	5	5	5
Concentrate parabens	1	1	1
Caffeine	-	2	2
[Cho][Phe]	-	-	0.2
Triethanolamine	q.s. to pH=4-6	q.s. to pH=4-6	q.s. to pH= 4-6
Deionized water	q.s. 100	q.s. 100	q.s. 100

Table 2: Qualitative and quantitative composition, % w/w, for O/W emulsions with and without caffeine and/or [Cho][Glu].

Compound	O/W Emulsions		
	Without caffeine/IL	**With caffeine and without IL**	**With caffeine and IL**
Crodafos CES®	6	6	6
MIP	2	2	2
BHT	0.1	0.1	0.1
Disodium EDTA	0.1	0.1	0.1
Propylene Glycol	5	5	5
PEG 400	5	5	5
Concentrate parabens	1	1	1
Caffeine	-	2	2
[Cho][Glu]	-	-	0.2
Triethanolamine	q.s. to pH=4-6	q.s. to pH=4-6	q.s. to pH= 4-6
Deionized water	q.s. 100	q.s. 100	q.s. 100

3.6.2. *Gels*

These formulations were also prepared in the presence and absence of ILs. The composition of the gels is shown in **Table 3**. Once again, all the raw materials were weighed and the deionized water and IL were placed in a flask. Then, the active was dissolved with stirring. When the drug was fully dissolved, the parabens concentrate and propylene glycol were added to the mixture. Finally, under vigorous stirring, the Carbopol® 940 was also added and the stirring was continued until a completely homogeneous formulation was attained.

Table 3: Qualitative and quantitative composition, % w/w, for gels with and without caffeine and/or the choline-based ILs.

Compounds	Gels			
	Without caffeine/IL	**With caffeine and without IL**	**Without caffeine and with IL**	**With caffeine/IL**
Carbopol® 940	0.5	0.5	0.5	0.5
Propylene Glycol	5	5	5	5
Concentrate parabens	1	1	1	1
Caffeine	-	2	-	2
[Cho][Phe] or [Cho][Glu]	-	-	0.2	0.2
Triethanolamine	q.s. pH=5	q.s. pH=5	q.s. pH=5	q.s. pH=5
Deionized water	q.s. 100	q.s. 100	q.s. 100	q.s. 100

3.7. Accelerated Stability Studies of the Topical Formulations

Organoleptic characteristics, pH and viscosity of all topical formulations were analysed at time zero and after 5 days. The prepared emulsions and gels were challenged with a centrifuge test and temperature cycles.

3.7.1 *Centrifuge Test*

In the centrifugation test, n = 3, approximately 5 g of each formulation was weighed and heated at 45 °C for 30 mins. Then, the samples were centrifuged for 30 mins at 7 200 x g.

3.7.2 *Temperature Cycles*

For the temperature cycles, n=3, the freeze-thaw stability was evaluated using 24 h temperatures cycles between -4 °C and 45 °C for 5 days.

3.8. Microscopic Analysis

Instability phenomena and the presence/absence of active crystals, in all the prepared emulsions with and without drug and/or IL, were analyzed by contrast phase microscopy. Briefly, an aliquot of emulsion was spread between a cover slip and a glass slide. The images were obtained using an OLYMPUS SC20 camera coupled to an inverted OLYMPUS CKX41 microscope with a 40x objective (Cerqueira, 2014).

3.9. Lipidic Implants

In previous studies our group has evaluated the incorporation of caffeine and ILs in lipidic implants (Antunes, 2015). Herein, the incorporation of the more lipophilic salicylic acid in lipidic implants, in the presence and absence of ILs, was also evaluated.

3.9.1 *Implants Preparation*

The lipidic implants were prepared by fusion and melding according the method of Kreye et al. with a few modifications (Antunes, 2015; Kreye, Siepmann, & Siepmann, 2008).

Briefly, the solid lipid was heated in a Becker with a water bath until melting. Drug, previously sprayed and sieved with a diameter under 100 µm, was added to the lipid, with continuous stirring, until its complete dispersion in the fused lipid. The fused lipid:drug mixture was pipetted with a sterile disposable plastic pipette. After cooling to RT, the implants were withdrawn from the pipette and stored in the desiccator to remove a possible excess of water. Before being used the implant was cut to the required size. The lipid:drug ratio in all prepared implants was 90:10, % w/w. The implants composition is described in **Table 4**.

Table 4: Composition, % w/w, for the Lipidic Implants with and without Salicylic Acid and/or the [Cho][Phe] IL.

Formulations	**% w/w**				
	Dynasan 118	**Gelucire 50/02**	**Sucrose**	**Ionic Liquid**	**Drug**
A	90.0	-	-	-	10
B	89.8	-	-	0.2	
C	80.0	10.0	-	-	
D	79.8	10.0	-	0.2	
E	80.0	-	10.0	-	
F	79.8	-	10.0	0.2	
G	100.0	-	-	-	-
H	99.8	-	-	0.2	
I	90.0	10.0	-	-	
J	89.8	10.0	-	0.2	
K	90.0	-	10.0	-	
L	89.8	-	10.0	0.2	

3.9.2 *Content Uniformity*

Content uniformity study, for the implant containing sucrose, was performed using formulation L, **Table 4,** with a lipophilic and a hydrophilic dye solution of 2.5 % w/w, Sudan III (Asmus, Gurny, & Möller, 2011) and Methylene Blue (Hyun, 2015), respectively. The formulation J, containing Gelucire 50/02 instead of Sucrose, had already been evaluated by our group in a previous work (Antunes, 2015).

3.9.3 *In vitro Drug Release*

Implants, n=5, were placed in 1.5 mL PBS pH 7.4 with 0.01 % w/w sodium azide at 37 °C with 0.3 x g. At predetermined time points, the medium was completely replaced with fresh PBS pH 7.4 and the drug content was measured by UV-Vis spectrophotometry. *Sink* conditions were kept in all experiments.

3.9.4 *Drug Content*

Implants were crushed and dispersed in 25 mL of absolute ethanol. Salicylic acid was completely dissolved, whereas implant excipients were dispersed. An aliquot was filtered, diluted and then drug quantification was performed by UV-Vis spectrophotometry at 295 nm, wavelength of maximum absorption for salicylic acid in absolute ethanol.

3.10. Statistical analysis

Differences in mean values of the results were evaluated by one-way analysis of variance, ANOVA, followed by Tukey's multiple comparison test. Values were expressed as mean ± standard deviation, SD. The differences between individual means were significant with at * $p<0.05$, ** $p<0.01$ and *** $p<0.001$.

All analyses were performed using GraphPad Prism 5® by GraphPad Software, USA.

4. Results and Discussion

In this work, caffeine and salicylic acid were used as hydrophilic and lipophilic models, respectively, and five ILs, three imidazole-based, [C2mim][Br], [C4mim][Br] and [C6mim][Br], and two choline-based, [Cho][Phe] and [Cho][Glu], were investigated as solubility and permeation promotors for both actives. The ability of ILs to enhance drug release from lipidic implants, was also explored.

The imidazole-based ILs, [C2mim][Br], [C4mim][Br] and [C6mim][Br], were kindly provided by CQB of Faculdade de Ciências da Universidade de Lisboa.

For the preparation of the two choline-based ILs derived from amino acids, small modifications were implemented to the synthesis already described in the literature (Gouveia et al., 2014), which allowed to afford higher yields (by 13% for [Cho][Glu] and by 20% for [Cho][Phe]). All studied ILs, shown in **Figure 4**, were viscous liquids at room temperature, with the exception of [C2mim][Br] which was a white solid. The ^{1}H-NMR spectroscopic data of the prepared ILs, [Cho][Glu] and [Cho][Phe], presented in **Appendix I**, are in agreement with previously described obtained data (Czekanski et al., 2014; Gouveia et al., 2014).

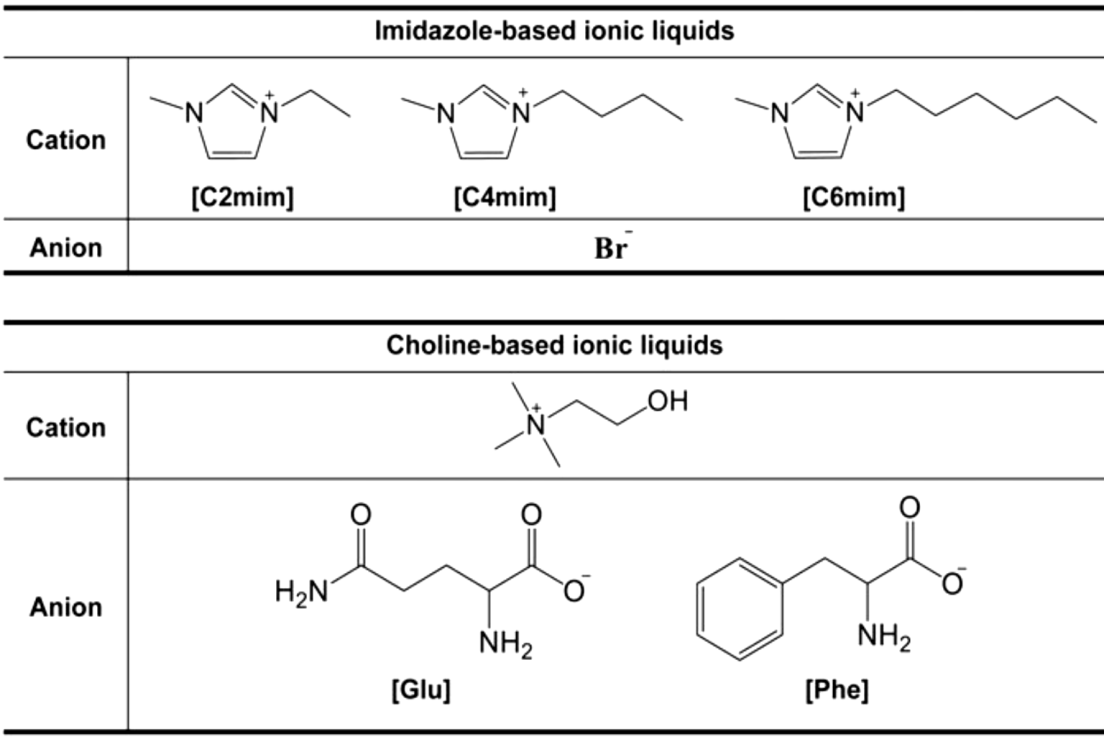

Figure 4: Structure of the studied ILs (Santos de Almeida et al., 2017).

4.1. Solubility Studies

Water is one of the most commonly employed solvents for pharmaceutical formulations. Hence, the influence of the prepared ILs on the solubility of caffeine and salicylic acid was evaluated in water. For this purpose, the solubility of caffeine and salicylic acid in water or in water:IL mixtures, in a proportion of 95:5, was evaluated at 25 °C and at 32 °C, as shown in **Figure 5**. Caffeine and salicylic acid solubility in deionized water at 25 °C were 20.02 ± 0.07 mg/mL and 2.07 ± 0.03 mg/mL, respectively, these results agree with the literature (Dias, Hadgraft, & Lane, 2007; Sigma-Aldrich, 2017).

For both studied actives, the choline-based ILs proved to be the best solubility enhancers. Nonetheless, this outcome was more significant for caffeine than for salicylic acid. In fact, for caffeine and when using the choline-based ILs, a two-fold solubility increase was observed at 25 °C and three-fold at 32 °C. With the imidazole-based ILs, results showed that [C6mim][Br] was the best solubility enhancer for caffeine. For Salicylic Acid, no significant differences in solubility were observed, in the presence of the imidazole-based ILs.

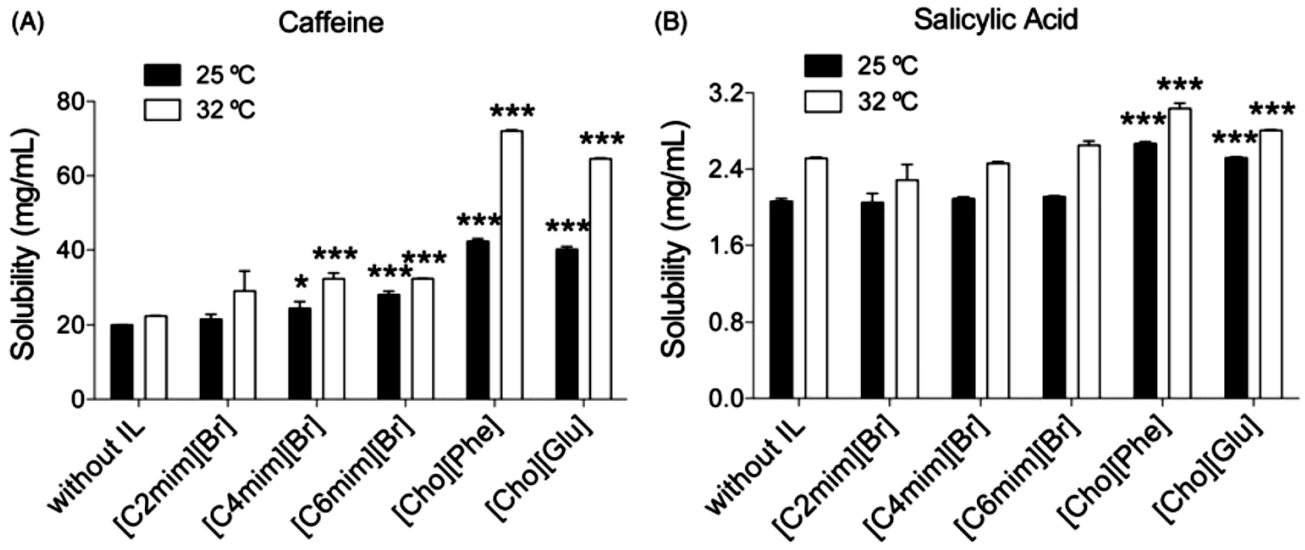

Figure 5: **A**. Caffeine and **B**. Salicylic acid solubility in water or water:IL mixtures, 95:5, % w/w, at 25 °C and 32 °C. n=3, mean ± SD and * p<0.05, ** p<0.01, *** p<0.001 in ANOVA, Tukey's Test.

Considering that the major solubility enhancement is observed for caffeine and in the presence of the less cytotoxic choline-based ILs, further solubility studies were carried out with this active in several concentrations of both ILs—0.1, 0.2, 0.5, 1.0, 3.0 and 5.0 % w/w—at both temperatures. These studies are shown in **Figure 6** and **7**.

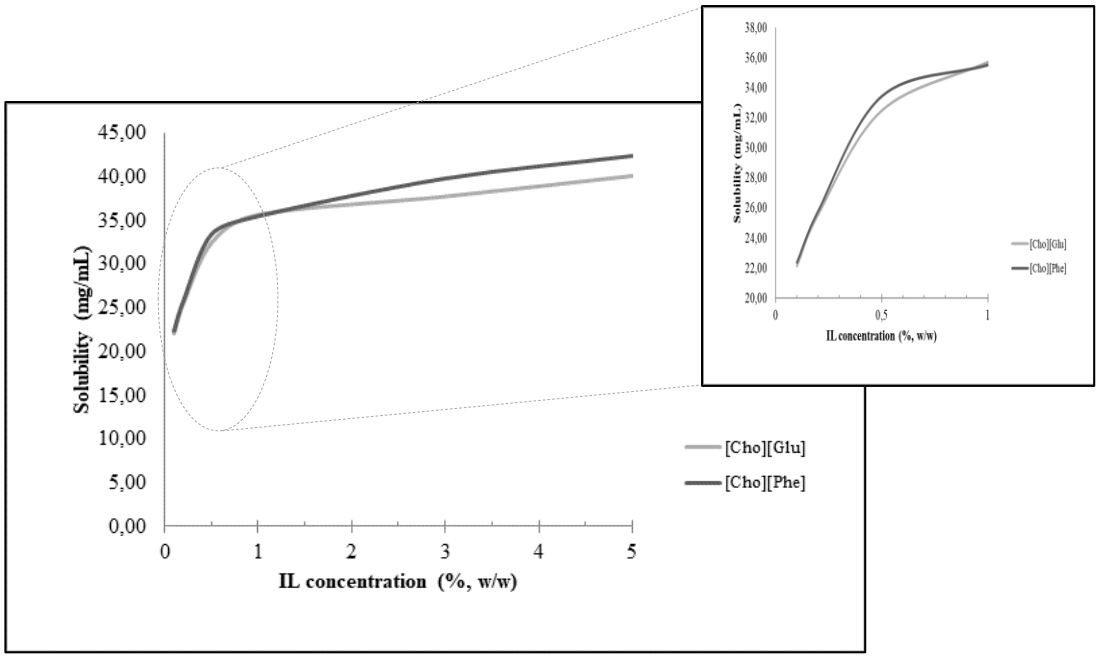

Figure 6: Caffeine solubility in different concentrations, % w/w, of the choline-based ILs at 25 °C. n=3, mean ± SD.

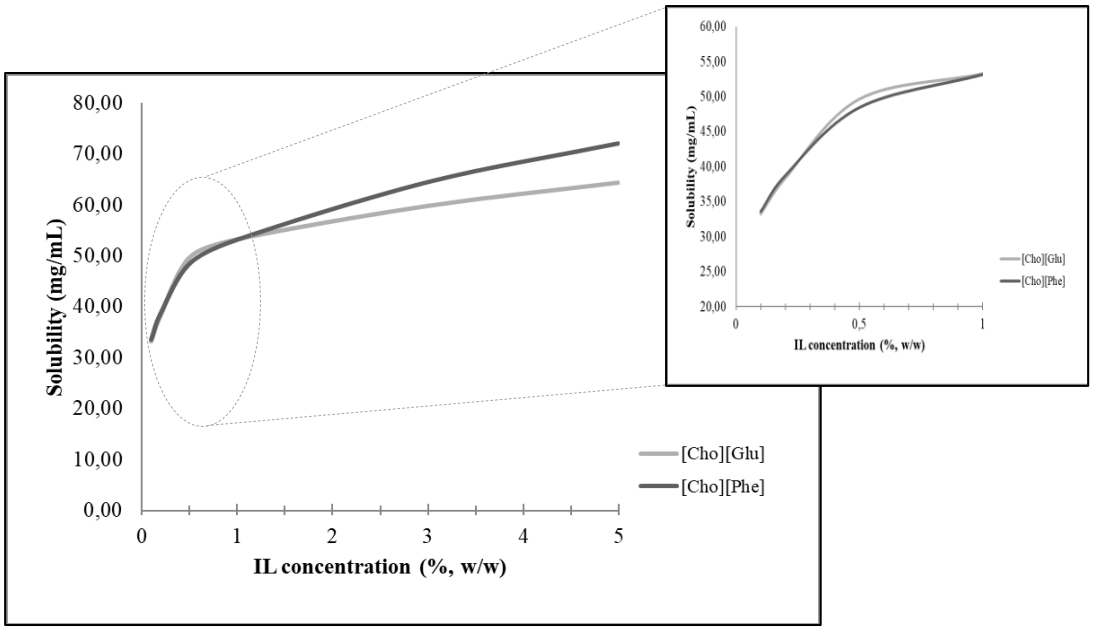

Figure 7: Caffeine solubility in different concentrations, % w/w, of the choline-based ILs at 32 °C. n=3, mean ± SD.

Once again, it was confirmed that caffeine solubility was higher in the presence of the ILs at all studied IL concentrations, and that at concentrations of IL higher than 1.0 % w/w, [Cho][Phe] was the best solubility enhancer at both temperatures.

Results also showed that caffeine solubility was greatly influenced by the ILs concentrations up to 0.5 % w/w and that at higher concentrations, even though the solubility continues to slightly increase, it was much less dependent on the IL content. This result evidences that even low concentrations of ILs impact caffeine solubility, which is particularly relevant since it shows that at non-toxic concentrations of IL, 0.2 % w/w, the desired enhancement in drug solubility is still achieved, has shown in **Figure 6** and **7**, and more particularly in **Table 5**.

Table 5: Caffeine solubility in water or water:ILs mixtures, 99.8:0.2 % w/w, at 25 °C and 32 °C. n=3, mean ± SD.

Solvent	Solubility, mg/mL			
	25 ºC		32 ºC	
	Mean	SD	mean	SD
Water	20.02	0.07	22.36	0.17
Water:[Cho][Glu]	25.69	0.02	29.67	0.35
Water: [Cho][Phe]	25.87	0.07	35.73	0.38

4.2. Permeation Studies

To investigate the influence of the prepared ILs on the studied actives permeation, solutions were used with drug excess as donor phases since their chemical potential is equal and a constant driving force is maintained. Additionally, in a system where the barrier properties of the membrane are unaffected by the vehicle and/or permeating solvent, the flux from a saturated solution will be independent from them (Rosado, Cross, Pugh, Roberts, & Hadgraft, 2003; Twist & Zatz, 1986).

For caffeine, the highest flux was observed with the more hydrophobic IL, [C6mim][Br]. Once again, dependence between permeation and the IL's alkyl chain size was noted amongst the imidazole-based ILs at **Figure 8A**. These results may be due to an increase in the caffeine partition membrane and/or an increase in diffusion. For salicylic acid, results

show that the permeation was slightly improved in the presence of [C6mim][Br] or [C2mim][Br], as it could be seen at **Figure 8B**. This may be due to [C2mim][Br] increasing the hydrophilicity of the vehicle, which would increase the partition of the more lipophilic permeant from the vehicle. On the other hand, a possible surfactant affect provided by the longer alkyl chain in [C6mim][Br] might have disturbed the skin membrane and thus also contributed to a higher flux of the active in the presence of this IL. These results can be supported with previous studies that say that imidazole-based ILs with alkyl chains longer than four carbons act as amphiphilic substances and display interface interaction (Fatemi & Izadiyan, 2011; Łuczak et al., 2008; Zhang et al., 2016). Significant differences in the permeation of the two model actives were not observed for the choline-based ILs. This may be an advantage, when a low permeation and a high solubility of the drug is sought, thus guaranteeing a lower incidence of adverse effects in topical formulations.

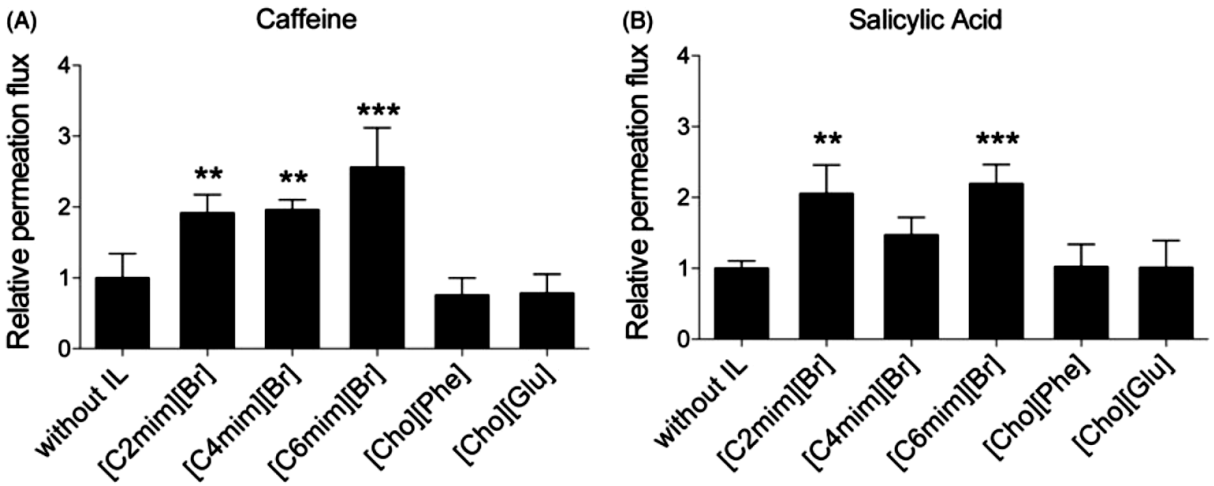

Figure 8: Relative permeation flux of saturated solutions of **A**. Caffeine and **B**. Salicylic acid in water or water:IL mixtures, 95:5 % w/w. $3 < n < 5$, mean ± SD and $^{**}p<0.01$ and $^{***}p<0.001$ with ANOVA, Tukey's test.

Nonetheless, to further assess which ILs may be more suitable as functional ingredients in DDS, it is necessary to evaluate their cytotoxicity, to understand their functionality at non-toxic concentrations.

4.3. Ionic Liquids Cytotoxicity

The cytotoxicity of the five studied ILs was characterized in human keratinocytes, HaCat cells. All ILs showed a clear concentration-dependent cytotoxicity, as shown in **Figures 9** and **10**.

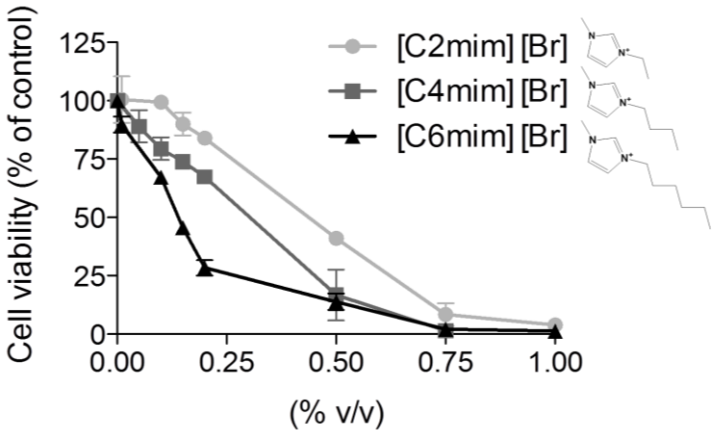

Figure 9: Cell viability of HaCat cells exposed to halogenated ILs during 24 h of incubation, MTT assay. Results are presented as mean ± SD, n=2.

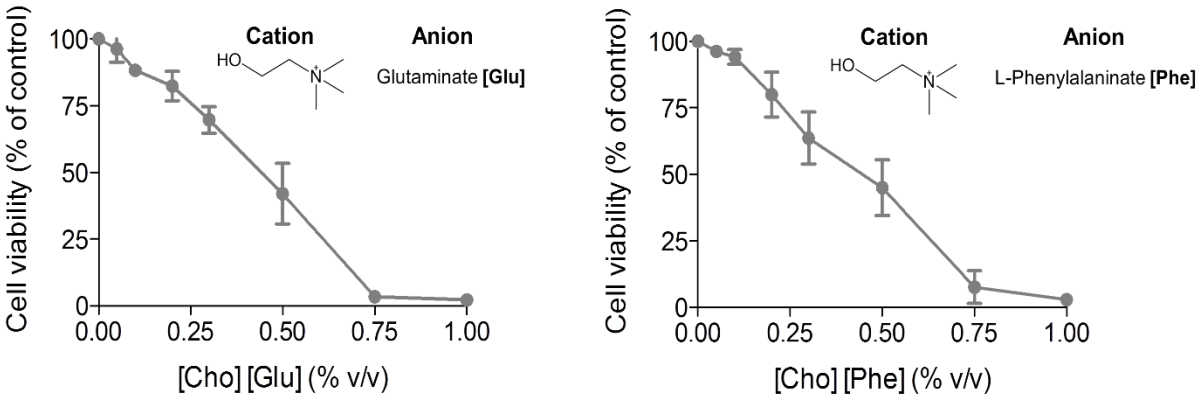

Figure 10: Cell viability of HaCat cells exposed to choline-based ILs during 24 h of incubation, MTT assay. Results are presented as mean ± SD, n=2.

The halogenated ILs showed a clear increase in cytotoxicity associated with the length of the alkyl chains, has may be seen in **Figure 9**. The half maximal inhibitory concentration, IC_{50}, values of halogenated ILs were 0.10 % V/V for hexyl, 0.30 % V/V for butyl and 0.44 % for ethyl analogue.

This findings are in agreement with previously published data (Romero, Santos, Tojo, & Rodríguez, 2008) and may be once again explained by the enhancement in surfactancy provided by the longer alkyl chains (Łuczak, Hupka, Thöming, & Jungnickel, 2008), which might have disturbed the cell membranes. Moreover, as a consequence of increase lipophilicity, [C6mim][Br] might have accumulated in cells at higher levels, exhibiting a higher cytotoxicity when compared to the less lipophilic analogues. Additionally, our results are also in agreement with previously published data concerning the cytotoxicity of [C6mim][Br] and [C4mim][Br] in human HeLa cells (Gouveia et al., 2014). Although these previous cytotoxicity assays were performed in a different cell line and under distinct experimental conditions, the hexyl derivative also presented higher cytotoxicity than the butyl analogue.

The two choline-based ILs, shown in **Figure 10**, exhibit a similar cytotoxic profile and proved to be less toxic than [C4mim][Br] and [C6mim][Br]. Their IC_{50} values were 0.43 % V/V for [Cho][Phe] and 0.42 % V/V for [Cho][Glu]. Furthermore, the cytotoxicity of the higher concentrations of amino acid derivates ILs may be ascribed to the alkaline properties of these ILs, which might disturb cell pH medium.

4.4. **Gravimetric studies:** Determination of the Percentage of imidazole-based ILs that permeated the skin

Since the halogenated ILs proved to be the most toxic ILs, it became relevant in this study to understand how much of these ILs permeated through the skin.

Gravimetric results, presented in **Table 6,** show that the more lipophilic [C6mim][Br] is, amongst the imidazole-based ILs, the IL that presents a higher permeation through the skin. Results also show that the longest the alkyl chain the higher these ILs permeate through the skin. This may be due to an enhancement of the ILs surfactant capacity, that may increase the

skin instability and thus promote the ILs' permeation. It should also be mentioned that for the more toxic [C4mim][Br] and [C6mim][Br] ILs, the permeation percentage is quite high, above 80 %, which reveals that it is fundamental to consider their cytotoxicity results when considering their use in topical delivery systems, since most of these ILs content will permeate the skin.

Table 6: Percentage, %, of imidazole-based ILs that permeated the skin. n=3 and mean ± SD.

Ionic Liquid	IL permeation %	
	Mean	**SD**
[C2mim][Br]	67.1	4.3
[C4mim][Br]	83.8	5.7
[C6mim][Br]	91.1	3.2

4.5. **Topical Formulations:** Preparation and Stability Studies

Within this study, 2.0 % w/w of caffeine, was incorporated into O/W emulsions and gels, in the presence or absence of the less toxic choline-based ILs, at an IL percentage that did not exhibit a marked decreased in cell viability, 0.2 % w/w.

4.5.1. *O/W Emulsions*

O/W emulsions proved to be stable regarding pH value, viscosity and organoleptic characteristics after the performed accelerated stability studies, as shown in **Table 7**.

Results showed that the formulations containing ILs had a lower viscosity**,** thus indicating that the ILs modified the rheology, which may be correlated with their properties as solvents (Anderson, Pino, Hagberg, Sheares, & Armstrong, 2003; Carda-Broch, Berthod, & Armstrong, 2003), since they have the capacity to dissolve both inorganic and organic

compounds. Additionally, O/W emulsions containing [Cho][Glu] required a larger amount of Crodafos CES®, 6.0 %, to stabilize the formulation, since this IL had a higher impact in decreasing the viscosity than [Cho][Phe], as shown in **Table 7**. Nevertheless, this change did not alter the pH values of the prepared samples and the used amount of Crodafos CES® was far from the upper usage limit.

Table 7: Temperatures cycles, n=3, for the O/W emulsions.

% Crodafos CES®	Formulation	Time Zero		Time Five	
		pH	Viscosity, mPa.s	pH	Viscosity, mPa.s
4	Without caffeine/IL	4.99	10 260	5.00	11 503
	With caffeine without IL	5.00	13 160	4.99	14 000
	With caffeine and [Cho][Phe]	4.99	12 630	4.99	12 900
6	Without caffeine/IL	4.99	17 130	5.00	19 100
	With caffeine and without IL	4.99	22 100	4.98	25 600
	With caffeine and [Cho][Glu]	5.00	19 200	5.01	22 030

4.5.2. *Gels*

To broaden the applicability of the choline-based ILs in different formulations, gels containing caffeine were also prepared. In this case, there was an observable difference in visual appearance when the active and the ILs were incorporated. The gel without caffeine or IL presented a translucent appearance with some air bubbles, possibly due to the incorporation of air during the preparation. In the presence of the drug and without IL, caffeine crystals were macroscopically observed. On the other hand, in the presence of caffeine and IL, the gel lost some transparency, which may be due to a decreased solubility of the polymer. However, caffeine crystals were not macroscopically and microscopically observable (data not shown), attesting that the ILs also enhanced the drug incorporation into the gels. Concerning the viscosity, a slight decrease was only detected in the gels containing the [Cho][Phe], but with no

impact on the gels stability. Stability results showed once again that the presence of ILs allowed a higher caffeine loading while maintain the gels stability, as shown in **Table 8**.

Table 8: pH and viscosity of the gels with and without caffeine and/or the choline-based ILs.

Formulation	pH	Viscosity, mPa.s
Without caffeine/IL	5.51	118 000
With caffeine without IL	5.52	122 000
With caffeine and [Cho][Phe]	5.45	117 000
With caffeine and [Cho][Glu]	5.46	123 000

4.6. Microscopic Analysis

Microscopic analysis showed that the incorporation of 2.0 % w/w of caffeine, in the absence of IL, resulted in drug crystallization after emulsion preparation, as shown in **Figure 11**. This is agreement with the caffeine solubility, which was determined to be 20 mg/mL in water at 25 °C, since a drug loading of 2.0 % w/w in the emulsion, represents a higher percentage of drug concentration in the aqueous phase, thus justifying the presence of crystals in the absence of the ILs. When the ILs were added to the aqueous phase of the emulsion, no drug crystals were visible, as may be seen in **Figure 11**, thus showing a higher drug incorporation into the emulsions containing ILs.

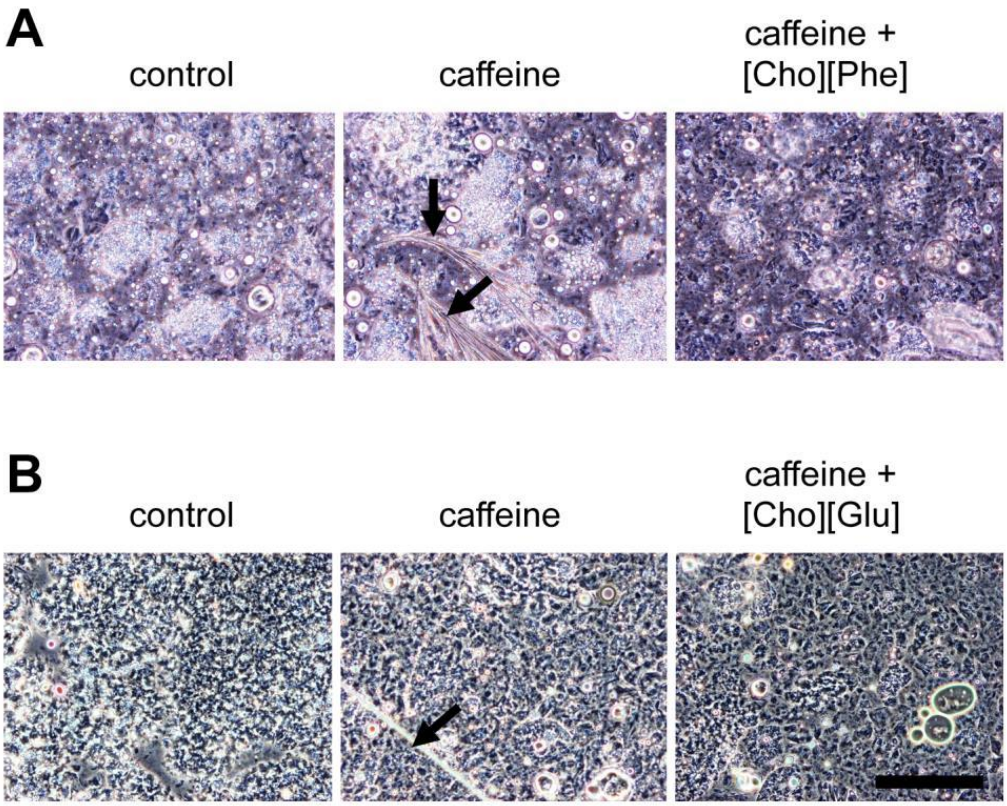

Figure 11: Microscopic analysis of O/W emulsions with and without caffeine and/or the IL: **A.** [Cho][Phe]; **B.**[Cho][Glu]. Arrows point to visible caffeine crystals. Scale bar = 100 µm.

4.7. Lipidic Implants

Lipidic implants present a great potential as parenteral controlled drug delivery systems since they provide drug protection, accurate and targeted release (Koennings et al., 2006; Kreye et al., 2008; Schwab, Sax, Schulze, & Winter, 2009). However, lipid implants have some disadvantages, such as inflexible drug release profiles, making it crucial to find ways to overcome this drawback. In this sense, the incorporation of ILs in these systems may be relevant to overcome this problem. In this context, in a previous work, developed by our group (Antunes, 2015), it was possible to show that the presence of IL may influence drug release from lipidic implants containing caffeine, a more hydrophilic drug. Following this, and to evaluate if ILs may also influence the release profile of lipophilic drugs, in the present study salicylic acid was incorporated in lipidic implants with or without IL and the performance of these systems was evaluated.

4.7.1. *Content Uniformity*

To evaluate if the incorporation of hydrophilic or lipophilic compounds influences the solids content of the prepared implants containing sucrose, in the presence of ILs, a content uniformity study was performed for formulation L, presented in **Table 4**.

All implants presented a homogeneous and even surface and dimensions were defined as 0.1 cm of diameter and 0.5 cm of length.

In the Content Uniformity assays, it was observed a great diffusion of both dyes solution in the lipidic implant, as shown in **Figure 12**. The studied implants presented a homogeneous dispersion in all batch. Thus, the results proved that this delivery system containing sucrose and ILs may be used to incorporate both hydrophilic and lipophilic drugs and, which is in agreement with previous work, that evaluated the content uniformity of lipidic implants containing Gelucire 50/02 and ILs (Antunes, 2015).

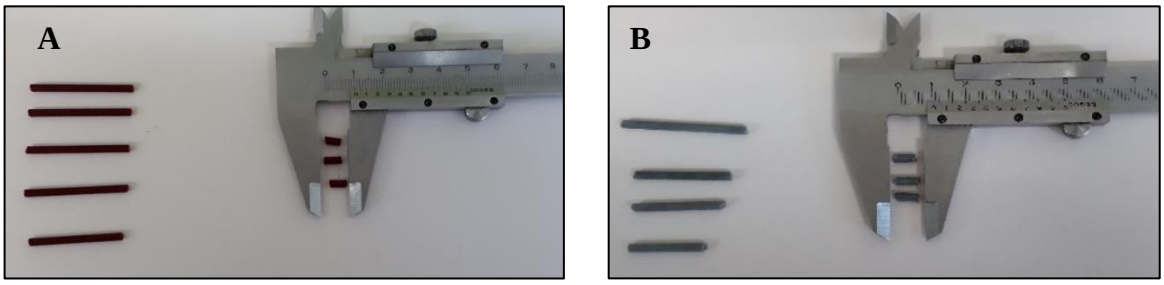

Figure 12: Lipidic Implants with 2.5 % w/w of **A.** Sudan III or **B.** Methylene Blue.

4.7.2. *In Vitro Drug Release*

The main goal of incorporating ILs in lipidic implants was to evaluate their influence in drug delivery. As shown in **Figure 13**, there was an initial burst release due to drug present at the surface of the implant, followed by a zero-order release profile. Also, the shape of the curves did not change, so the *in vitro* drug release's mechanism was equal for all batches.

The lowest release was observed in the presence of Dynasan 118®, which could be explained by its high lipophilicity and low wettability (Amaral, 2016; Antunes, 2015). These two characteristics may hinder the salicylic acid release, since it is a lipophilic drug and it is more comfortable for this drug to stay in the implant than to be release to a lipophobic medium, such as PBS. It is also relevant to mention that the drug release performance of the formulation

containing Dynasan 118® and Sucrose, was similar to the formulation only containing Dynasan 118®.

However, formulations contaning Gelucire 50/02, both with and without IL, presented the highest drug release. These results may be a consequence of Gelucire 50/02 being a release promotor and allowing the implant to degrade over time. The lower release of Dynasan 118:Gelucire 50/02:IL than Dynasan 118:Gelucire 50/02 may be due to an interaction between the drug and the implant containing IL, which shows that the presence of ILs may influence the drug release as desired.

Furthermore, when concerning formulations with Sucrose, these presented a lower drug release than Gelucire 50/02, despite Sucrose being more degradable, which was noticeble since the implants were more fragmented in the end of the study, the drug release was lower than for the implants containing Gelucire 50/02. This result shows that using sucrose has a higher influence on the implants degradability, but not on drug release.

Finally, in general results showed that the presence of the IL enhances drug release, because all formulations containing IL had higher drug release when compared with the formulation containing Dynasan 118® alone.

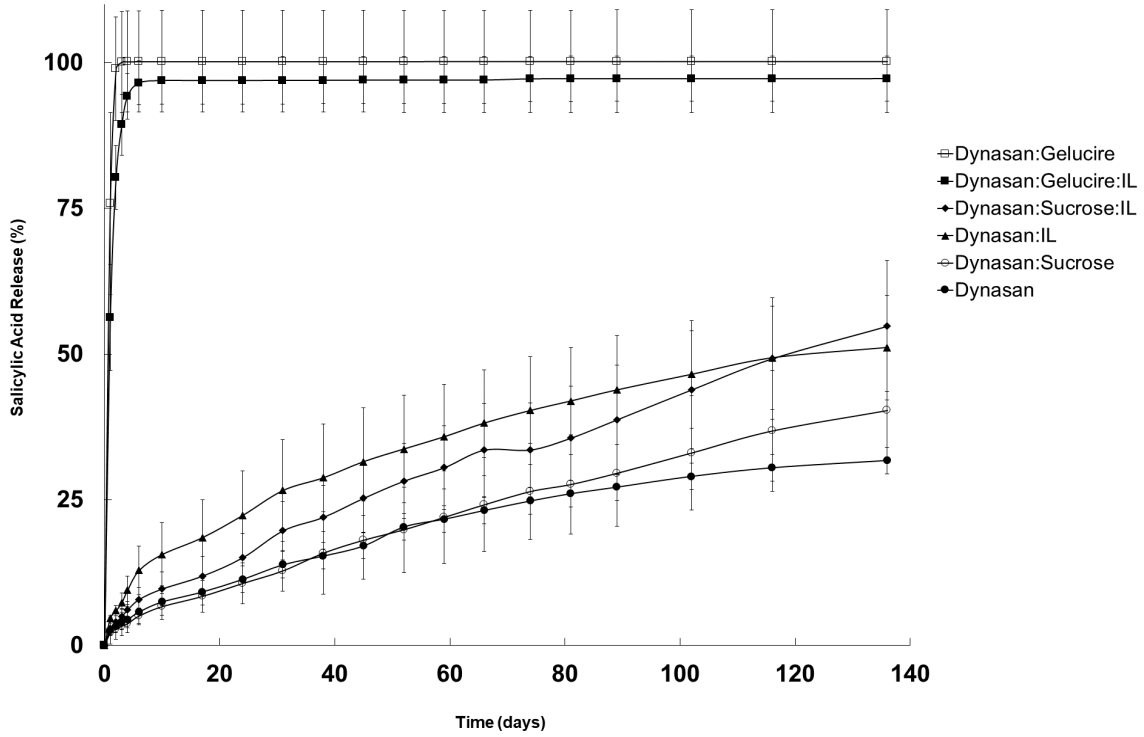

Figure 13: Effect of implants composition on drug release, mean ± SD and n=5.

4.7.3. *Drug Content*

Results showed an appropriate content uniformity and a satisfactory homogeneity in all batches containing salicylic acid, as shown in **Figure 14**, where no statically significant differences were observed in the drug content for all the studied batches.

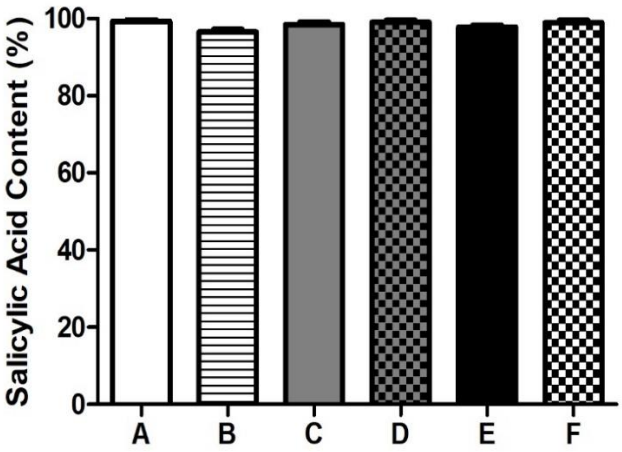

Figure 14: Drug content of the prepared implants with **A.**Dynasan 118, **B.** Dynasan 118:IL, **C.** Dynasan 118:Gelucire 50/02, **D.** Dynasan 118:Gelucire 50/02:IL, **E.** Dynasan 118:Sucrose and **F.** Dynasan 118:Gelucire 50/02:IL. n=3 and mean ± SD. Statistically analysed by ANOVA, Tukey's test.

5. Conclusion

Five different ILs were studied—three imidazole-based ILs, [C2mim][Br], [C4mim][Br] and [C6mim][Br], and two choline-based ILs, [Cho][Phe] and [Cho][Glu]—as solubility and/or permeation promotors of the hydrophilic and lipophilic active models, caffeine and salicylic acid respectively. The influence of the ILs on drug release from lipidic implants was also evaluated.

The choline-based ILs proved to be the best solubility promoters for both actives, although a more prominent effect was observed for the more hydrophilic caffeine. It was also shown that lower concentrations of these ILs, up to 0.5 % w/w, had a higher influence on caffeine solubility. Regarding permeation, the choline based ILs showed no significant impact on the skin permeation, for both actives. This fact may be useful to guarantee a lower incidence of adverse effects in topical formulations where low permeation of the active is sought.

For the halogenated ILs, it was observed that the size of the alkyl chain impacted their ability to enhance the caffeine solubility and permeation. When concerning salicylic acid, these ILs did not impact the drug solubility. On the other hand, for this active, drug permeation was enhanced in the presence of both [C2mim][Br] and [C6mim][Br], while [C4mim][Br] showed no impact on this parameter. Nonetheless, the alkyl chain length also influenced the ILs cytotoxicity, with the [C6mim][Br] presenting the higher cytotoxicity. [Cho][Phe] and [Cho][Glu] showed lower cytotoxicity when compared with [C4mim][Br] and [C6mim][Br].

Because of the higher toxicity of the imidazole-based ILs, it was also evaluated the amount of these ILs that permeated through the skin through gravimetric studies. Results showed that the longest the alkyl chain the more this ILs permeate through the skin, probably be due to an enhancement of the ILs surfactant capacity.

Furthermore, for the more toxic [C4mim][Br] and [C6mim][Br] ILs, the permeation percentage is higher than 80%, revealing that it is vital to consider their cytotoxicity when considering their use Drug delivery systems.

Results clearly showed that between the imidazole- and the choline-based ILs, the latter are more suitable to incorporate into drug delivery systems, since they had higher impact in drug solubility and had a lower cytotoxicity. Also, since a more prominent enhancement in solubility was observed for caffeine with the less cytotoxic [Cho][Phe] and [Cho][Glu] ILs,

O/W emulsions and gels were prepared containing this more hydrophilic active and choline-based ILs.

When preparing the topical formulations, it was considered the IL percentage that did not exhibit a marked decrease in cell viability. All prepared emulsions and gels were stable after stress stability studies, which was a good indicative that the choline-based ILs did not negatively affect the integrity of the prepared topical formulations.

Finally, lipidic implants, in the presence and absence of ILs, were successfully prepared and drug content uniformity was achieved. In general implants containing ILs presented a higher drug release.

In conclusion, this study clearly shows that the less toxic choline-based ILs, are better suited as functional ingredients in drug delivery systems. This statement was proven since even at low non-toxic concentrations, these salts enhanced drug solubility and facilitated the incorporation of higher drug concentrations in topical formulations, while keeping their organoleptic properties, stability and safety and they also led to a higher drug release from lipidic implants while maintaining a drug content uniformity.

6. References

Alba, M. N., Gerenutti, M., Yoshida, V. M. H., & Grotto, D. (2017). Clinical comparison of salicylic acid peel and LED-Laser phototherapy for the treatment of *Acne vulgaris* in teenagers. *Journal of Cosmetic and Laser Therapy*, *19*(1), 49–53.

Allen, T. M. (2004). Drug Delivery Systems: Entering the Mainstream. *Science*, *303*(5665), 1818–1822.

Almeida, T. S. de, Júlio, A., Caparica, R., Rosado, C., Fernandes, A. S., Saraiva, N., … Mota, J. P. (2015). Ionic liquids as solubility/permeation enhancers for topical formulations: Skin permeation and cytotoxicity characterization. *Toxicology Letters*.

Almeida, T. S. de, Júlio, A., Mota, J. P., Rijo, P., & Reis, C. P. (2017). An emerging integration between ionic liquids ans nonotechnology: general uses and future prospects in drug delivery. *Therapeutic Delivery*, *6*(8), 461–473.

Álvarez, M. S., Esperança, J. M. S. S., Deive, F. J., Sanromán, M. Á., & Rodríguez, A. (2015). A biocompatible stepping stone for the removal of emerging contaminants. *Separation and Purification Technology*, *153*, 91–98.

Amaral, V. (2016). *Implantes Lipídicos: Desenvolvimento, Libertação in vitro de fármacos e estabilidade*. Universidade Lusófona de Humanidades e Tecnologias.

Anderson, J. L., Pino, V., Hagberg, E. C., Sheares, V. V, & Armstrong, D. W. (2003). Surfactant solvation effects and micelle formation in ionic liquids. *Chemical Communications (Cambridge, England)*, (19), 2444–2445.

Antunes, A. (2015). *Influência de um líquido iónico na libertação de cafeína em implantes lipídicos*. Lisboa.

Arif, T. (2015). Salicylic acid as a peeling agent: a comprehensive review. *Clinical, Cosmetic and Investigational Dermatology*, *8*, 455–461.

Asmus, L. R., Gurny, R., & Möller, M. (2011). Solutions for lipophilic drugs: A biodegradable polymer acting as solvent, matrix, and carrier to solve drug delivery issues. *International Journal of Artificial Organs*, *34*(2), 238–242.

Carda-Broch, S., Berthod, A., & Armstrong, D. W. (2003). Solvent properties of the 1-butyl-3-methylimidazolium hexafluorophosphate ionic liquid. *Analytical and Bioanalytical Chemistry*, *375*(2), 191–199.

Cerqueira, S. S. (2014). *In vitro techniques to assess of SOD mimetics in breast cancer cell spread and adhesion*. Universidade Lusófona de Humanidades e Tecnologias.

Czekanski, L., Santos de Almeida, T., Portugal Mota, J., Rijo, P., & Araújo, M. E. M. (2014). Synthesis of benzoazole ionic liquids and evaluation of their antimicrobial activity. *Biomedical and Biopharmaceutical Research*, *11*(2), 227–235.

De Santis, S., Masci, G., Casciotta, F., Caminiti, R., Scarpellini, E., Campetella, M., & Gontrani, L. (2015). Cholinium-amino acid based ionic liquids: a new method of synthesis and physico-chemical characterization. *Phys. Chem. Chem. Phys.*, *17*(32), 20687–20698.

Dias, M., Hadgraft, J., & Lane, M. E. (2007). Influence of membrane-solvent-solute interactions on solute permeation in skin. *International Journal of Pharmaceutics*, *336*(1–2), 108–114.

Dobler, D., Schmidts, T., Klingenhoefer, I., & Runkel, F. (2012). Ionic liquids as ingredients in topical drug delivery systems. *International Journal of Pharmaceutics*, *441*(1–2), 620–627.

Earle, M. J., Esperança, J. M. S. S., Gilea, M. A., Canongia Lopes, J. N., Rebelo, L. P. N., Magee, J. W., … Widegren, J. A. (2006). The distillation and volatility of ionic liquids. *Nature*, *439*(7078), 831–834.

Fatemi, M. H., & Izadiyan, P. (2011). Cytotoxicity estimation of ionic liquids based on their effective structural features. *Chemosphere*, *84*(5), 553–563.

Fernandes, A. S., Serejo, J., Gaspar, J., Cabral, F., Bettencourt, A. F., Rueff, J., … Oliveira, N. G. (2010). Oxidative injury in V79 Chinese hamster cells: Protective role of the superoxide dismutase mimetic MnTM-4-PyP. *Cell Biology and Toxicology*, *26*(2), 91–101.

Ferré, S. (2016). Mechanisms of the psychostimulant effects of caffeine: implications for substance use disorders. *Psychopharmacology*, *233*(10), 1963–1979.

Frade, R. F., & Afonso, C. A. (2010). Impact of ionic liquids in environment and humans: an overview. *Human and Experimental Toxicology*, *29*(12), 1038–54.

Frizzo, C. P., Gindri, I. M., Tier, A. Z., Buriol, L., Moreira, D. N., & Martins, M. A. P. (2013). Pharmaceutical Salts : Solids to Liquids by Using Ionic Liquid Design. In *Ionic Liquids-New Aspects for the Future* (pp. 557–580).

Ghandi, K. (2014). A Review of Ionic Liquids , Their Limits and Applications. *Green and Sustainable Chemistry*, *4*(February), 44–53.

Gouveia, W., Jorge, T. F., Martins, S., Meireles, M., Carolino, M., Cruz, C., … Araújo, M. E. M. (2014). Toxicity of ionic liquids prepared from biomaterials. *Chemosphere*, *104*, 51–56.

Herman, A., & Herman, A. P. (2012). Caffeine's mechanisms of action and its cosmetic use. *Skin Pharmacology and Physiology*, *26*(1), 8–14.

Hough, W. L., Smiglak, M., Rodríguez, H., Swatloski, R. P., Spear, S. K., Daly, D. T., … Rogers, R. D. (2007). The third evolution of ionic liquids: active pharmaceutical ingredients. *New Journal of Chemistry*, *31*(8), 1429.

Hyun, D. C. (2015). A Polymeric Bowl for Multi-Agent Delivery. *Macromolecular Rapid Communications*, *36*(16), 1498–1504.

Jain, K. K. (2008). Drug Delivery Systems – An Overview. In *Drug Delivery Systems* (Vol. 437, p. 251).

Jung, S., Kim, M. H., Park, J. H., Jeong, Y., & Ko, K. S. (2017). Cellular Antioxidant and Anti-Inflammatory Effects of Coffee Extracts with Different Roasting Levels. *Journal of Medicinal Food*, *20*(6), 626–635.

Klessig, D. F., Tian, M., & Choi, H. W. (2016). Multiple targets of salicylic acid and its derivatives in plants and animals. *Frontiers in Immunology*, *7*(MAY), 1–10.

Koennings, S., Garcion, E., Faisant, N., Menei, P., Benoit, J. P., & Goepferich, A. (2006). In vitro investigation of lipid implants as a controlled release system for interleukin-18. *International Journal of Pharmaceutics*, *314*(2), 145–152.

Kreye, F., Siepmann, F., & Siepmann, J. (2008). Lipid implants as drug delivery systems. *Expert Opinion on Drug Delivery*, *5*, 291–307.

Kubota, K., Shibata, A., & Yamaguchi, T. (2016). The molecular assembly of the ionic liquid/aliphatic carboxylic acid/ aliphatic amine as effective and safety transdermal permeation enhancers. *European Journal of Pharmaceutical Sciences*, *86*, 75–83.

Lawrence, M. J., & Rees, G. D. (2000). Microemulsion-based media as novel drug delivery systems. *Advanced Drug Delivery Reviews*, *45*(1), 89–121.

Lekakh, O., Mahoney, A. M., Novice, K., Kamalpour, J., Sadeghian, A., Mondo, D., … Tung, R. (2015). Treatment of acne vulgaris with salicylic acid chemical peel and pulsed dye laser: A split face, rater-blinded, randomized controlled trial. *Journal of Lasers in Medical Sciences*, *6*(4), 167–170.

Łuczak, J., Hupka, J., Thöming, J., & Jungnickel, C. (2008). Self-organization of imidazolium ionic liquids in aqueous solution. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *329*(3), 125–133.

Lupi, O., Semenovitch, I. J., Treu, C., Bottino, D., & Bouskela, E. (2007). Evaluation of the effects of caffeine in the microcirculation and edema on thighs and buttocks using the orthogonal polarization spectral imaging and clinical parameters. *Journal of Cosmetic Dermatology*, *6*(2), 102–107.

Marrucho, I. M., Branco, L. C., & Rebelo, L. P. N. (2014). Ionic liquids in pharmaceutical applications. *Annual Review of Chemical and Biomolecular Engineering*, *5*, 527–46.

Mitkare, S. S., Lakhane, K. G., & Kokulwar, P. U. (2013). Ionic liquids: Novel Applications in Drug Delivery. *Research Journal of Pharmacy and Technology*, *6*(11), 1274–1278.

Mizuuchi, H., Jaitely, V., Murdan, S., & Florence, A. T. (2008). Room temperature ionic liquids and their mixtures: Potential pharmaceutical solvents. *European Journal of Pharmaceutical Sciences*, *33*(4–5), 326–331.

Nelson, J. (1985). *Chemistry: The Central Science*. (Prentice-Hall, Ed.) (3th ed.).

Pubchem: Open chemistry database. (n.d.-a). Caffeine Database. Retrieved 26 August 2017, from https://pubchem.ncbi.nlm.nih.gov/compound/2519#section=Top

Pubchem: Open chemistry database. (n.d.-b). Salicylic Acid Database. Retrieved 26 August 2017, from https://pubchem.ncbi.nlm.nih.gov/compound/salicylic_acid#section=Top

Qiu, Z., & Texter, J. (2008). Ionic liquids in microemulsions. *Current Opinion in Colloid and Interface Science*, *13*(4), 252–262.

Romero, A., Santos, A., Tojo, J., & Rodríguez, A. (2008). Toxicity and biodegradability of imidazolium ionic liquids. *Journal of Hazardous Materials*, *151*(1), 268–273.

Rosado, C., Cross, S. E., Pugh, W. J., Roberts, M. S., & Hadgraft, J. (2003). Effect of vehicle pretreatment on the flux, retention, and diffusion of topically applied penetrants in vitro. *Pharmaceutical Research*, *20*(9), 1502–1507.

Santos de Almeida, T., Júlio, A., Saraiva, N., Fernandes, A. S., Araújo, M. E. M., Baby, A. R., … Mota, J. P. (2017). Choline- versus imidazole-based ionic liquids as functional ingredients in topical delivery systems: cytotoxicity, solubility, and skin permeation studies. *Drug Development and Industrial Pharmacy*, *0*(0), 1–8.

Schwab, M., Sax, G., Schulze, S., & Winter, G. (2009). Studies on the lipase induced degradation of lipid based drug delivery systems. *Journal of Controlled Release*, *140*(1), 27–33.

Shamshina, J. L., Barber, P. S., & Rogers, R. D. (2013). Ionic liquids in drug delivery. *Exper. Opin. Drug Deliv.*, *10*(10).

Sigma-Aldrich. (2017). Salicylic acid | Sigma-Aldrich. Retrieved 3 September 2017, from http://www.sigmaaldrich.com/catalog/product/sigma/s5922?lang=pt&region=PT

Stoimenovski, J., MacFarlane, D. R., Bica, K., & Rogers, R. D. (2010). Crystalline vs. ionic liquid salt forms of active pharmaceutical ingredients: A position paper. *Pharmaceutical Research*, *27*(4), 521–526.

Tiwari, G., Tiwari, R., Bannerjee, S., Bhati, L., Pandey, S., Pandey, P., & Sriwastawa, B. (2012). Drug delivery systems: An updated review. *International Journal of Pharmaceutical Investigation*, *2*(1), 2.

Twist, J., & Zatz, J. (1986). Influence of solvents on paraben permeation through idealized skin model membranes. *Journal of the Society of Cosmetic Chemists*, *444*(December), 429–444.

Velasco, M. V. R., Tano, C. T. N., Machado-Santelli, G. M., Consiglieri, V. O., Kaneko, T. M., & Baby, A. R. (2008). Effects of caffeine and siloxanetriol alginate caffeine, as anticellulite agents, on fatty tissue: Histological evaluation. *Journal of Cosmetic Dermatology*, *7*(1), 23–29.

Vignoli, J. A., Bassoli, D. G., & Benassi, M. T. (2011). Antioxidant activity, polyphenols, caffeine and melanoidins in soluble coffee: The influence of processing conditions and raw material. *Food Chemistry*, *124*(3), 863–868.

Wagemaker, T. A. L., Rijo, P., Rodrigues, L. M., Maia Campos, P. M. B. G., Fernandes, A. S., & Rosado, C. (2015). Integrated approach in the assessment of skin compatibility of cosmetic formulations with green coffee oil. *International Journal of Cosmetic Science*, *37*(5), 506–510.

Zech, O., Thomaier, S., Bauduin, P., Rück, T., Touraud, D., & Kunz, W. (2009). Microemulsions with an ionic liquid surfactant and room temperature ionic liquids as polar pseudo-phase. *Journal of Physical Chemistry B*, *113*(2), 465–473.

Zhang, D., Wang, H., Cui, X., Wang, C., Zhang, D., Wang, H., … Wang, C. (2016). Evaluations of imidazolium ionic liquids as novel skin permeation enhancers for drug transdermal delivery. *Pharmaceutical Development and Technology*, *7450*(January), 1–10.

Zhu, W., Guo, C., Yu, A., Gao, Y., Cao, F., & Zhai, G. (2009). Microemulsion-based hydrogel formulation of penciclovir for topical delivery. *International Journal of Pharmaceutics*, *378*(1–2), 152–158.

7. Glossary

Choline-based Ionic Liquids: ionic liquids in which the cation is choline.

Drug Delivery System: a formulation or a medical device that introduces the drug in the body and increases the efficacy and safety of the drug controlling the rate, time and place of its release.

Halogenated Ionic Liquids: ionic liquids in which the anion is a halogen.

Imidazole-based Ionic Liquids: ionic liquids in which the cation is imidazolium.

Ionic Liquids: salts with organic cation and an organic or inorganic anion.

8. Scientific Outputs

Articles

Santos Almeida, T., Júlio, A., Saraiva, N., Fernandes, A.S., Araújo, M.E.M., Baby, A.R., Rosado, C., Portugal Moa, J. (2017). Choline- versus imidazole-based ionic liquids as functional ingredients in topical delivery systems: cytotoxicity, solubility and skin permeation studies. *Drug Development and Industrial Pharmacy, in press*. http://dx.doi.org/10.1080/03639045.2017.1349788

Santos Almeida, T., Júlio, A., Mota, J.P., Rijo, P., Reis, C. (2017). An emerging integration between ionic liquids and nanotechnology: general uses and future prospects in drug delivery. *Therapeutic Delivery*, 8(6), 461-473. DOI: 10.4155/tde-2017-0002.

Santos de Almeida, T., Júlio, A., Caparica, R., Rosado, C., Fernandes, A. S., Saraiva, N., Ribeiro, M., Araújo, M. E., Baby, A. R., Costa, J. G., Portugal Mota, J. (2015). Ionic liquids as solubility/permeation enhancers for topical formulations: skin permeation and cytotoxicity characterization. *Toxicol. Lett.*, S293. http://dx.doi.org/10.1016/j.toxlet.2015.08.841

Oral Communications

Júlio, A., Caparica, R., Fernandes, A.S., Saraiva, N., Araújo, M.E., Baby, A.R., Fonte, P., Rosado, C., Portugal Mota, J., Santos de Almeida, T. (2017*). Ionic liquids as functional ingredients in drug delivery systems*. Oral communication showed in Encontro Ciência 2017, Lisbon.

Santos de Almeida, T., Júlio, A., Pereira, M., Caparica, R., Pereira, N. Antunes, A. Rocha, F., Saraiva, N. Fernandes, A., Araújo, M.E. Baby, A. R., Reis, C., Rosado, C., Mota. J. (2016). *Functional ingredients in delivery systems*. Oral communication showed in II Jornadas CBIOS, Lisbon.

Posters

Júlio, A., Raposo, M., Portugal Mota, J.*, Santos de Almeida, T.* (*Shared senior authorship) (2017). *Ionic Liquids as Functional Ingredients in Lipidic Implants*. Poster showed in XXV Encontro Nacional da Sociedade Portuguesa de Química, Lisbon.

Júlio, A., Raposo, M., Antunes, A., Silva, H., Portugal Mota, J.*, Santos de Almeida, T.* (*Shared senior authorship) (2017). *Influence of Ionic Liquids in Drug Release from Lipidic Implants*. Poster showed in 6th FIP Pharmaceutical Sciences World Congress, Stockholm.

Júlio, A., Antunes, C., Caparica, R., Araújo, M.E., Saraiva, N., Fernandes, A., Baby, A.R., Rosado, C., Portugal Mota, J.*, Santos de Almeida, T.* (*Shared senior authorship) (2016). *Amino Acid Based Ionic Liquids as Functional Ingredients in Topical Formulations Containing Caffeine*. Poster showed in II Jornadas CBIOS, Lisbon.

Santos de Almeida, T., Júlio, A., Caparica, R., Rosado, C., Fernandes, A. S., Saraiva, N., Ribeiro, M., Araújo, M. E., Baby, A. R., Costa, J. G., Portugal Mota, J. (2015). *Ionic liquids as solubility/permeation enhancers for topical formulations: skin permeation and cytotoxicity characterization*. Poster showed in 51st Congress of the European Societies of Toxicology, Oporto.

Santos de Almeida, T., Júlio, A., Caparica, R., Fernandes, A.S., Saraiva, N., Araújo, M.E., Baby, A.R., Rosado, C., Portugal Mota, J. (2015). *Ionic liquids as solubility/permeation enhancers in topical drug delivery systems*. Poster showed in 19th European Symposium of Organic Chemistry, Lisbon.

Santos de Almeida, T., Júlio, A., Caparica, R., Ribeiro, M., Rocha, F., Araújo, M.E., Baby, A.R., Rosado, C., Portugal Mota, J. (2015). *Study of the applicability of ionic liquids as excipients in topical formulations*. Poster showed in V Congresso Nacional de Ciências Dermatocosméticas/IV Congresso da Sociedade Portuguesa de Ciências Cosmetológicas, Lisbon.

Portugal Mota, J., Júlio, A., Araújo, M.E., Rolim, A., Rosado, C., Santos de Almeida, T. (2014). *Ionic liquids as functional solvents/vehicles for topical and dermocosmetic products*. Poster showed in 28th Congress of the International Federation of Societies of Cosmetic Chemists, Paris.

Santos de Almeida, T.*, Júlio, A., Caparica, R., Ribeiro, M., Pereira, M., Pereira, N., Rocha, F., Araújo, M.E.M., Baby, A.R., Rosado, C., Saraiva, N., Portugal Mota, J.* (*Shared senior authorship) (2014). *Ionic liquids as functional ingredients in topical drug delivery systems*. Poster showed in I Jornadas CBIOS, Lisbon.

Júlio, A., Caparica, R., Ribeiro, M., Rocha, F., Rosado, C., Araújo, M.E.M., Baby, A.R., Santos de Almeida, T.*, Portugal Mota, J.* (*Shared senior authorship) (2014). *Ionic liquids as functional solvents in topical drug delivery systems*. Poster showed in I Jornadas CBIOS, Lisbon.

Appendix

Appendix I: ^{1}H-NMR Spectrums of the Synthetized Choline-based ILs

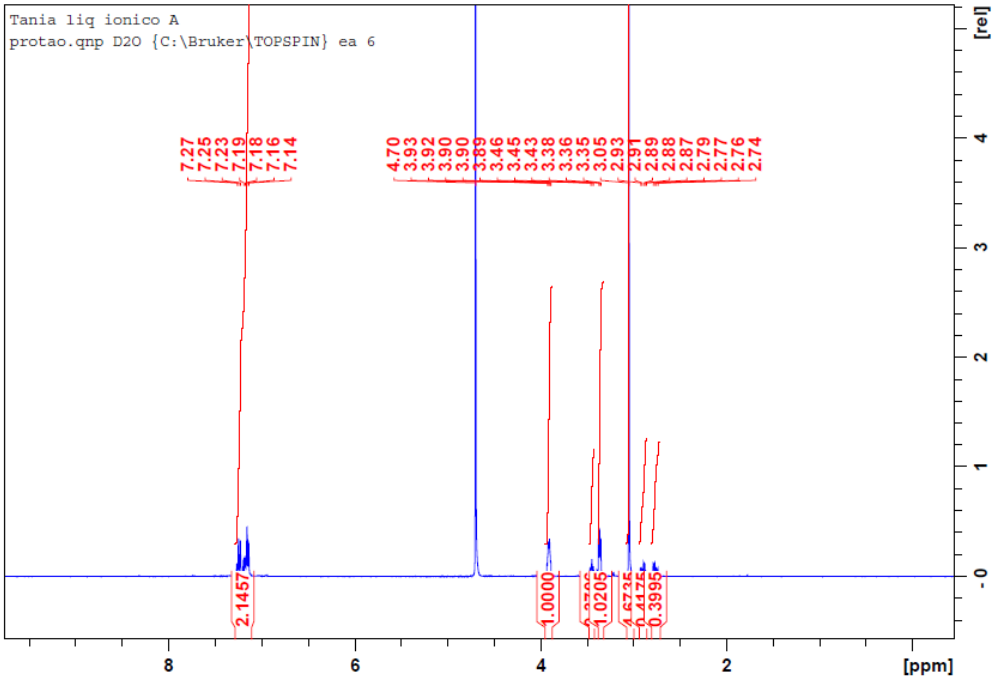

Figure 15: ^{1}H NMR Spectrum of the synthetized [Cho][Glu].

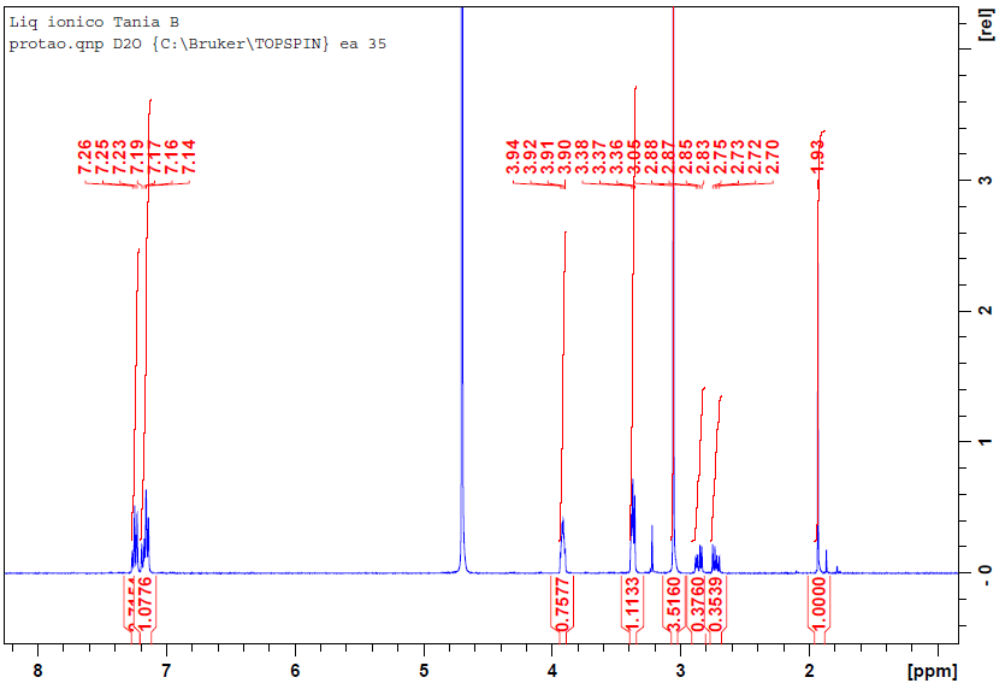

Figure 16: ^{1}H NMR Spectrum of the synthetized [Cho][Phe].

Appendix II: Physical and Chemical Properties of the Model Drugs

Active	Molecular Formula	Molecular Weight, g/mol	Melting Point, °C	Density, g/cm^3	Water Solubility at 25 °C, mg/mL
Caffeine	$C_8H_{10}N_4O_2$ [a]	194.19 [a]	238 [a]	1.23 [a]	20.02 ± 0.07[b]
Salicylic Acid	$C_7H_6O_3$[c]	138.12 [c]	158 [c]	1.44 [c]	2.07 ± 0.03 [c]

a. Sigma-Aldrich, 2017a; **b**. Dias et al., 2007; **c**. Sigma-Aldrich, 2017b